Author:
Cole, Laura M

Title:
Cortisol effects on pupil size and locus coeruleus activity

Cortisol effects on pupil size and locus coeruleus activity

By Laura Cole

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Health Sciences

Bristol Medical School
March 2021

Word Count: 39401

Abstract

The regulation of behaviour is a higher-order cognitive function that is guided by attention. Intrinsic and extrinsic factors, such as behavioural relevance and physical salience, as well as the reward value of an event or stimulus can modulate behaviour by influencing attention. In stress, attention is biased towards bottom-up salience and behaviour is modulated so that is stimulus-driven and conducive to survival, rather than being goal-directed. The Locus Coeruleus (LC) is a small norepinephrine (NE) nucleus residing in the dorsal pons that has an essential role in arousal and attention, as well as strong links with the stress responsive hypothalamic-pituitary-adrenal (HPA) axis. In a manner akin to the attentional control of behaviour, the LC responds to both top-down and bottom-up salience in animals, so theories have proposed a role for the LC in attentional control. However, whether this function translates to humans is unclear due to the challenges of imaging the brainstem, which prevent the non-invasive investigation of the LC.

The measure of pupil size provides a non-invasive index of LC activity, as stimuli with top-down and bottom-up salience evoke short bursts of firing in the LC as well as transient pupil dilations. In addition, a change in arousal induces coincident fluctuations in baseline LC activity and baseline pupil size. This thesis reports a series of experiments, in which human LC function was explored in human volunteers by implementing an adapted auditory oddball task with concurrent pupillometry. Transient pupil dilations were evoked by task relevant and low probability events, and the effect of task relevance on the pupil was amplified by incentive salience when a reward manipulation was added to the task. In addition to the transient effect on pupil dilation, reward incentives evoked an increase in baseline pupil size. This evidence indicates a role for the human LC in the processes underlying these different types of salience.

Cortisol is the main end product of the HPA axis in humans and administering cortisol receptor antagonists disrupted the transient effect of incentive salience on the pupil compared with placebo. In contrast, an antagonist of the NE beta adrenoceptor enhanced the transient effect of incentive salience on the pupil. In addition, administration of a stress-level dose of cortisol disrupted the tonic but not the phasic effect of incentive salience on the pupil. This suggests that cortisol and NE modulate the underlying processes of incentive salience, such as extrinsic motivation, via the LC. Implications for a maladaptive stress system, including the LC, is discussed in relation to the development of apathy, with disrupted attentional control and extrinsic motivation. In addition, this technique has allowed me to demonstrate both cortisol and NE regulation of LC activity. Therefore, this thesis demonstrates that human LC function can be investigated with the non-invasive measure of pupillometry.

COVID-19 statement

My research activities were substantially affected by the outbreak of COVID-19. My data collection was put on hold during the first lockdown. Given the lengthy period of time between when I was told to stop my research and resume, several participants had relocated and therefore withdrew from my clinical research project. As some of these participants had already started on the drug treatments, I was unable to re-recruit to make up these numbers. Therefore, 24 participants completed this study rather than 30.

In addition, resuming my research activities was extremely challenging given the additional safety measures that were put in place during the pandemic, the use of one of my research sites for the COVID-19 vaccine trial, an essential member of my research team being placed on furlough and a period of isolation. As a result, I did not have time to conduct another planned piece of research, despite receiving an extension. Therefore, this thesis would have included another results chapter that was prevented by the outbreak of COVID-19.

Finally, the pandemic impacted on my data analysis, as my data extraction script did not execute on my laptop, which I used to work from home. Ultimately, I had to send all of my data with the script to my supervisor who was able to run the extraction for me. Whilst this allowed me to conduct my data analysis, it greatly reduced the amount of time that I was able to dedicate to thoroughly considering and interpreting my data, as this process ended up taking weeks longer than it would have, in the last 2-3 months of my Ph.D.

To my parents

Acknowledgments

I'd like to thank the Ludichrist group, the Lightman group and my research team for their help and support throughout my Ph.D. Thanks to my supervisors Stafford Lightman and Iain Gilchrist, particularly for your patience and humour, and to Rosie Clark for helping me to decipher MATLAB code!

I wouldn't be here without the support of my family and friends. I'd like to thank Paul and Maisy for keeping me sane and my brother *Mr* Tommy Cole for his wise words and for being an endless source of inspiration. Thanks also to my grandparents, aunts, uncles and cousins.

Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE:

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Our environment contains too much information for us to process at the same time. Therefore, an attentional control system is needed to guide internal resources towards important stimuli within our environment and shift the internal control state to facilitate goal-directed or stimulus-driven cognition and behaviour (Corbetta & Shulman, 2002; de Wit & Dickinson, 2009). Important stimuli include those that relate to internal goals, beliefs and values, as well as external reward incentives. These types of stimuli capture attention in a top-down manner, facilitating goal-directed cognition and behaviour. In contrast, stimulus-driven events are physically salient and automatically capture attention in a bottom-up manner (Fecteau & Munoz, 2006). This not only includes events that visually stand out but those that automatically capture attention due to high salience, such as a sudden loud noise or the presence of a predator (Aston-Jones & Cohen, 2005). Rapid attentional capture by these types of stimuli promotes survival by enabling the fast identification of environmentally significant events and facilitating automatic, stimulus-driven and habit-based behaviours (Anderson, 2013; Evans et al, 2019). This ability to shift between control states in response to a constantly changing environment and internal goals is essential for the expression of adaptive and goal-directed behaviour (Dajani & Uddin, 2015; Goschke & Bolte, 2014).

Because of its central role, a dysfunctional attentional control system can result in abnormal attention, cognition, motivation and behaviour. Therefore, an important question for researchers is: which parts of the brain modulate attentional control? Cumulative evidence indicates the involvement of the locus coeruleus (LC), a small NE brainstem nucleus with a major role in arousal and attention, and strong links with the stress system.

This thesis will explore the role of the LC in bottom-up and top-down processes using pupillometry, the measure of pupil size, as an indirect marker of human LC activity. It will also investigate the connection between the LC and the stress system, which can become maladaptive in disease.

Definitions

The term *bottom-up* refers to processes that are driven by the sensory information from a stimulus (i.e., stimulus-driven). In contrast, *top-down* processes are goal-directed and driven by higher-order cognition. According to dual-system theories of control state, the attentional control system is dichotomous, with the top-down and bottom-up system competing for dominance (de Wit & Dickinson, 2009). Within the top-down category of attentional control, the literature often refers to both behaviourally relevant information and reward incentives as being goal-directed or having top-down salience, without clearly stating whether said events are goal-directed or have top-down salience because they relate to internal behavioural goals or external rewards. This is problematic because behavioural goals and reward incentives are separate cognitive constructs and recruit disparate brain regions (Awh et al., 2012). Therefore, it is important to distinguish between them. In this thesis I will do so by explicitly stating whether goal-directed or top-down events and effects are related to *internal* goals or *external* rewards unless they involve a combination of the two. In addition, I will refer the two types of top-down salience as *behavioural* and *incentive* salience.

The locus coeruleus

The locus coeruleus norepinephrine system

The central NE system consists of two ascending pathways: The dorsal and ventral NE bundles, both of which originate in the brainstem. The ventral bundle arises from brainstem nuclei in the pons and medulla, and projects to the amygdala, hypothalamus, midbrain, medulla and spinal cord. The dorsal NE bundle originates from a subset of LC neurons, collectively referred to as *A6*. The LC resides in the dorsal pontine tegmentum, and projects to the cerebral cortex, hippocampus and cerebellum, as well as brain regions innervated by the ventral bundle including the amygdala, hypothalamus and spinal cord (Szabadi, 2013). These broad-reaching projections are at odds with the relatively small size of the LC, which is one of the smallest nuclei in the brain, measuring approximately 14.5 mm in length, 2.5 mm in diameter and 2.0 mm in height (Fernandes et al., 2012). In addition, the LC is the primary site of NE synthesis in the central nervous system (Szabadi, 2013). NE exerts effects

on its targets by binding to G-protein coupled receptors: the alpha and beta adrenoceptors (Ramos & Arnsten, 2007).

Locus coeruleus neurons have two characteristic patterns of firing: phasic and tonic, which have separate but related functions. Tonic firing is made up of irregular but continuous, low frequency activity (1-3 Hz) that, according to animal studies, appears to correlate with changes in arousal across the sleep-wake cycle (Aston-Jones & Bloom, 1981; Hobson et al., 1975). For example, tonic activity increases across the sleep-wake continuum: through the stages of sleep to the transition from sleep to waking, and in states of waking, through low to high arousal states, such as automatic, habitual activities to mentally challenging tasks and conditions of stress. This suggests that the function of tonic activity in the LC is the modulation of arousal across the sleep-wake cycle (Foote et al., 1980; Hobson et al., 1975). In contrast, phasic firing consists of transient bursts of higher frequency activity (8-10 Hz) (Aston-Jones & Bloom, 1981; Foote et al., 1980). Animal studies have shown that phasic activity in the LC is rapidly evoked by top-down and bottom-up salience (Akaike, 1982; Aston-Jones & Bloom, 1981; Bouret & Richmond, 2015; Foote et al., 1980).

Animal studies of locus coeruleus function

Animal studies have explored the role of the phasic LC response. These investigations report transient bursts of activity in response to infrequent, novel, noxious, reward related, task relevant and unexpected events (Aston-Jones & Bloom, 1981; Bouret & Sara, 2004; 2005; Bouret & Richmond, 2015; Dayan & Yu, 2006; Hirata & Aston-Jones, 1994; Rajkowski et al., 1994; Vankov et al., 1995). Whilst this list of evoking stimuli might seem quite broad, studies have found that phasic responses in the LC are selective for these characteristics, as they are absent for other stimuli, which are presented within the same experiment as a comparison. For example, compared with task-relevant events, absent or rapidly habituated LC responses have been reported for similar sensory events that act as distractors and so are not task relevant (Aston-Jones et al., 1994). Several review articles have put all of this evidence together and proposed theories of phasic LC function.

Early theories postulate roles related to sensory processing, as stimuli that evoke phasic LC activity come from many different sensory modalities (discussed by Berridge & Waterhouse,

2003 and Kalwani et al., 2014). However, this view was discredited when studies found that the phasic LC response is plastic and follows changes in task utility. For example, when a former non-target stimulus becomes a target, the phasic response for the former target is abolished and emerges instead for the new target (Aston-Jones et al., 1997; Rajkowski et al., 2004). In most cases, a target is only a target when it is associated with a task objective. Therefore, it is task relevant and possesses behavioural salience. The plasticity of the phasic response with its sensitivity to behavioural salience is suggestive of a more complex role for the phasic LC response associated with higher-order cognitive processes related to behaviour. Therefore, several research groups have asked whether the phasic response functions in an internal cognitive process leading up to a motor event, such as the decision to action a behaviour, or even the motor event itself (Aston-Jones et al., 1994; Clayton et al., 2004; Kalwani et al., 2014; Rajkowski et al., 2004). However, this question has been confounded by the use of target stimuli by these studies. For example, most of the time, a target stimulus is behaviourally relevant because there is a response required to that stimulus, such as a button press. Since the stimulus presentation and ensuing behavioural response are typically close together in time, it is difficult to distinguish between the attentional and motor contribution to the phasic LC response. Therefore, it is unclear whether the phasic response to behavioural relevance is actually a result of the motor preparation or execution rather than behavioural salience.

The phasic LC response can also be evoked by bottom-up salience, such as unexpected and novel events (Aston-Jones & Bloom, 1981; Dayan & Yu, 2006). In addition, there is evidence that the phasic response to target stimuli is reduced if the target has high probability, suggesting that the phasic LC response is sensitive to the combination of bottom-up and top-down salience (Aston-Jones et al., 1994; Rajkowski et al., 2002). However, several research groups have stressed the importance of behavioural salience for task-evoked LC responses, and theories of LC function postulate that this, rather than bottom-up salience, drives the phasic LC response (Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Sales et al., 2019). This controversy arose due to evidence showing early, unselective phasic responses to target and non-target stimuli, followed by absent phasic responses to low probability non-target stimuli compared with large phasic responses to target stimuli (Rajkowski et al., 2004). Similarly, other studies have reported a habituated phasic response with repeated

presentations of bottom-up salience such as novelty, unless the stimulus additionally possesses top-down salience (Aston-Jones & Bloom, 1981; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005). However, it is also true that bottom-up salience can decline with repeated presentations of a stimulus, especially if a stimulus has bottom-up salience because it is novel and unexpected, as the stimulus becomes increasingly less novel and more expected over time. In contrast, top-down salience does not decline with repeated presentations, unless behavioural or task contingencies change. Therefore, the finding that the phasic LC response habituates to bottom-up salience does not disprove a role for the LC related to stimulus-driven events. In sum, evidence suggests that the phasic LC response is evoked by bottom-up salience, but the response may habituate over time as the evoking event becomes less salient and less relevant to behaviour.

Given that the phasic LC response is evoked by bottom-up and top-down salience, several research groups have proposed a role for the LC in saliency attribution or processing (e.g., Corbetta et al., 2008; Vazey et al., 2018). Mechanisms involved in saliency attribution and processing are functionally related to attentional control, which regulates the selection of salient stimuli and facilitates the reorientation of internal cognitive and behavioural resources accordingly (Bouret & Sara, 2004; Itthipuripat et al., 2017; Melloni et al., 2012). Therefore, the LC could indirectly facilitate adaptive behaviour by influencing attentional control. Furthermore, this could explain why the phasic LC response is strongly linked to behaviour, as mentioned above (e.g., Clayton et al., 2004). Most of the literature has suggested similar roles for the LC in modulating attention, cognition, motivation and behaviour. Thus, on the whole, research in this field suggests that the LC responds to important environmental events, including those that are physically and behaviourally salient because it has a direct or indirect role in the attentional control of behaviour (Bari et al., 2020; Bouret & Sara, 2004; Corbetta et al., 2008; Rajkowski et al., 1993; Sara & Bouret, 2012; Ventura et al., 2008).

Theories of locus coeruleus function

There are several all-encompassing theories of LC function that piece together the roles of phasic and tonic LC activity into one, unified model. Perhaps the most influential model is the adaptive gain theory by Aston-Jones and Cohen (2005). According to this theory, frontal

brain regions constantly evaluate task utility and send corresponding information to the LC. In response, the LC releases NE at cortical targets, regulating the gain of processing (signal-to-noise ratio) in the local neuronal circuits that facilitate task performance. Phasic and tonic rates of firing modulate the gain of these circuits according to the external environment by facilitating or disengaging task-relevant processes. In this way, particular patterns of phasic and tonic firing encode particular control states. Specifically, firing patterns predominated by phasic activity and intermediate tonic activity (phasic mode) promote task engagement, whereas high (tonic mode) or low rates of tonic firing are respectively associated with distractibility and drowsiness, promoting task disengagement and environmental exploration of new sources of reward. The phasic LC response acts as an attentional filter, which becomes biased for behaviourally relevant stimuli. Events that possess bottom-up salience, including novel and unexpected events, might evoke a phasic response in the LC if they are highly salient and elicit a reorientation of internal resources, such as a sudden loud sound or a threat.

The network reset theory of LC function takes a slightly different view of the same collection of evidence, postulating that phasic bursts of activity in the LC are elicited by important environmental stimuli and act as a reset signal for the active functional networks underlying ongoing task activities (Bouret & Sara, 2005). The phasic LC response prompts the reorganisation of large neuronal networks, causing a cognitive shift in task set in accordance with the eliciting stimulus. According to the network reset theory, active functional networks are not only reset but also maintained by phasic LC activity. Therefore, phasic firing in the LC can sustain or disrupt active functional networks in line with environmental demands. Tonic activity is also thought to contribute to network reset (Bouret & Sara, 2005).

The adaptive gain theory and network reset theory propose distinct functions for the phasic LC response in attentional filtering and regulating active functional networks. However, both theories propose that phasic LC activity can be evoked by bottom-up and top-down salience, and that changes in phasic and tonic firing rates modulate the mode of control (e.g., a task engaged and disengaged mode). In this way, the adaptive gain theory and network reset theory are only subtly different in that they respectively propose that firing

rates in the LC regulate motivation and task switching. These processes are related and ultimately interact to modulate the appropriate control of internal resources in a constantly changing environment.

Stress function

Stress is defined as "a state of real or perceived threat to homeostasis" (Smith & Vale, 2006, p. 383). Under stress, the normal function of the LC changes. It is widely agreed that the LC-NE system facilitates the cognitive and behavioural components of the stress response by stimulating adaptive changes in control state (Morris et al., 2020). Stress activation of the LC increases tonic firing and suppresses phasic firing. In line with the adaptive gain theory, evidence suggests that this causes a surge in arousal and distraction, suppressing the activation of the top-down control system (i.e., goal-directed processes; Elam et al., 1985; McCall et al., 2015; Valentino, 1988; Valentino & Foote, 1987; 1988). The result is overriding bottom-up control, with enhanced environmental scanning and the capture of attention by stimulus-driven events. Together, these changes promote survival by facilitating threat detection, as well as stimulus-driven and habit-based behaviours that conserve energy whilst enabling fight-or-flight responses (Braunstein-Bercovitz et al., 2001; Henckens et al., 2010; 2012; Hermans et al., 2014; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017; Schwabe & Wolf, 2009; 2011; van Marle et al., 2009; 2010). The adaptive modulation of phasic and tonic LC mode in stress is coordinated through reciprocal connections with the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA Axis

Underlying mechanisms

The HPA axis is made up of a complex set of interactions between the three main structures: the hypothalamus, the pituitary gland and the adrenal gland. The HPA axis is under control of two systems: the circadian pacemaker, and the stress system, which activates the HPA axis via inputs from the medial amygdala, the central nucleus of the amygdala, the lamina terminalis, the LC and various other brain stem nuclei. In contrast, inhibitory inputs from the hippocampus suppress HPA axis activity (Aguilera et al., 1995; Cunningham et al., 1990; Dong et al., 2001; Matheson et al., 1971; Kolbe et al., 2015; Nicolaides et al., 2014; Petrovich & Swanson, 1997).

Stimulation of the HPA axis initiates a cascade of tightly coordinated events. Upon activation, neurons originating in the paraventricular nucleus (PVN) of the hypothalamus release corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophysial portal circulation at the median eminence. Corticotrophin-releasing hormone and AVP stimulate corticotroph cells in the anterior pituitary gland to release of adrenocorticotrophic hormone (ACTH), which travels in the systemic circulation and targets glucocorticoid secreting cells within the zona fasciculata of the adrenal cortices. This results in the secretion of glucocorticoids from the adrenal cortex (Nicolaides et al., 2014) .

In non-stress conditions, glucocorticoid secretion follows an hourly, pulsatile (ultradian) rhythm, superimposed upon a diurnal circadian rhythm, which is conserved in all mammals including humans, with blood concentrations falling throughout the day, then building up overnight to peak on waking (Weitzman et al., 1971). Stressors evoke a surge in glucocorticoid secretion, much larger than that under circadian and ultradian control (Krieger et al., 1971; Weitzman et al., 1971; Windle et al., 1998; Young et al., 2004).

Since glucocorticoids are lipophilic, they readily pass through the phospholipid bilayer of cell membranes and bind to their receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), which typically reside in the cytoplasm (Timmermans et al., 2019). The GR is ubiquitous, present in most cell and tissue types, whereas the expression of MR is more specific (Reul & de Kloet, 1985). As a result, glucocorticoids can influence almost all organ systems, and they have a profound effect on the brain where they mediate neuronal differentiation, synaptic plasticity, arousal, cognition, emotion and behaviour (Fitzsimons et al., 2013; Kadmiel & Cidlowski, 2013; Karssen et al., 2001; Thau et al., 2019). In addition, glucocorticoids coordinate internal homeostatic mechanisms, such as metabolic, cardiovascular, immune and neuroendocrine activities (Sapolsky et al., 2000).

The precise effect that glucocorticoids exert on their targets depends on several factors. For example, as MR has a high affinity and GR has a low affinity for glucocorticoids, MR and GR are preferentially bound at different glucocorticoid concentrations. Therefore, the ratio of MR/GR signalling may change over the circadian cycle, or even the course of a single stress

or ultradian glucocorticoid pulse. Furthermore, although MR and GR bind to the same glucocorticoid response elements on DNA, they bind distinct sets of genes, with a small 30% overlap (Datson et al., 2001). As a result, glucocorticoids exert distinct effects via their receptors at different phases of a stress or smaller ultradian pulse (McMaster et al., 2011). Therefore, a blunted ultradian rhythm in disease alters relative GR and MR binding and induces more constant gene transcription, whereas pulses of glucocorticoids induce fluctuations in receptor occupancy, leading to differential and tissue-specific gene expression and phenotype. Indeed, research has demonstrated that pulsatile profiles of glucocorticoids produce fundamentally different transcription programs than that of smooth profiles (Stavreva et al., 2009). In addition, the effects of glucocorticoids also vary across brain regions because, as mentioned, whereas GR is present in most parts of the brain, MR is predominantly found in limbic areas, such as the amygdala, hippocampus and prefrontal cortex (Reul & de Kloet, 1985).

Fast and slow effects

Glucocorticoids exert their effects via fast, non-genomic mechanisms and slower, genomic mechanisms (Dallman, 2005). The traditional effects of glucocorticoid signalling occur via genomic pathways, which depend on MR or GR mediated transcription and *de novo* protein synthesis (Karst et al., 2005). Via this pathway, the ligand bound receptor translocates from the cytoplasm to the nucleus. Here, it influences gene expression indirectly via interactions with other transcription factors or directly by binding to specific DNA sites, such as glucocorticoid response elements, and by activating or repressing the transcription of specific gene sets (Mifsud & Reul, 2016).

The faster, non-genomic pathway occurs independently of DNA binding and *de novo* protein synthesis (Karst et al., 2005). These non-genomic or fast effects occur within seconds to minutes of glucocorticoid exposure, a time scale that is too fast to be accounted for by slower genomic mechanisms (Wiegert et al., 2005). Perhaps the most well-known example of these fast effects is the rapid suppression of the HPA axis by glucocorticoids, occurring within 5-10 minutes of corticosterone (the primary glucocorticoid in rodents) infusion in rats (Hinz & Hirschelmann, 2000).

Animal studies and a few human studies have demonstrated fast effects of glucocorticoids on cognition, emotion and behaviour. Interestingly, these effects tend to have the opposite directionality of the later, slow effects of glucocorticoids. Thus, the effects of glucocorticoids vary over time. The consensus is that the fast effects stimulate rapid, adaptive changes in cognition and behaviour, such as survival behaviours in stress, whereas the slow effects restore homeostasis (e.g., de Kloet et al., 2005; Henckens et al., 2010; 2012). However, there has been little research of these fast effects in humans. Therefore, there is a gap in our understanding of how the effects of cortisol (the primary glucocorticoid in mammals) on human cognition, emotion and behaviour vary over time.

Links with the autonomic nervous system and the locus coeruleus

The HPA axis is one of the two neuroendocrine stress response systems, together with the autonomic nervous system (ANS). Both of these systems are highly interconnected. Their activation by stress follows a highly coordinated, temporal sequence. Because the HPA axis is an endocrine system, its effects take minutes to emerge, rather than seconds, such as those of the ANS. This delay is due to the use of hormonal messengers, which travel through systemic circulation, a much slower mode of transport than neuronal signal transmission. As a result, the first effects of the stress response are those of the ANS, which are short-lived, followed by those of the HPA axis by glucocorticoids, which are more sustained (Ulrich-Lai & Herman, 2009).

The ANS is innervated by a number of inputs, many of which similarly project to the HPA axis and activate the stress response. The major activating stress signals arise from the central nucleus of the amygdala, the LC and the ventrolateral medulla (Jones & Yang, 1985; Ulrich-Lai & Herman, 2009). Upon activation, the ANS promotes rapid physiological changes through its sympathetic branch and by increasing circulating levels of epinephrine and NE, which are secreted principally from the adrenal medulla as well as sympathetic nerve terminals. The result is a rapid activation of the fight-or-flight response, which increases blood pressure, heart rate, peripheral vasoconstriction and energy mobilisation. The parasympathetic branch serves to protect homeostasis by modulating the duration of the sympathetic response (Ulrich-Lai & Herman, 2009).

The LC is an important part of the stress response and regulator of the ANS. It sends direct projections to the sympathetic preganglionic neurons in the spinal cord and parasympathetic preganglionic neurons in the brain stem and spinal cord (Lewis & Coote, 1990; Unnerstall et al., 1984). In stress, the LC is activated by inputs from the PVN of the HPA axis (Valentino, 1988; Valentino & van Bockstaele, 2008). As mentioned, stress activation of the LC causes an increase in tonic firing and central NE. The result is enhanced sympathetic activity and an increase in CRH synthesis in the PVN and glucocorticoid secretion from the adrenal gland (Ward et al., 1976). In this way, the HPA-axis promotes LC activity, and the LC-NE system promotes HPA axis activity. In addition, the LC expresses both MR and GR, and its activity is regulated by glucocorticoids (Avanzino et al., 1987; Makino et al., 2002). It has been suggested that fast effects of glucocorticoids on the brainstem stimulate and sustain a state of behavioural arousal during stress (Aston-Jones & Bloom, 1981; Grant et al., 1988; Rasmussen et al., 1986).

The ANS and the LC work synergistically with the HPA axis in the physiological response to stress. The interactions described above coordinate the peripheral (fight-or-flight) and central (cognitive and behavioural) arms of the stress response to bring about an adaptive state of physiological, cognitive and behavioural preparedness.

The HPA axis in disease

In some circumstances, the stress response becomes abnormal and stimulates maladaptive physiological, cognitive and behavioural changes (de Kloet et al., 2005). Pathological stress systems have been described in numerous diseases, including depression, post-traumatic stress disorder, Alzheimer's disease, schizophrenia, Parkinson's disease and stroke (e.g., Bradley & Dinan, 2010; Herrero et al., 2015; Keller et al., 2017; Olsson et al., 1992; Pomara et al., 2003; Speer et al., 2019). Reports of underlying pathology include hypercortisolemia, hypocortisolemia, impaired HPA axis inhibition, dysregulation of HPA axis activity, abnormal circadian cortisol and a blunted cortisol awakening response (e.g., Bao & Swaab, 2019; Carroll et al., 2007; Hatfield et al., 2004; Kwon et al., 2015; Rohleder et al., 2004). These abnormalities are clearly linked to the HPA axis, and perhaps areas of the brain that regulate or are regulated by HPA axis activity, such as the LC. Although the ANS is also a major part of the stress response, this system has not been so widely associated with disease. Symptoms

of ANS disorders are well-defined and typically characterised by peripheral symptoms. For example, bradycardia can result from abnormal parasympathetic activation or sympathetic withdrawal, and hypotension results from sympathetic withdrawal (Kitchen et al., 2006; Mano & Iwase, 2003). In contrast, the HPA axis in disease is associated with maladaptive neuroplastic changes in the brain (Radley et al., 2015). Symptoms tend to be related to cognition and behaviour, implicating the diseased HPA axis in psychiatric mechanisms (Stokes & Sikes, 1991).

The HPA axis in reward

Evidence indicates a link between the HPA axis and reward (Bao & Swaab, 2019; Bunce et al., 2015; Kinner et al., 2016; Koob & Kreek, 2007; Montoya et al., 2014; Putman et al., 2010). This link was initially hypothesised after reports describing HPA axis and cortisol dysregulation in affective disorders and addiction, as the common theme between these diseases could be a dysfunctional reward system (Bogdan & Pizzagalli, 2006; Carroll et al., 2007; Kwon et al., 2015; Lovallo, 2006). For example, in depression an aberrant reward system could impair motivated reward-seeking behaviours. In contrast, in addiction it could bias motivation in favour of addictive behaviours (Whitton et al., 2015). In line with this notion, Putman et al. (2010) found that the administration of 40 mg (a high, stress-dose) oral hydrocortisone (synthetic cortisol) increased risky decision-making in men, suggesting that exogenous cortisol biases behaviours in favour of reward-seeking. In turn, this suggests that, with a maladaptive HPA axis, the normal function of cortisol could change and promote abnormal reward-seeking behaviours, such as in addiction.

Reward and motivation

Definitions

Motivation is a complex cognitive construct. It is the biasing, top-down influence driven by external rewards or internal values and beliefs, such as behavioural goals (Ryan & Deci, 2000). In addition, bottom-up mechanisms can bias motivation if they are subsequently perceived as being behaviourally relevant, such as a threat (Sussman et al., 2016). For clarity, I will define motivation according to the factor that is driving it—that is, extrinsic motivation (rewards) or intrinsic motivation (behavioural goals).

Extrinsic motivation or wanting a reward, must be distinguished from reward itself, which is the hedonic effect that comes with reward delivery (Berridge & Robinson, 2016). In research, the investigation of reward has been broken up into distinct stages, mainly comprising a reward cue step, and a reward delivery step. Reward cues can act as reward incentives and these terms are often used interchangeably in the literature (Zhang et al., 2009). However, they are not one and the same. The term *reward incentive* relates to the motivational value associated with the reward cue, whereas *reward cue* refers to the stimulus that is presented to signal upcoming reward (Robinson et al., 2014). This reward can be high or low or even completely negligible. Therefore, a reward cue does not inherently possess incentive salience and drive extrinsic motivation: if a reward cue signals low or absent reward, especially compared with a reward cue that signals a larger reward, it is less likely to be perceived as having incentive salience. In contrast, stimuli that signal large rewards generally have incentive salience. However, the extent to which a given reward is rewarding varies to some degree across individuals, thus the degree of incentive salience attributed to a given reward cue can vary across individuals (Robinson et al., 2014). However, generally the greater or more intense a reward is, the more rewarding it is perceived as being. For example, high monetary rewards are typically perceived as more rewarding than low monetary rewards, thus reward cues that signal high monetary rewards typically have high incentive salience.

Therefore, reward cues can have incentive salience but do not always, and reward incentives are more closely linked to the wanting or motivational part of the reward-seeking process than reward per se. In contrast, reward delivery is closely associated with the actual rewarding or hedonic effect (Berridge & Robinson, 2016). Despite this, the literature often associates both effects observed at the presentation of reward cues and those observed at reward delivery with reward. This is a confusing generalisation, given that these steps reflect motivation and reward, which are separate constructs and so are likely underpinned by distinct neuronal circuitry. In the present thesis, I will refer to the terms *reward cue* as the sensory stimulus used to signal reward, and *reward incentive* as the motivational value associated with the reward cue. In addition, I will distinguish between the motivational (presentation of reward cues) and rewarding (reward delivery) stages of reward investigations.

Finally, reward sensitivity is ill-defined in the literature, having been given a number of different definitions, such as reward anticipation and the tendency for extrinsic motivation to bias behaviour in favour of a reward (Depue & Collins, 1999; van Hulst et al., 2015). In this thesis, like Van Lippevelde et al. (2020), I will define reward sensitivity as the propensity to detect reward signals in the environment, as this definition is similar to several others in the literature (e.g., Fauth-Bühler, 2019). Reward sensitivity can be gaged by measuring incentive salience and is a marker of extrinsic motivation, as it indicates the extent to which internal resources are biased to reward.

The locus coeruleus in reward

As mentioned, phasic responses in the LC have been observed for behaviourally relevant stimuli, such as targets (Aston-Jones et al., 1994; Kalwani et al., 2014; Rajkowski et al., 2004). Target stimuli represent a behavioural goal, and although reward incentives recruit comparatively disparate brain regions, they similarly represent a goal, albeit reward related. Therefore, behavioural and incentive salience can capture attention and bias behaviour in a similar goal-directed, top-down manner (Awh et al., 2012). As a result, it is plausible that, like target stimuli, reward incentives activate a phasic LC response, especially if the LC functions in the attentional control of behaviour. Indeed, there is some evidence of a link between phasic LC activity and reward incentives. This suggests a role for the LC related to both internal and external goals, such as goal-directed behaviour for both types of events.

Early electrophysiological animal studies of LC function presented rewards with target stimuli during task training to encourage animals to make a behavioural response to targets. However, these studies concluded that recorded phasic LC responses were evoked by behavioural salience rather than reward delivery (e.g., Aston-Jones et al., 1994; 1997). A few more recent animal studies were specifically carried out to investigate the relationship between the LC and reward-related events (reward cues and delivery). The majority of these studies reported phasic LC responses to reward incentives but not reward delivery (Bouret & Richmond, 2015; Varazzani et al., 2015). This suggests that phasic LC responses occur as a result of incentive salience and extrinsic motivation rather than the actual rewarding effect.

One research group carried out a series of studies investigating the relationship between LC activity and reward in monkeys using electrophysiological recordings (Bouret & Richmond, 2015; Jahn et al., 2018; Varazzani et al., 2015). For example, in one paper from this group, Varazzani et al. (2015) trained monkeys to perform a series of squeezes on a grip for reward delivery (drops of water), and found that when monkeys made these squeezes, the required amount of force covaried with phasic LC activity. This research team went on to implement the same reward task on monkeys administered with clonidine, which reduces central NE (Jahn et al., 2018). The treated monkeys squeezed the grip with less force, expending less effort during the reward task. Therefore, whilst they still engaged in the task, monkeys demonstrated less effortful engagement, indicating that a reduction in central NE disrupted extrinsic motivation.

Similarly, Bouret and Richmond (2015) recorded LC activity in monkeys whilst manipulating the size of expected reward (drops of water). They found that LC activity was higher at cue onset when the cue signalled a larger reward. In contrast, when monkeys released a lever to receive the reward after the reward cue was presented, LC activity was higher if smaller rewards were expected. Bouret and Richmond (2015) explained these paradoxical results by proposing a role for the LC in mobilising the resources required to perform goal-directed actions for rewards. For example, cue presentation stimulated the LC, which mobilised the amount of energy proportional to the expected reward size. Prior to lever release, slightly more energy is required to reach threshold for movement, so if this threshold remains constant across all trials, the supplementary amount of energy required to reach threshold is inversely related to the total energy mobilised on cue onset. Therefore, more energy is required just before action onset when smaller reward cues are presented. Regardless of whether the LC is explicitly involved in mobilising energy resources for goal-directed, reward-seeking responses, the evidence clearly suggests that phasic LC activity is more closely related to extrinsic motivation and reward-seeking behaviour than the actual rewarding effect.

The locus coeruleus norepinephrine system in addiction

Additional evidence for the role of the LC in reward comes from addiction studies.

Some of the earliest NE addiction studies investigated the effect of opiates on the LC in animals. They found that opiate withdrawal caused hyperactivity in the LC in rodents (Akaoka & Aston-Jones, 1991). This hyperactivity was reported to drive the expression of physical withdrawal symptoms, such as motor hyperactivity, mastication and piloerection (Maldonado & Koob, 1993). In addition, evidence has demonstrated the importance of central NE for opiate-seeking behaviours. For example, studies have shown that central NE depletion abolishes the preference for morphine-related contexts in mice, as well as the stress-induced reinstatement of heroin-seeking behaviour in rats (Sahraei et al., 2004; Shaham et al., 2000). Whilst historically it was thought that activity in the dopaminergic system was necessary for opiate abuse, some studies have reported conflicting results (Dworkin et al., 1988; Pettit et al., 1984). Therefore, another neurotransmitter system, such as NE, is likely to be involved.

In psychostimulant studies of addiction, the LC-NE system is essential for drug-induced locomotor activity and sensitisation (an increased drug effect with repeated doses; Drouin et al., 2002). Like the opiate addiction studies, investigations have reported that NE depletion abolishes the preference for amphetamine-related contexts, as well as the reinstatement of psychostimulant self-administration in mice, with an emphasis on the stress-induced reinstatement of drug-seeking behaviours (Davis et al., 1975; Erb et al., 2000; Ventura et al., 2003). For example, the reinstatement of stress-induced (foot shock) cocaine-seeking behaviour is abolished in rats by clonidine, a presynaptic alpha adrenoceptor agonist that reduces sympathetic activity and central NE (Erb et al., 2000). Similarly, NE has been implicated in the stress-induced reinstatement of other drugs of abuse (Lê et al., 2011; Leri et al., 2002; Shaham et al., 2000; Shepard et al., 2004). This suggests that the combined effects of NE and stress are required for this reinstatement of drug-seeking behaviour.

Since the LC is closely linked with the endogenous stress system and is essential for the stress response, it is unsurprising that some LC-NE effects, whether adaptive or maladaptive, are specifically triggered by stress, such as the stress-induced reinstatement of addictive behaviours. Indeed, Schwabe et al. (2010; 2012) demonstrated that a stress-induced shift in control state, from a goal-directed to a stimulus-driven mode is dependent

on both NE and cortisol, implicating the LC and the HPA axis. They administered healthy human male and female volunteers with a 20 mg stress dose of hydrocortisone, or 20 mg yohimbine (an antagonist of the presynaptic alpha adrenoceptor that increases central NE) or both. Participants were then trained on a task to receive two different food outcomes, one of which was subsequently devalued by consumption to satiety. A following session of extinction training showed whether participants displayed goal-directed or habitual behaviour, as food devaluation should have changed behaviour accordingly, so that participants were less inclined to make goal-directed actions for delivery of the devalued food. This behavioural modulation in line with task goals is indicative of goal-directed rather than habitual behaviour. In contrast, responses for the devalued food would be indicative of habitual behaviour. Schwabe et al. (2010; 2012) found that hydrocortisone and yohimbine rendered behaviour habitual, whereas monotherapy with hydrocortisone or yohimbine resulted in goal-directed behaviour. As mentioned, this suggests that the combined effects of cortisol and NE are required for the stress-induced shift in control mode towards a habitual, stimulus-driven state. Otherwise, behaviour remains goal-directed.

With this in mind, perhaps the HPA axis and LC function in reward by facilitating the appropriate attentional control of goal-directed (including reward-seeking) or stimulus-driven states. As such, a pathological HPA axis and/or LC, disrupts the adoption of appropriate control states and in doing so promotes maladaptive reward-seeking behaviours. If we relate this to the addiction studies that report a stress-triggered reinstatement in drug-seeking by NE, it suggests that this reinstatement occurs due to the combined effects of NE and cortisol, which drive a pathological shift in control state in favour of drug-seeking behaviour rather than stimulus-driven behaviour in stress. Therefore, perhaps cortisol brings about a change in control state via the LC by stimulating changes in LC phasic and tonic mode.

Apathy

Similarly, cumulative evidence indicates that a pathological mechanism involving the LC and cortisol promotes the development of apathy (Bouret & Richmond, 2015; Jahn et al., 2018; Kaye & Lightman, 2006; Putman et al., 2010; Schwabe et al., 2010; 2012; Tiemensma et al., 2014). Historically, apathy has lacked a universal definition, although it is now widely agreed

that it encompasses a group of amotivation syndromes, characterised by a profound lack of motivation with functional impairment. Classification also requires the expression of at least 2 out of 3 of the following symptom clusters: impaired goal-directed behaviour, cognition and emotion (Robert et al., 2009; 2018).

A lack of clinically valid measures of apathy has somewhat restricted its research (e.g., Carrozzino, 2019). This is problematic because apathy is highly prevalent, common to many diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression and traumatic brain injury (e.g., den Brok et al., 2015; Ishizaki et al., 2011; Kant et al., 1998; Kiang et al., 2003; Nobis et al., 2018). In addition, apathy occurs with functional impairment, which has a substantial negative impact on quality of life (Robert et al., 2009; 2018). Despite this, there are no effective treatments available on prescription for apathy (e.g., Theleritis et al., 2019). There is even an absence of any prospective treatment, as there has been very little research of the molecular mechanisms of apathy, especially universal mechanisms shared by different diseases (Heron et al., 2018). Therefore, there are no drug targets. Research in this field could lead to the discovery of novel treatment strategies, with the enhancement in the quality of life of thousands of patients worldwide.

With this in mind, a good starting point for uncovering the underlying mechanisms of apathy is to investigate patients who commonly present with apathy and show well-defined pathophysiology. For example, patients with primary adrenal insufficiency, which is characterised by insufficient cortisol, often present with apathy (Cleghorn, 1951; Farah et al., 2015; Tiemensma et al., 2014). Although these patients receive cortisol replacement therapy, the normal function of cortisol is still lost, as the therapy does not replicate the physiological ultradian rhythms of cortisol. As a result, treated patients with primary adrenal insufficiency still display symptoms, such as apathy (Tiemensma et al., 2014). This evidence suggests that abnormal cortisol activity is involved in apathy pathophysiology. What's more, when patients with primary adrenal insufficiency are deprived of cortisol replacement therapy, they lose the pressor response to stressors, a cardiovascular sympathetic reflex that elevates blood pressure (Kaye & Lightman, 2006). This suggests that primary adrenal insufficiency patients show abnormal sympathetic and cortisol activity. Therefore, perhaps both of these abnormalities are involved in the underlying mechanism of apathy.

Since the LC is a cortisol target that promotes sympathetic activity, it is a good candidate brain region for the study of apathy. Precisely how dysfunctional LC activity could result in apathy is unclear, although as mentioned it could promote maladaptive attentional control, resulting in abnormal cognition, motivation and behaviour—in other words, an abnormal control state.

Conceptual frameworks have been useful in identifying prospective avenues for research by defining key processes in motivated behaviour that could become dysfunctional in apathy. For example, Husain and Roiser (2018) outlined a framework for apathy centred around effort-based decision-making for rewards. According to this framework, the decision-making process involves several steps, including the generation of options for behaviour, option selection, anticipation of action or reward, action execution and effort, the hedonic or rewarding effect and a learning phase, which reinforces behaviour and guides future option selection. Abnormalities within any one of these stages could result in or contribute to the development of an apathetic-type syndrome.

Evidence from the LC literature could suggest a role for the LC-NE system in option generation and selection, given the proposed role of the LC in the attribution of salience and a behavioural decision-making process; the anticipatory phase, which involves motivational arousal; or initiating action and effort, given the proposed role of the LC in mobilising energy resources for goal-directed actions (Aston-Jones & Cohen, 2005; Bouret & Richmond, 2015; Jahn et al., 2018; Vazey et al., 2018). At present, it is not possible to say whether disrupting any one of these steps involves the pathological LC and results in apathy, as investigation of the LC in motivation, particularly extrinsic motivation, is still in its early stages. However, this framework does support apathy research focusing on the LC and the relationship between the LC and salience or motivation. In addition, in terms of experimental design, the framework posits that reward anticipation could become disrupted in apathy, so it supports the assessment of apathy by measuring reward sensitivity, as it suggests that extrinsic motivation is reduced in apathy. In support of this notion, Rochat et al. (2013) found that increasing reward insensitivity correlated with symptoms of apathy in stroke patients. This is useful because, as mentioned, present measures of apathy include self-reports, which lack clinical validity and reliability (Carrozzino, 2019).

Pupillometry

The challenges of investigating the human locus coeruleus

Compared with animal studies, which have the use of more invasive techniques, investigation of the human LC has been limited by the particular challenges of imaging the brain stem (Turker et al., 2019). Functional imaging of the brain is typically conducted using blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI), which has a spatial and temporal resolution that is limited by the haemodynamic response. Because the brainstem is so densely packed with nuclei and comprises a relatively small volume, brainstem nuclei tend to have small cross-sections compared with other brain regions (Butler & Taube, 2015; Fernandes et al., 2012; Watson, 2012). Unfortunately, the spatial resolution of BOLD fMRI is not sufficient to distinguish between adjacent brainstem nuclei. Furthermore, the precise shape and location of the LC varies across individuals, so the use of published coordinates and brain atlases in locating the LC is problematic (Keren et al., 2009; Swallow et al., 2003; Turker et al., 2019). Despite this, the majority of studies investigating human LC function have used such localisation techniques (K. Liu et al., 2017). This has led to the investigation of unreliable regions of interest, which are likely to capture surrounding brain stem nuclei as well as the LC. Indeed, the LC and surrounding nuclei appear homogenous in T1-weighted images. Thus, accurate identification of the voxels containing the LC is difficult (Swallow et al., 2003; Turker et al., 2019). What's more, the brainstem has a topological arrangement, which groups nuclei with similar functions (Fernández-Gil et al., 2010). As a result, the LC has overlapping functions with surrounding nuclei, such as the inferior colliculus and the nucleus incertus, both of which also function in arousal and control state (Ryan et al., 2011; Turker et al., 2019). Therefore, researchers cannot distinguish the LC from surrounding nuclei by implementing a task that is selective for LC activation.

Another challenge for fMRI studies of the LC is minimising physiological noise. The brainstem is located close to major arteries and the ventricles, which introduce signals from cardiac-related artifacts and cerebrospinal fluid, respectively. It is also closer to the lungs than the rest of the brain, increasing the susceptibility of fMRI to respiratory artifacts. As a result, brainstem fMRI suffers from a poor signal-to-noise ratio (Harvey et al., 2008).

Attempts to combat physiological noise include a process called spatial smoothing, which reduces noise effects by averaging across adjacent voxels (Mikl et al., 2008). However, for small brainstem nuclei, spatial smoothing can prevent the identification of the true region of interest. In addition, as the LC is so close to the fourth ventricle, smoothing could actually introduce more noise into the signal. In investigations of the human LC, spatial smoothing has been used inconsistently (K. Liu et al., 2017; Turker et al., 2019).

Most investigations of human LC function have used single-echo fMRI, which measures the magnetic resonance signal in each voxel once per acquisition (K. Liu et al., 2017). In contrast, multi-echo fMRI measures the magnetic resonance signal at least two times per acquisition, reducing the influence of physiological noise and signal dropout from regions of interest (Turker et al., 2019). Investigations of the human LC with fMRI have been inconsistent in their use of single echo denoising techniques (K. Liu et al., 2017). Their failure to implement a unified approach has led to unreliable data across the research field. Indeed, Turker et al. (2019) compared different fMRI acquisition (single and multi-echo) and denoising techniques, reporting various estimates of LC function obtained by these approaches.

Recent research suggests that neuromelanin-sensitive fMRI is a more effective means of localising the LC, as the LC contains a pigment called neuromelanin, which has paramagnetic properties, whereas surrounding nuclei do not (Sasaki et al., 2006). This approach increases the contrast of neuromelanin-containing voxels in the LC compared with adjacent nuclei, allowing the identification of the LC from surrounding nuclei (Keren et al., 2009; Turker et al., 2019). However, neuromelanin-sensitive imaging is expensive and very few fMRI studies investigating the LC have implemented this approach (Turker et al., 2019).

Due to the challenges of imaging the LC in humans, a large part of this research field has used pupillometry, the measure of pupil size, to investigate human LC function instead.

Pupillometry studies of the locus coeruleus

Like the firing rates of LC neurons, pupil responses can be high amplitude and transient (phasic, pupil dilatory responses) or lower in amplitude and sustained (tonic, baseline changes in pupil size). The rationale behind using pupillometry to investigate LC activity

comes from cumulative findings, which have shown that baseline pupil size, like tonic LC activity, is sensitive to changes in arousal and control state, and that phasic pupil responses occur for some of the stimulus properties that evoke phasic activity in the LC, such as bottom-up and top-down salience (e.g., Beatty, 1982; Qiyuan et al., 1985). Therefore, findings from human pupillometry studies have been compared with those of electrophysiological animal studies of the LC (Aston-Jones et al., 1994; 1997; Beatty, 1982; Qiyuan et al., 1985; Rajkowski et al., 1994; 2004). It is thought that, since the onset of arousal- and stimulus-evoked changes in LC activity occur at similar points in time as evoked changes in pupil size, they must share a common neuronal pathway. Therefore, arousal- and stimulus-evoked changes in pupil size provide a marker of LC activity.

Most comparisons by human studies have focused on LC responses predicted by the adaptive gain theory (e.g., Chiew & Braver, 2013; Gabay et al., 2011; Gilzenrat et al., 2010; Murphy et al., 2011, 2014). To recap, the adaptive gain theory posits that LC phasic mode, which occurs at intermediate levels of tonic activity, promotes the exploitation of current behavioural rewards and therefore task engagement. In contrast, high and low tonic activity promote the exploration of alternative rewards and therefore task disengagement. Disengagement occurs at low levels of tonic activity due to drowsiness, and at high levels of tonic activity due to extreme arousal and distractibility. Since phasic mode is thought to facilitate task engagement, it should be associated with improved behavioural performance compared with high and low tonic activity, which is associated with poor performance (Aston-Jones & Cohen, 2005). Therefore, the adaptive gain theory predicts phasic or transient pupil dilatory responses with relatively small baseline pupils during optimal task engagement and performance, and smaller or absent phasic pupil responses with relatively large, or even smaller baseline pupils during poor task engagement and performance.

Task paradigms implemented by several human pupillometry studies of the LC have been specifically designed to investigate whether indicators of task engagement (mostly behavioural performance) influence phasic pupil responses and especially baseline pupil size. Researchers then manipulate task engagement whilst measuring the effects on behavioural performance and pupil size (and therefore LC activity), to see whether there is a correlation. A demonstrated association is used to infer a relationship between behavioural

performance and LC function. For example, in the first of a series of experiments, Gilzenrat et al. (2010) found that smaller baseline pupil size and larger phasic pupil responses were associated with superior task performance in a simple pitch discrimination task implemented in humans. This suggests that phasic LC mode, which is marked by phasic LC activity (and therefore phasic pupil responses) and low tonic LC activity (small baseline pupils) is associated with task engagement. However, very low tonic activity in the LC (smallest baseline pupil size) is associated with drowsiness, task disengagement and therefore poor behavioural performance. Similarly, Murphy et al. (2011) carried out a simple auditory task in humans and found that pupil size correlated with task performance. Specifically, they reported that large phasic pupil responses, evoked by targets, correlated with poor task performance, as did the largest and smallest recoded baseline pupil size. In contrast, intermediate baseline pupil size was associated with superior task performance, as predicted by the adaptive gain theory. The correlations between baseline pupil size and task performance support the adaptive gain theory; however, the correlation between phasic pupil responses and task performance does not.

Another experiment by Gilzenrat et al. (2010) involved a simple pitch discrimination task, with added concepts of cost (increasing task difficulty) and reward, which were implemented to manipulate task engagement. For example, the authors postulated that reward would promote engagement, as would task difficulty (as greater difficulty requires greater mental effort) up to a point, after which task disengagement would occur. In anticipation of this, the authors introduced an escape button which, once pressed, allowed participants to truly escape from the task before starting the same task again, beginning with low but constantly increasing difficulty and reward values. It was expected that escape button presses would occur when increasing reward became increasingly unattainable due to task difficulty, which increased up to a point at which pitch discrimination was impossible as the two tones were identical. Pupillometry data from these tasks revealed that baseline pupil size increased up to and peaked upon escape, then fell over the next few trials at the beginning of the subsequent task. Similarly, phasic pupil responses increased and then diminished near escape. These pupil responses are indicative of task disengagement as predicted by the adaptive gain theory. After escape, baseline pupil size decreased and phasic pupil responses increased, indicative of phasic LC mode and task engagement. This

supports the adaptive gain theory and suggests that pupil responses and LC activity are influenced by factors that affect task engagement and therefore a goal-directed control state.

In contrast to these human pupillometry studies, most of the animal studies in this field investigated the processes underlying the phasic LC response (Aston-Jones et al., 1994, 1997; 1999; Clayton et al., 2004; Kalwani et al., 2014; Rajkowski et al., 1994; 2004; Vazey et al., 2018). In addition, although the adaptive gain theory posits that changes in the magnitude and frequency of phasic and tonic activity in the LC alters task engagement and therefore control state, it also suggests that a phasic response locked to a salient (top-down or bottom-up) event reflects an attentional filtering process, biased towards important environmental stimuli (Aston-Jones & Cohen, 2005). Few human pupillometry studies have investigated this hypothesis by manipulating top-down and bottom-up salience whilst measuring the effects on pupil size, especially in the context of a simple sensory task (e.g., O'Bryan & Scolari, 2021; Liao et al., 2016). For example, although Liao et al. (2016) investigated the effects of salience on phasic pupil responses, they implemented a task in which participants ignored auditory or visual stimuli, whilst responding to stimuli in the other modality by making various button presses. This is just one example of many, in which human pupillometry studies have implemented more complex task paradigms than those used by the animal studies.

This is problematic because pupillometry is an indirect measure of LC activity, thus any change to the task paradigm compared with the animal studies could lead to the recruitment of disparate brain regions and a change in the mechanisms underlying the pupil response. To mitigate this problem, it is important to first carry out a similar task paradigm to those implemented by animal studies. Once reliable pupil responses to top-down and bottom-up salience have been established, more complex task manipulations can be implemented to see how they affect the established pupil responses. Furthermore, given that human pupillometry studies often use more complex paradigms than animal studies and that many have focused on predicted changes in baseline pupil size by the adaptive gain theory, there is a gap in the literature for human pupillometry studies of the LC that simply

replicate the task design of electrophysiological animal studies, which reported phasic LC responses to top-down and bottom-up salience.

The oddball task

Much of the LC electrophysiological and pupillometry literature has used the oddball task to investigate the relationship between LC activity and pupil size (Aston-Jones et al., 1994; Aston-Jones & Cohen, 2005; Gilzenrat et al., 2010; Hong et al., 2014; Liao et al., 2016; Murphy et al., 2011, 2014; Rajkowski et al., 2004). In its most simplistic form, this task involves presenting an infrequent target (oddball) stimulus amongst frequent distractor stimuli. For example, Rajkowski et al. (1994) presented oddball stimuli with a 10-20% probability to monkeys. Typically, subjects are tasked with identifying the oddball stimulus and they must signal its presentation by making some sort of behavioural response, such as a button press. Most oddball tasks are auditory or visual, in which case, the oddball stimulus might differ from distractors in auditory frequency, colour or shape.

The oddball task is well suited to investigations of LC function, as it provides stimuli that possess top-down (behavioural) and bottom-up salience, characteristics that have been shown to evoke phasic responses in the LC (Aston-Jones et al., 1994; Rajkowski et al., 1994). For example, the target oddball stimulus has behavioural salience because it is task relevant, whereas bottom-up salience can be manipulated by adjusting stimulus probability. Therefore, measuring stimulus-evoked, phasic changes in pupil size by a low probability oddball stimulus provides a marker of phasic LC activity in response to bottom-up and top-down salience. In addition, by collecting epochs of baseline pupil size outside of stimulus presentation, investigators can assess tonic LC activity, which indexes arousal and control state (Aston-Jones & Cohen, 2005; Gilzenrat et al., 2010).

Most of the early animal studies, which measured LC activity via electrophysiological recordings, implemented a visual version of the oddball task—for example, Rajkowski et al. (2004) presented visual oddballs on a computer monitor, in the form of two-dimensional vertical or horizontal rectangles. However, presenting visual stimuli in human pupillometry studies is problematic as the pupil constricts in response to light exposure. Therefore, a visual oddball task could evoke changes in pupil size between trials by luminance rather

than task manipulations. Consequently, the majority of human pupillometry studies have implemented auditory oddball tasks, in which the oddball stimulus differs from distractors in auditory frequency (Gilzenrat et al., 2010; Hong et al., 2014; Liao et al., 2016; Murphy et al., 2011, 2014).

An important question for pupillometry research is whether the characteristic LC responses to visual oddball stimuli exist for auditory oddball stimuli. One electrophysiological animal study presented auditory tones to rats and reported coinciding phasic LC responses in a passive listening task (Aston-Jones & Bloom, 1981). However, as this task was passive, unlike most oddball tasks, there was no target stimulus nor any type of behavioural response, so the auditory stimulus did not have top-down salience. Furthermore, stimulus probability was not manipulated, so the auditory stimulus did not have bottom-up salience. These are important properties for evoking LC activity. Having said this, Aston-Jones and Bloom (1981) described a stimulus novelty effect on the LC by the auditory tones, with phasic responses habituating over time as the stimulus was repeated. This suggests that the phasic response seen here could have been the result of a stimulus novelty or bottom-up salience effect, which in turn suggests that phasic LC responses can be evoked by salient auditory tones. In support of this, a human fMRI study, which used neuromelanin-sensitive imaging, reported LC activation in response to auditory oddball presentations (Mather et al., 2020). This validates the implementation of auditory oddball tasks in pupillometry research of the LC.

Evidence of a causal link between the locus coeruleus and pupil

Precisely how LC activity leads to changes in pupil size is unclear, and evidence of a causal relationship between the LC and the pupil has been lacking until recently. For example, before 2015, only one study had compared electrophysiological recordings of LC activity with pupil size in the same animal. This was an unpublished animal study carried out by Rajkowski et al. (1993) who reported a relationship between tonic LC activity and baseline pupil size (they did not examine phasic LC activity). Since this research remains unpublished, it is difficult to determine the reliability of this data. Even so, pupillometry studies of LC activity persisted. In 2016, research led by Siddhartha Joshi investigated the causal link between the LC and pupil size by carrying out single unit recordings from several brain regions (the inferior colliculus, superior colliculus, anterior cingulate cortex and posterior

cingulate cortex), with simultaneous pupillometry in monkeys (Joshi et al., 2016). Whilst the authors found associations between neuronal activity and pupil size for several brain regions, non-luminance changes in pupil size most reliably correlated with the LC. For example, electrical stimulation of the LC evoked changes in pupil size at every site tested, whereas results were more variable for other brain regions, such as the inferior and superior colliculus. This evidence is supported by similar electrical stimulation of the LC in rats (Y. Liu et al., 2017).

Joshi et al. (2016) also presented unexpected auditory tones to monkeys during passive fixation and found that phasic responses in the LC showed a trial-by-trial correlation with pupil size. In contrast, whilst the same tones evoked responses in other regions, the responses did not vary trial-by-trial. Only activity in the LC covaried with pupil size both over long (several seconds) and short (single spikes) timescales, suggesting that phasic and tonic LC responses correlate with phasic and tonic changes in pupil size.

Two other animal studies comparing LC activity with pupil size reported a relationship between phasic LC activity and phasic pupil responses. The first recorded LC activity and pupil size in monkeys performing a reward task (Varazzani et al., 2015). The authors reported coinciding phasic LC responses and phasic pupil responses at the presentation of a reward cue and action onset (actions were made for reward delivery). Phasic responses in the LC also occurred sporadically during passive tasks, supposedly as a result of rapid fluctuations in control state. The second study reported similar findings, with coinciding phasic LC activity and phasic pupil responses in rats during quiet waking and locomotion (Reimer et al., 2016). In addition, there have been human fMRI studies of LC activity that have compared LC activity with changes in pupil size (Murphy et al., 2014). Whilst such investigations typically report a correlation between task evoked LC activity and pupil size, fMRI investigations of LC activity have been unreliable, as discussed in detail above. However, this collective evidence supports a relationship between phasic LC activity and phasic pupil responses.

Some studies have sought out the pathway that connects the LC with the pupil (Joshi et al., 2016; Y. Liu et al., 2017). The most obvious link is the ANS, which recruits the LC and

constricts or dilates the pupil via respective parasympathetic and sympathetic contributions. For instance, the pupil is constricted by the pupillary sphincter muscle, which receives excitatory parasympathetic input from neurons of the ciliary ganglion. In turn, the ciliary ganglion is innervated by the Edinger-Westphal nucleus, residing in the midbrain. Therefore, pupil constriction is driven by the parasympathetic activity of Edinger-Westphal neurons (or inhibition of the dilatory sympathetic input). The pupillary dilator muscle receives excitatory sympathetic input from neurons of the superior cervical ganglion. In turn, the superior cervical ganglion is innervated by neurons of the intermediolateral cell column of the spinal cord. Therefore, pupil dilation occurs when the sympathetic neurons of the superior cervical ganglion stimulate the pupillary dilator muscle to contract or when parasympathetic input of the pupillary sphincter muscle is inhibited (McDougal & Gamlin, 2015). Indeed, a recent investigation confirmed that pupil dilation evoked by the LC is dependent on inhibitory pathways from the LC that project to the ipsilateral and contralateral parts of the Edinger-Westphal nucleus. These pathways suppress the parasympathetic activation of pupil constriction by the Edinger-Westphal nucleus, thus dilating the pupil (Y. Liu et al., 2017; Nobukawa et al., 2021).

Altogether, the evidence suggests that the LC dilates the pupil through autonomic connections. In addition, whilst pupil size is not a direct measure of LC activity, the literature provides substantial evidence to support the use of pupillometry as a marker of LC activity.

Pupillometry studies of the locus coeruleus and reward

I am interested to see if there is any evidence that supports a relationship between pupil size and incentive salience, given the purported role of the LC in the attentional control of goal-directed behaviour for external rewards. This evidence will provide further support for the use of pupillometry in investigations of LC function in reward processes, such as extrinsic motivation.

Several human pupillometry studies have investigated the effect of extrinsic motivation on pupil size, reporting phasic pupil responses to reward cues, with larger dilations evoked by larger reward incentives (Chiew & Braver, 2014; Dix & Li, 2020; Gilzenrat et al., 2010; Manohar & Husain, 2015). For example, Manohar and Husain (2015) implemented a

monetary incentive task, in which participants were presented with an auditory reward cue, then ignored a distractor and made a saccade to a target location in order to obtain a 0p, 10p or 50p reward. Participants exhibited larger phasic pupil dilations when presented with the 50p reward incentive. Dix and Li (2020) reported similar phasic pupil responses by reward incentives. Their interpretation of these findings is particularly interesting as it suggests a role for the LC in extrinsic motivation. Specifically, they proposed that larger phasic pupil responses in high reward conditions reflected a greater effect of incentive salience on the pupil, resulting from an enhanced resource allocation to extrinsic motivation. Notably, this interpretation is strikingly similar to the hypothesised role of the LC in reward by electrophysiological animal studies. To reiterate, Bouret and Richmond (2015) suggested that the LC mobilises the resources required to perform goal-directed actions. Together, these pieces of evidence suggest that the phasic LC response and the phasic pupil response to incentive salience are similar. Therefore, pupillometry can be used to investigate the effect of incentive salience on LC activity.

Evidence for an association between baseline pupil size and incentive salience is variable, with some studies reporting an increase in baseline pupil size by larger reward incentives, and others reporting no change in baseline pupil size (Chiew & Braver, 2014; Gilzenrat et al., 2010; Walsh et al., 2019). Most of those that have reported a change in baseline pupil size by incentive salience have explained findings by relating them to changes in control state predicted by the adaptive gain theory (e.g., Gilzenrat et al., 2010). For example, larger rewards motivate task engagement and improved behavioural performance (unless they are used as a task distractor), thereby promoting a goal-directed, reward-biased control state. In this way, these studies comply with the LC literature, which propose that tonic LC activity covaries with control state and linearly with arousal (Aston-Jones & Bloom, 1981; Aston-Jones & Cohen, 2005; Chiew & Braver, 2014; Foote et al., 1980; Hobson et al., 1975). However, the reason for the inconsistent effects of incentive salience on baseline pupil size is unclear.

In sum, studies that have investigated the relationship between pupil size and reward mostly report an association between the phasic pupil response and incentive salience, which is a marker of reward sensitivity and closely associated with extrinsic motivation. In

contrast, the association between baseline pupil size and incentive salience is less clear. These findings parallel electrophysiological recordings of phasic and tonic LC activity in reward tasks. Therefore, this evidence provides further support for a link between the LC and pupil, and pupillometry can be used to investigate human LC function in extrinsic motivation.

Research plan

This thesis reports a series of pupillometry experiments that administer different versions of an auditory oddball task to human volunteers. In Chapter 2, I report three experiments. One of the main objectives of these experiments was to design and implement a similar oddball paradigm on humans compared with the early animal studies of LC function. If the recorded pupil responses to task stimuli resemble the phasic and tonic LC responses reported by animal studies, this would validate the use of my oddball task in combination with pupillometry as a marker of human LC activity. The remaining experiments in this chapter explore the task components that drove the pupil response and developed a reward manipulation for the oddball task. These experiments were implemented to develop and test an oddball task in order to validate its use for the investigation of human LC function, particularly in motivation, in the later experiments reported in Chapter 3 and 4.

Chapter 3 reports a clinical study that implemented the oddball task with a reward manipulation and concurrent pupillometry in healthy human volunteers. The main objective was to investigate the underlying mechanisms of salience effects on the pupil, especially behavioural and incentive salience as indicators of intrinsic and extrinsic motivation. As mentioned earlier in this chapter, evidence suggests that these mechanisms could involve cortisol and NE signalling, although there has been very little research in this field (Bouret & Richmond, 2015; Jahn et al., 2018; Kaye & Lightman, 2006; Putman et al., 2010; Robert et al., 2008; 2019; Schwabe et al., 2010; 2012; Tiemensma et al., 2014). Therefore, I administered cortisol and NE receptor antagonists to participants and investigated the effects of salience on the pupil with these treatments compared with placebo. A difference in the phasic and/or tonic pupil response to salience between a treatment and placebo could indicate cortisol or NE involvement in the underlying processes of behavioural, incentive or bottom-up salience. These findings could have implications for the treatment of

apathy, which is characterised by a profound lack of motivation with marked reward insensitivity (Adam et al., 2013; Marin, 1991; Martínez-Horta et al., 2014; Muhammed et al., 2016; Rochat et al., 2013).

Chapter 4 reports another experiment that implemented an oddball task with a reward manipulation and concurrent pupillometry in healthy human volunteers. The main objective was to investigate whether a stress dose of cortisol differentially altered the effects of salience on the pupil over time, as an indicator of distinct fast and slow effects of cortisol. To test this, I administered a stress-level dose of hydrocortisone on healthy human volunteers and examined pupil responses to salience on both the rising and falling phase of the ensuing stress pulse of cortisol. A difference in the effects of salience on the pupil between the rising and falling phase would indicate that cortisol exerted distinct fast and slow effects. The motivation for this study was the lack investigation of the fast and slow effects of cortisol in humans, especially on reward sensitivity and extrinsic motivation. In addition, this study allowed the assessment of cortisol effects on LC activity, which is indexed by the phasic and tonic pupil response (Joshi et al., 2016; Y. Liu et al., 2017). This is an important area of research as although the LC and HPA axis are highly interconnected there has been very little investigation of how this connection influences cognition and behaviour in humans (e.g., Schwabe et al., 2010; 2012). These findings could have implications for disease marked by a maladaptive stress system.

Chapter 2

Introduction

In this chapter, I report three experiments that investigate pupil sensitivity to different types of salience in various versions of an oddball task with concurrent pupillometry. The first experiment was designed to investigate whether the pupil shows the characteristic phasic and tonic responses to oddball task stimuli reported by electrophysiological animal studies of the LC. Phasic and tonic LC responses are reflected by task-evoked, phasic pupil responses and in epochs of baseline pupil size. Therefore, I indirectly measured phasic and tonic changes in LC activity by examining phasic pupil responses and changes in baseline pupil size, respectively. The second experiment was designed to investigate why oddball stimuli evoke phasic pupil responses: is it due to the aspect of top-down, behavioural salience or the associated motor response, which is generated to signal identification of the target stimulus? The third experiment investigated the effect of adding a reward manipulation to the oddball task on the pupil. Here, my aim was to develop a reward task that showed reliable pupil responses to incentive salience, to validate the use of this task with pupillometry in the later experiments reported in Chapter 3 and 4. The findings reported in this chapter have implications for LC function, which has received relatively little attention in human studies compared with other parts of the brain, due to the challenges of examining LC activity with non-invasive techniques.

Literature review

As discussed in Chapter 1, the LC is the primary source of NE in the CNS and the sole source in the cortex (Samuels & Szabadi, 2008). Despite this, there has been little investigation of the LC in humans due to its small size compared to cortical structures, strong physiological noise and the proximity of surrounding brainstem nuclei. These issues prevent traditional non-invasive techniques, such as fMRI from obtaining accurate measurements of the LC (Turker et al., 2019). However, researchers have identified pupillometry as an alternative measure of LC activity (Joshi et al., 2016).

Both phasic LC activity and phasic pupil responses can be evoked by bottom-up salience (e.g., Aston-Jones & Bloom, 1981; Dayan & Yu, 2006; Qiyuan et al., 1985; Vankov et al.,

1995). These phasic pupil responses scale with subjective salience, so that larger dilations follow the presentation of more salient stimuli (Liao et al., 2016). The range of salient stimuli that have been used to evoke these phasic responses is quite broad; however, perhaps the most shared characteristic of these stimuli is low probability. Such stimuli are salient because they tend to stand out compared with more frequently presented, expected stimuli. So, as stimulus probability decreases, bottom-up saliency increases, and so does the phasic pupil response (Qiyuan et al., 1985). Similar phasic LC responses by bottom-up salience have been reported in animal studies (Aston-Jones & Bloom, 1981; Dayan & Yu, 2006; Vankov et al., 1995). However, as mentioned in Chapter 1, the relationship between phasic LC activity and bottom-up salience has largely been overlooked by the literature in favour of behavioural salience, as the consensus is that there is a stronger relationship between behavioural salience and phasic LC activity than bottom-up salience. This notion has been driven by the finding that phasic responses to bottom-up salience habituate unless the stimulus is also behaviourally relevant (Aston-Jones & Bloom, 1981; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005). It has been suggested that the phasic response is only evoked by bottom-up salience if the stimulus is so highly salient that it elicits a behavioural orienting response. Indeed, highly salient events generally cause a reorientation of internal resources and behaviour (Sara & Bouret, 2012).

Evidence from both human pupillometry studies and animal studies with electrophysiological LC recordings indicates that presentations of target stimuli evoke phasic activity in the LC (Aston-Jones et al., 1994; Gilzenrat et al., 2010; Liao et al., 2016; Rajkowski et al., 1994). In most cases, a target is only a target (and so has top-down, behavioural salience) when there is a response required to that stimulus. As a result, in most laboratory tasks, the target stimulus is often temporally confounded with a motor response, such as a button press, which must be made on target presentation for successful task completion. This is typical for oddball tasks, which have been implemented in many of the studies mentioned above. In such instances, phasic responses to the target oddball stimulus may be a consequence of this motor output rather than the behavioural salience per se. Evidence from pupillometry studies suggests that the onset of the pupil response and motor output are closer than that of stimulus presentation, indicating that these phasic responses are at least more likely to be related to behaviour rather than sensory processing (Clayton et al.,

2004). However, in these instances, it is unclear whether an overt behavioural response is required to evoke a phasic change in LC activity or pupil size, or whether behavioural salience alone is sufficient. The latter would implicate the LC in intrinsic motivation rather than motor preparation or execution.

As mentioned in Chapter 1, compared with target stimuli, reward incentives possess a similar but distinct type of top-down salience, as they also motivate goal-directed behaviour (Awh et al., 2012). Therefore, perhaps like target stimuli, reward incentives evoke phasic LC and pupil responses. Indeed, recent studies have reported phasic LC responses to reward incentives. For example, Bouret and Richmond (2015) found that reward cues modulated phasic LC responses, with larger responses for cues that signalled larger rewards. Pupillometry studies have also reported associations between reward incentives and phasic (and sometimes tonic) changes in pupil size. Like LC activity, pupil size increases in response to reward cues, with larger dilations for cues predicting greater rewards (Chiew & Braver, 2014; Dix & Li, 2020; Gilzenrat et al., 2010; Manohar & Husain, 2015). Most reward studies have focused on the dopaminergic system, but the above evidence opens a novel avenue of investigation, as it indicates that the LC, which is a major NE nucleus, has a role in extrinsic motivation.

The present study addresses some of the issues discussed above directly by implementing an adapted auditory oddball task combined with pupillometry in groups of human volunteers. In Experiment 1, I investigated the contribution of bottom-up salience (stimulus probability) and behavioural salience to the pupil response. However, as participants manually responded to the target oddball stimulus, I could not determine whether any change in pupil size related to behavioural salience or motor output. So, in Experient 2, I compared two conditions, one in which the participants manually responded to the target and one which they silently counted the total number of targets instead. Finally, to investigate the relationship between incentive salience and pupil size (and LC activity by proxy), in Experient 3, I compared pupil responses to reward cues in high and low reward conditions.

General method

Participants

All participants had normal or corrected-to-normal vision and were paid for participation. Informed consent was obtained for all studies, and all were approved by the Faculty of Health Sciences Research Ethics Committee, University of Bristol, UK. Participants were recruited from the University of Bristol community via poster advertisements, webpage advertisements and email lists.

Materials

All experiments were written using Psychophysics Toolbox v3 (Brainard, 1997) running under Matlab 2015a (The MathWorks, Inc., Natick). The computer display was a ViewPixx 22.5-inch liquid crystal display screen, with a resolution of 1920 x 1200 pixels (VPixx Technologies, Inc); viewing distance was 60 cm. Pupil area (left pupil) and eye position were recorded at a sampling rate of 500 Hz by a tower-mounted Eyelink 1000 (SR Research, Canada) eye tracker, which has a typical operating spatial resolution of 0.5^{o} or better. Head movement was minimised using a chin and forehead rest. Manual responses were recorded via a computer keyboard. See Figure 2.1 for pupillometry set up.

Figure 2.1

Pupillometry Set Up

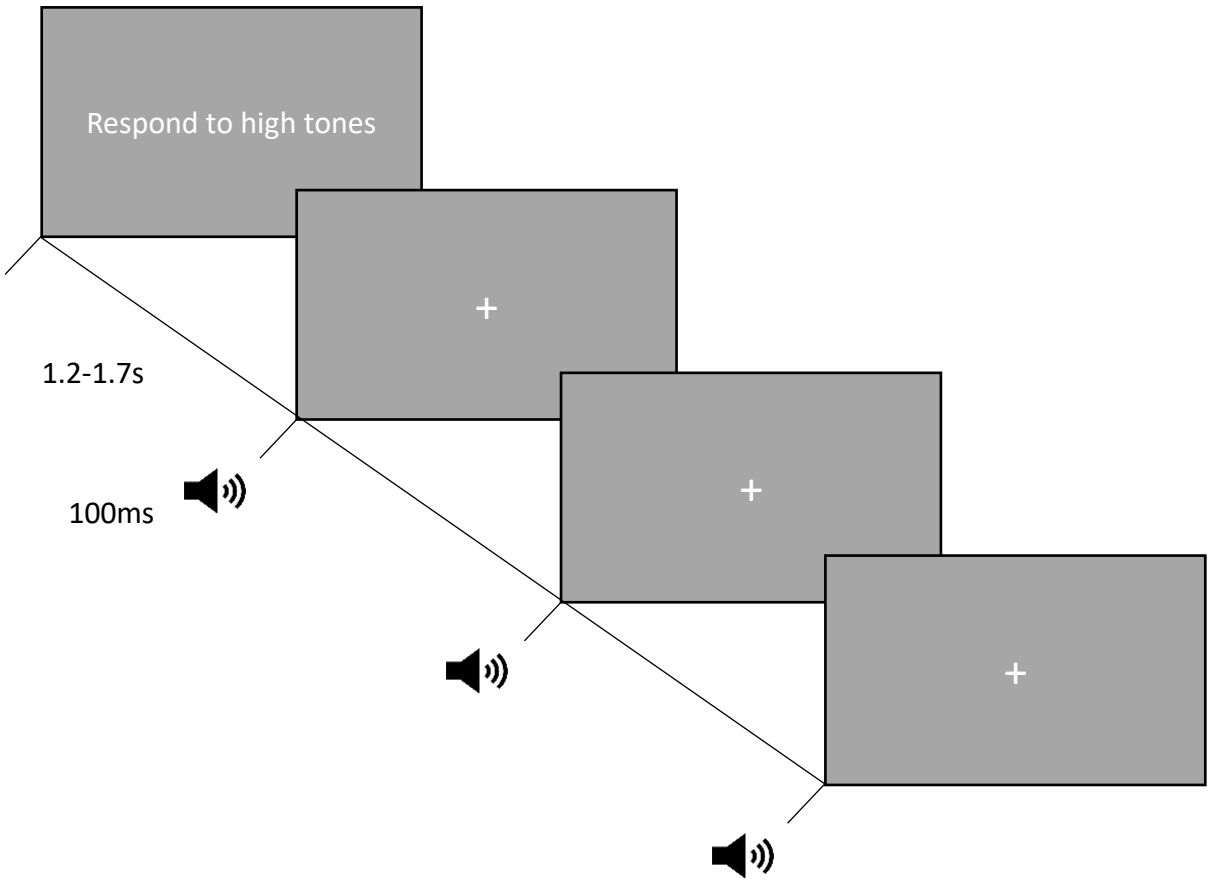
Design

In each of the following experiments, participants were asked to carry out an auditory oddball task (see Figure 2.2 for a diagram of the oddball task design). In the task, participants were played a sequence of short tones (100 ms) and required to listen for, and respond to, a rarely occurring tone (the oddball target), which occurred alongside another rarely occurring tone (deviant tone) and a frequently occurring tone (standard tone), neither of which required a response. The standard tone had a frequency of 1,400 Hz and the oddball and deviant were either 1,200 or 1,600 Hz (counterbalanced within or between participants, depending on the experiment), and the interstimulus interval was randomly varied between 1.2 and 1.7 s (uniformly distributed). In a given block, 8% of the trials were deviant and 8% were oddball tones and 84% were standard tones. Participants were instructed to maintain fixation on a fixation cross that was present at the centre of the

screen throughout each block of trials. Trial order within a block was randomised. Each testing session was made up of a practice block and experimental blocks.

Figure 2.2

Diagram showing the Oddball Paradigm



Note. The figure shows the instruction screen followed by three example trials, which present a 100ms auditory tone of the oddball (8%), deviant (8%), or standard (84%) trial type. Each trial is separated by 1.2-1.7s.

Data processing and statistical analysis

The raw pupil data was subjected to the following pre-processing. I coded as *missing* data where the eye tracker detected a blink using the default Eyelink setting. Around these detected blinks, there were additional periods of instability in the recording pupil size, which I coded as *missing* pupil data 200 ms either side of the blink onset and offset. The recorded pupil size also depends on the eye position and so I coded as *missing* all pupil samples where the eye position was two degrees of visual angle away from the central fixation point. I then carried out a linear interpolation across the missing data based on the first and the last recorded pupil measurement. In these studies, I was interested in event-related changes in pupil size that occurred within about 5 s of the onset of the tone. Some participants tend to have long term changes in pupil size across the whole block duration probably due to changes in overall arousal levels. To remove this effect, I next carried out a linear detrending of the data across the duration of the block of trials. Using a number of different artificial pupils, which were laser printed on paper, I converted the arbitrary units of pupil size produced by the Eyelink eye tracker into pupil size in mm^2.

For each trial (tone), I next extracted the pupil size data time locked to the tones, from 500 ms before the tone onset to 4,000 ms after the tone onset. For each participant, I then averaged these responses for each Trial Type (Oddball, Deviant or Standard). To allow for statistical analyses, I averaged the pupil response in each Trial Type, for each participant, across three 500 ms epochs. The first of these Epochs was the *Pre-stimulus Baseline Epoch* (-500 to 0 ms). The second of these Epochs was the *Stimulus Response Epoch* which was placed in the data to capture the peak response to the stimulus across all the Trial Types within a particular experiment. The third of these Epochs was the *Post-stimulus Baseline Epoch* (3,500 to 4,000 ms).

Experiment 1

Participants

There were 27 participants. Three participants were excluded because they made more than 20% manual response errors on the task, resulting in a final sample of 24 participants (15 females; age range 18-69).

Design

Trial Type (i.e., task relevance and stimulus probability) was manipulated within an auditory oddball paradigm to investigate the effect of Trial Type on pupil area. Participants carried out 1 practice block of 25 trials followed by 2 experimental blocks of 250 trials. In one block, the high tone (1,600 Hz) was defined as the Oddball Trial Type, and in the other the low tone (1,200 Hz) was defined as the Oddball Trial Type. Block order was counterbalanced between participants.

Procedure

Participants were seated in front of a computer monitor and a tower-mounted eye-tracking system. They were instructed to place their chin on a chinrest, fixate on a central fixation cross and respond to the oddball stimulus by pressing any key on the computer keyboard in front of them.

Once participants indicated that they were ready, all lights were switched off, and they completed the practice block, followed by pupil calibration and validation measures, and the first experimental block.

As soon as block 1 was initiated, instructions appeared on the screen to remind participants to respond to the oddball stimulus. All visual stimuli were then removed from the screen other than the fixation cross. At the end of block 1, participants were allowed a one-minute break (in the dark) before commencing block 2 of the task, which followed the same procedure as block 1.

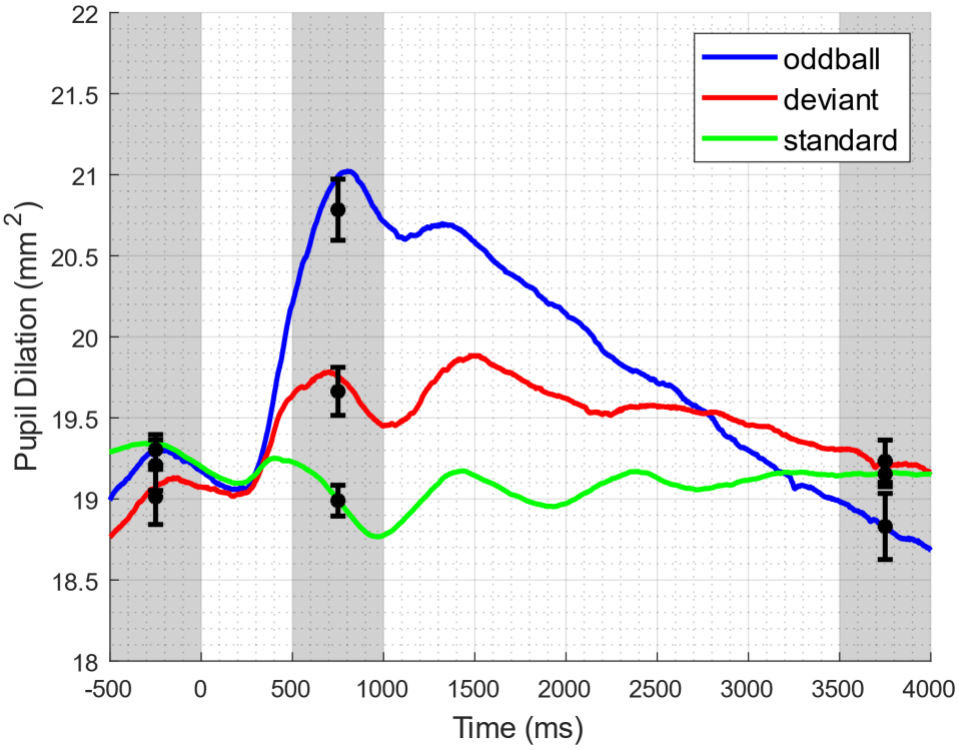
Results

For each Trial type, 4500 ms of the mean pupil data (500 ms of Pre Stimulus Baseline pupil data and 4000 ms of pupil data from the onset of the stimulus, as described above) was averaged again across all participants to give the grand average. Grand average pupil responses for the three Trial Types are plotted in Figure 2.3. The figure shows a clear response to the stimulus onset for the Oddball Trial Type alongside a smaller response to the Deviant when compared to the Standard Trial Type. The peak of these responses is around 750 ms post onset so the Stimulus Response Epoch in this experiment was defined

as being between 500 and 1,000 ms. All three Epochs are shown shaded in grey in Figure 2.3, with the mean pupil area for each Epoch and Trial Type plotted in black.

Figure 2.3

Grand Average Pupil Responses to the Oddball, Deviant and Standard Stimuli Averaged in Experiment 1.



Note. The Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. The mean pupil area for each Epoch is plotted in black with error bars to show the range.

I carried out a repeated measures Analysis of Variance (ANOVA) on the average pupil size with the following factors: Trial Type (Oddball, Deviant, Standard); Epoch (Pre-stimulus Baseline, Stimulus Response, Pre-stimulus Baseline) and Block (Block 1, Block 2). There was a significant main effect of Trial Type- $F(2, 46) = 7.78$, $p = 0.001$; partial ETA squared= 0.253), Block - $F(1, 23) = 14.98$, $p = 0.001$; partial ETA squared = 0.394 and Epoch - $F(2, 46) = 32.53$, $p < 0.001$; partial ETA squared = 0.586. The only significant interaction was between Trial Type and Epoch - $F(4, 92) = 25.35$, $p < 0.001$; partial ETA squared = 0.524. The main effect of Block reflects an overall tendency for the pupil to be more dilated in Block 1 ($M = 20.47$ mm^2) than Block 2 ($M = 18.23$ mm^2).

In order to explore the interaction between Trial Type and Epoch, first the two baseline Epochs were compared using a repeated measures ANOVA on the average pupil size with two factors: Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-stimulus Baseline, Post-Stimulus Baseline). There was no significant effect of Trial Type $F(2, 46) = 1.28$, $p = 0.287$; partial ETA squared = 0.053, nor Epoch $F(1, 23) = 1.57$, $p = 0.223$; partial ETA squared = 0.064. However, there was a significant interaction between Trial Type and Epoch $F(2, 46) = 3.40$, $p = 0.042$; partial ETA squared = 0.129. This resulted from the contrast between a smaller pupil in the Pre-Stimulus Baseline than the Post-Stimulus Baseline for Oddball and Standard Trial Types and the opposite pattern, of a larger pupil in the Pre-Stimulus Baseline than the Post-Stimulus Baseline, for the Deviant Trial Type (see Figure 2.3).

To investigate the effect of Trial Type on pupil size within the Stimulus Response Epoch a one-way repeated measures ANOVA was carried out on the average pupil size with Trial Type (Oddball, Deviant, Standard) as the factor. There was a significant effect of Trial Type $F(2, 46) = 43.10$, $p < 0.001$; partial ETA squared = 0.652. Post hoc testing revealed significant differences in pupil area between all levels of Trial Type (Paired Samples t-test: [oddball - deviant: $t(23) = 5.98$, $p < 0.001$; oddball - standard: $t(23) = 7.44$, $p < 0.001$; deviant - standard: $t(23) = 4.65$, $p < 0.001$]). This demonstrates that the differences between the pupil responses observed in Figure 2.3 are significant within the Stimulus Response Epoch.

Discussion

Within the Stimulus Response Epoch, there was a reliable effect of Trial Type with a greater pupil dilation in the Oddball Trial Type, followed by the Deviant and Standard Trial Type (see Figure 2.3). The response to the Deviant Trial Type when compared with the Standard gives a measure of the effect of stimulus probability when the stimulus is task irrelevant. The low stimulus probability in this Trial Type will have resulted in this stimulus having an increased salience in a bottom-up (task-irrelevant) manner. The reliable difference between the Standard and Deviant Trial Types confirms that the pupil is responsive to such bottom-up salience. The difference in the pupil response between the Deviant and Oddball Trial Types measures the effect on the pupil response of the target being task relevant, while all other features are kept constant (i.e., stimulus probability and so bottom-up salience). The reliable difference between the Oddball and Deviant Trial Types suggests that there is also an effect on the pupil response of task relevance or top-down, behavioural, salience. Together these comparisons suggest that the pupil response to the Oddball Trial Type is a combination of both bottom-up and top-down salience signals and that this phasic response to the oddball stimuli is sensitive to stimulus probability and task relevance.

Consistent with these results, de Gee et al. (2014, 2017) showed that phasic pupil responses are stronger for targets than distractors in equiprobable setups. In addition, Strauch et al. (2020) reported larger phasic pupil responses to frequent target stimuli than infrequent distractor stimuli, and even larger responses to infrequent target stimuli. This suggests that, although the pupil responds to both stimuli—task relevant and infrequent task-irrelevant events—the pupil response is greatest for task-relevant target stimuli. However, these factors can combine to amplify the pupil response further so that as infrequent target stimuli produce an even larger response as shown in the current experiment.

I also found a significant effect of Epoch. However, comparison of the two baseline phases revealed no significant effect of Epoch, suggesting that the pupil area was similar between the two and that the main effect of Epoch stemmed from the Stimulus Response Epoch. Although apparent from looking at Figure 2.3, this result confirms that the task stimuli increased pupil area above baseline, resulting in a phasic pupil response.

Pupil area decreased across Block 1 and 2. This could be explained by a decrease in alertness over time, as alertness is positively correlated with pupil size (Hou et al., 2005). This suggests that the participants may become fatigued throughout the task, so in Experiment 2 I decided to reduce the number of trials per block and allow participants to have a break in between blocks.

The current experiment seems to show clear evidence of a task relevant effect on the pupil response, and I have so far been attributing this effect to the top-down salience of the stimulus for this Trial Type. However, as discussed in the Introduction, it remains possible that elevated pupil response to the oddball stimulus is a result of the motor preparation or motor execution associated with the manual response. To investigate this, in Experient 2 I adjusted the oddball task by adding a counting block, in which participants were asked to silently count oddball stimulus presentations and compared this directly with a condition where the participants were required to respond to each Oddball Trial Type as before.

Experiment 2

Participants

There were 25 participants. Five participants were excluded for producing error rates of 20% or more in 1 or more experimental blocks, or due to missing pupil data, resulting in a final sample of 20 participants (14 females; age range 18-29).

Design

Trial Type (Oddball, Deviant, Standard) and Response Type (Manual, Counting) were manipulated within an auditory oddball paradigm to investigate their effects on pupil area. I expected to find a difference in pupil area between Response Types, and between Trial Types.

Participants carried out 1 practice block of 25 trials followed by 4 experimental blocks of 125 trials. In two of the experimental blocks, participants were asked to count the number of Oddball Trial Types that they heard and to state the total count at the end of the block (Counting Response Type); in the other two blocks, participants made manual responses to

the Oddball instead (Manual Response Type). The two blocks for each Response Type were administered consecutively, so that each participant completed all blocks for one Response Type before completing the other, and the block order was counterbalanced between participants, so that half of the participants completed the Counting Response Type first. The auditory frequency of the Oddball Trial Type was also counterbalanced between participants so that the low tone (1,200 Hz) was Oddball for half of the participants, and the high tone (1,600 Hz) was the Oddball for the others.

Procedure

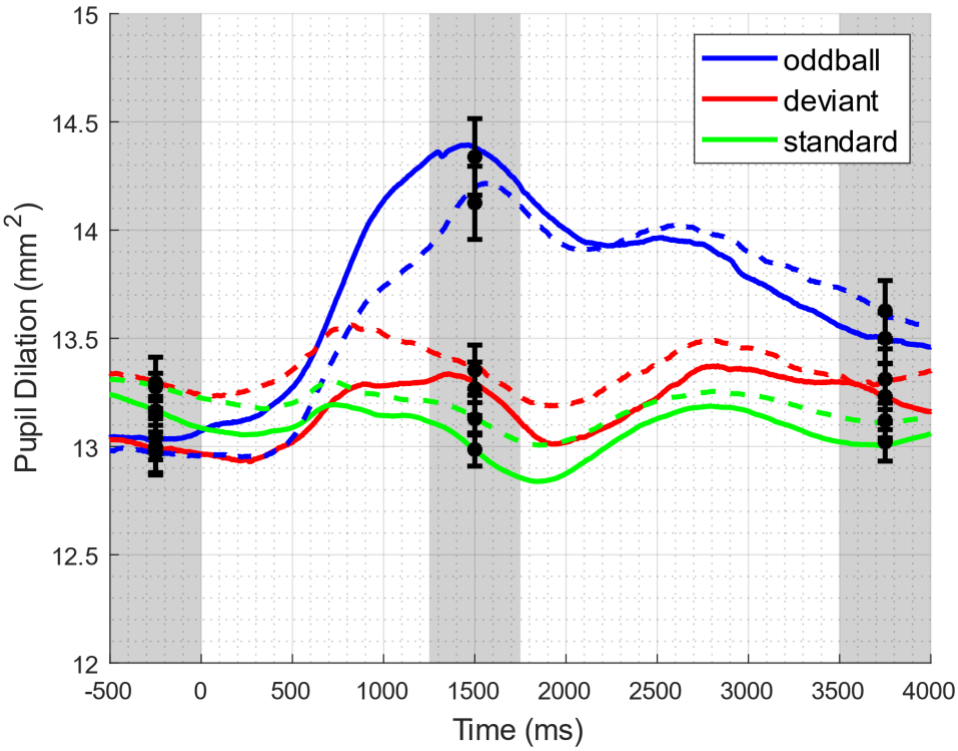
The procedure was the same as Experiment 1, with the exception that between blocks 2 and 3, participants were reminded that the task rules had changed, and that for the subsequent blocks, they should silently count the number of oddball tones rather than manually respond, or vice versa.

Results

Grand average pupil responses in the three Trial Types and two Response Types are plotted in Figure 2.4. The figure shows a large pupil response in the Oddball Trial Type, with smaller responses in the Deviant and Standard Trial Type, respectively. This pattern is clear for both Response Types. The peak of these pupil responses is around 1,500 ms, so the Stimulus Response Epoch was defined as 1,250-1,750 ms. In Figure 2.4, the Pre-Stimulus, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. For both Response Types, the mean pupil response for each Epoch and Trial Type is plotted in black

Figure 2.4

Grand Average Pupil Responses to the Oddball, Deviant and Standard Stimuli for Both Response Types in Experiment 2



Note. The Trial Types in the Manual Response Type are the solid lines, and the Trial Types in the Counting Response Type are the Dashed lines. The Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. The mean pupil area for each Epoch is plotted in black with error bars to show the range.

I carried out a repeated measures ANOVA on the average pupil size with the following factors: Response Type (Manual, Counting); Trial Type (Oddball, Deviant, Standard); and Epoch (Pre-stimulus baseline, Stimulus Response, Post-stimulus baseline). There was a significant main effect of Trial Type - $F(2, 38) = 21.905$, $p < 0.001$; partial ETA squared = 0.536 and Epoch - $F(2, 38) = 27.702$, $p < 0.001$; partial ETA squared = 0.593, but no significant effect of Response Type (Manual: $M = 13.07$ mm^2, $SD = 4.69$ mm^2; Counting: $M = 13.18$ mm^2, $SD = 4.12$ mm^2) - $F(2, 19) = 0.029$, $p = 0.866$; partial ETA squared = 0.002. There was a significant interaction between Trial Type and Epoch - $F(4, 76) = 31.429$, $p < 0.001$; partial ETA squared = 0.623.

To investigate the interaction between Trial Type and Epoch, the two baseline Epochs were compared using a repeated measures ANOVA on the average pupil size with two factors: Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Post-Stimulus Baseline). There was a main effect of Epoch - $F(1, 19) = 12.038$, $p = 0.003$; partial ETA squared = 0.388 but not Trial Type - $F(2, 38) = 2.637$, $p = 0.085$; partial ETA squared = 0.122. There was a significant interaction between Trial Type and Epoch - $F(2, 38) = 10.608$, $p < 0.001$; partial ETA squared = 0.358. This interaction resulted from contrasting patterns of a larger pupil for the Oddball Trial Type than the Deviant and Standard for the Post-Stimulus Baseline Epoch, and a larger pupil for the Standard Trial Type than the Deviant and Oddball for the Pre-Stimulus Baseline Epoch.

To investigate the effect of Trial Type on pupil size within the Stimulus Response Epoch, a one-way repeated measures ANOVA was carried out on the average pupil size within this Epoch, with Trial Type (Oddball, Deviant, Standard) as the factor. There was a significant effect of Trial Type - $F(2, 38) = 51.424$, $p < 0.001$; partial ETA squared = 0.730. Post hoc testing revealed significant differences between all levels of Trial Type (Paired Samples t-test: [Oddball - Deviant: $t(19) = 7.054$, $p < 0.001$; Oddball - Standard: $t(19) = 8.555$, $p < 0.001$; Deviant - Standard: $t(19) = 2.713$, $p = 0.014$]).

Discussion

I found a significant effect of Trial Type in the Stimulus Response Epoch, with a greater dilation for the Oddball Trial Type, followed by the Deviant and Standard. There was no effect of Response Type, so the effect of the oddball stimulus on the pupil is not a result of motor preparation or execution, but of task relevance, or top-down, behavioural salience. Whilst these findings are in line with the previous investigations that have reported task-evoked, phasic pupil dilations in the absence of overt behavioural responses, I have not been able to find a study that has directly compared pupil responses to targets in the presence and absence of a motor event (Einhäuser et al., 2008; Swallow et al., 2019). Therefore, previous studies could not say definitively that pupil responses to target stimuli were the result of a top-down salience effect rather than a motor effect. In contrast, my results provide a direct comparison of pupil responses in the presence and absence of motor output.

I also found a significant effect of Epoch. Comparison of the two baseline phases revealed a significant effect of Epoch, with greater pupil size in the Post-Stimulus Baseline Epoch than the Pre-Stimulus Baseline Epoch. This suggests that pupil size did not return to baseline after the phasic response to the oddball and deviant stimuli in the Stimulus Response Epoch. Therefore, the main effect of Epoch stems from the task-evoked pupil responses in the Stimulus Response Epoch and a lag in return to baseline, which is evident in the Post-Stimulus Baseline Epoch.

Compared with Experiment 1, the pupil responses to the oddball and deviant stimuli are slightly delayed. As the experimental blocks in the Manual Response Type involved exactly the same task paradigm as before, the increased latency of the phasic response could be due to individual differences in pupil response latency, rather than a difference in cognition.

My results suggest that pupil size is sensitive to task relevance or behavioural salience. As I am using pupil size as a marker of LC activity, these findings implicate the LC in the bottom-up and top-down control of attention. Like task or behavioural relevance, which possesses top-down salience and influences (intrinsic) motivation, reward incentives possess top-down salience and influence (extrinsic) motivation. Precisely how these top-down salience effects compete or interact to influence attention is unclear. Although there is some conflicting evidence, one study found that stimuli associated with greater rewards receive more attention than behaviourally relevant stimuli associated with low reward (Hickey et al., 2010). Therefore, perhaps the addition of a reward component to the oddball task would alter the effect of top-down salience by task relevance on the pupil by amplifying or interfering with it. I am especially interested in this notion given recent evidence that reward incentives evoke phasic changes in LC activity and the pupil (Chiew & Braver, 2014). In Experiment 3, I investigated the relationship between pupil size and reward incentives in human volunteers.

Experiment 3

Participants

Twenty-five participants took part in the experiment. Five participants were excluded for producing error rates above 20%, or due to missing pupil data, so the final sample contained 20 participants (12 females; age range 19-45). Errors were defined as making manual responses to the deviant or standard stimulus, or not responding to the oddball stimulus. Slow responses to the oddball stimulus (>0.7s) were not classified as errors.

Design

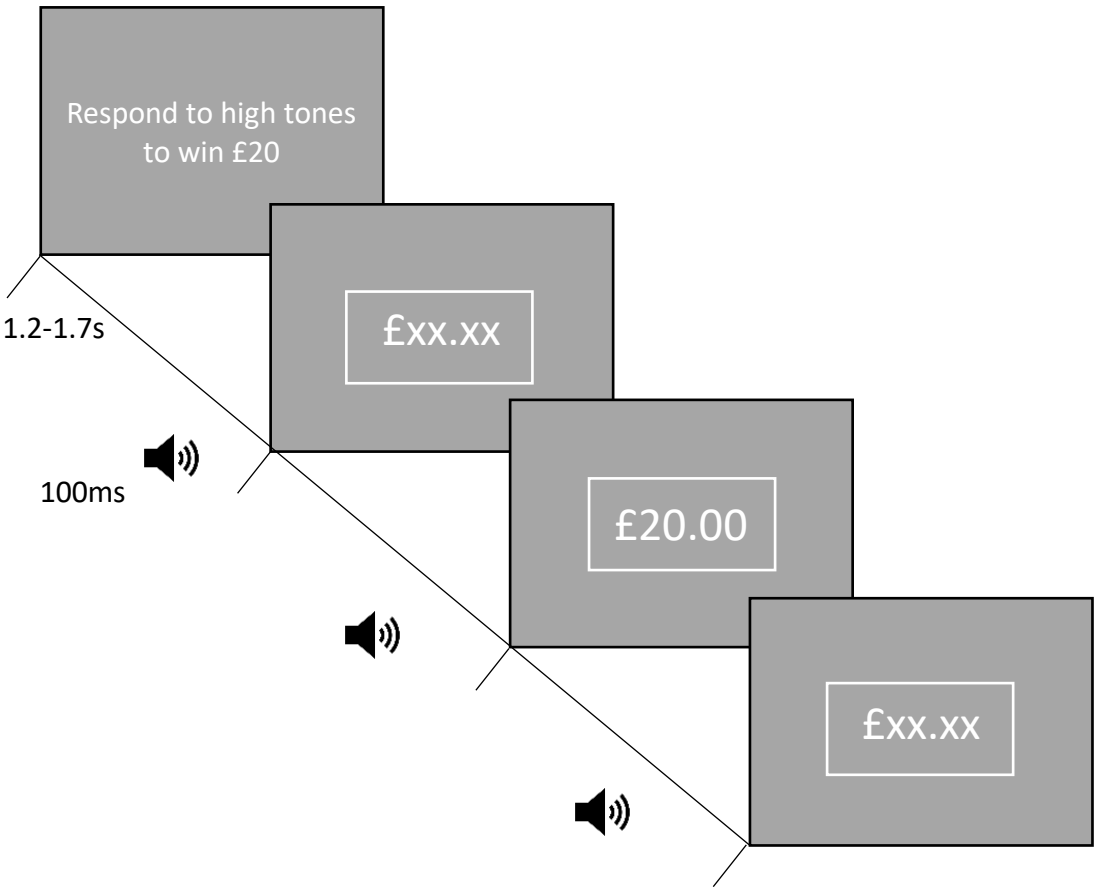
I manipulated Trial Type (Oddball, Deviant, Standard) and Reward Condition (Low, High) within an auditory oddball paradigm to investigate the effects on pupil area (see Figure 2.5 for a diagram of the oddball task paradigm). Participants carried out 1 practice block of 25 trials followed by 4 experimental blocks of 125 trials. In two of the experimental blocks, the oddball stimulus was associated with 20p; in the other two it was associated with £20. On-screen instructions conveyed the amount of reward associated with the oddball stimulus prior to each block, and participants were instructed to make quick manual responses to the oddball stimulus if they wanted to have a chance of winning the associated reward. Visual stimuli were added to the task to provide participants with visual feedback: if participants responded in time to the oddball stimulus (<0.7s), *£00.20* (in low reward blocks) or *£20.00* (in high reward blocks) appeared on-screen within a fixation box. If participants did not respond in time, *£00.00* was displayed instead. Visual stimuli made up of the same number of pixels were presented with and between each trial so that there was no difference in luminance throughout the task and importantly, between trials.

At the end of the task, one of the oddball trials from the whole task was selected at random, and if the participant had responded within 0.7 s to the selected oddball stimulus, they received the associated monetary reward in cash. High and low reward blocks were alternated within participants, so that no participant completed 2 high reward nor 2 low reward blocks consecutively, and the block order was counterbalanced between participants, with half of the participants completing a high reward block first and the other half completing a low reward block first. Like Experiment 2, half of the participants were

told to respond to the low tone (1,200 Hz) and the other half were told to respond to the high tone (1,600 Hz).

Figure 2.5

Diagram showing the Oddball Paradigm with a Reward Manipulation.



Note. The diagram shows the introductory instruction screen, followed by three example trials in the High Reward Condition. Visual stimuli consisting of the same number of pixels were presented in a fixation box with each auditory trial.

Procedure

The procedure was like the previous experiments, with some added changes related to the reward manipulation. As part of this, participants were informed about the amount of reward associated with the oddball stimulus prior to each block. Additionally, after

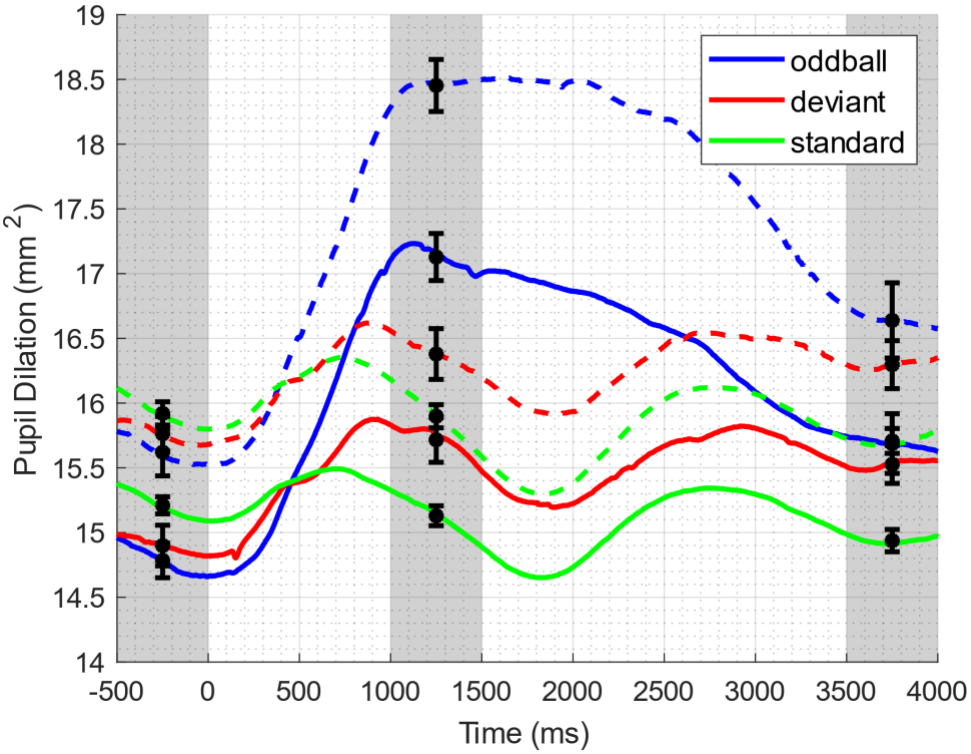
completing all four experimental blocks, a lottery incentive reward system was executed, and if the participants won a monetary reward, they received the cash immediately.

Results

Grand average pupil responses for the 3 Trial Types and 2 Reward Conditions are plotted in Figure 2.6, which depicts a clear pupil response to the Oddball Trial Type and smaller responses to the Deviant and Standard, respectively. This pattern is clear for both Reward Conditions, although overall the pupil is more dilated in the High Reward Condition. The peak of the pupil responses is around 1,250 ms, so the Stimulus Response Epoch was defined as 1,000-1,500 ms. In Figure 2.6, the Pre-Stimulus, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. For both Reward Conditions, the mean pupil response for each Epoch and Trial Type is plotted in black.

Figure 2.6

Grand Average Pupil Responses to the Oddball, Deviant and Standard Stimuli for both Reward Conditions in Experiment 3



Note. The High Reward Condition is represented by the dashed line, the Low Reward Condition is represented by the solid line. The Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. The mean pupil area for each Epoch is plotted in black with error bars to show the range.

I carried out a repeated measures ANOVA on the average pupil size with the following factors: Reward Condition (Low, High); Trial Type (Oddball, Deviant, Standard); and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). There was a significant main effect of Reward Condition – $F(1, 19) = 7.31$, $p = 0.014$; partial ETA squared = 0.278, Trial Type - $F(2, 38) = 38.80$, $p < 0.001$; partial ETA squared = 0.671 and Epoch - $F(2, 38) = 42.76$, $p < 0.001$; partial ETA squared = 0.692. There was a significant interaction between Trial Type and Epoch - $F(4, 76) = 35.18$, $p < 0.001$; partial ETA squared = 0.649, and Trial Type, Epoch and Reward Condition - $F(4, 76) = 2.65$, $p = 0.039$; partial ETA squared = 0.122.

To investigate the effect of Trial Type and Reward Condition on pupil size within the Stimulus Response Epoch, a two-way repeated measures ANOVA was carried out on the

average pupil size within this Epoch, with Trial Type (Oddball, Deviant, Standard) and Reward Condition (Low, High) as the factors. There was a significant effect of Trial Type - $F(2, 38) = 97.78$, $p < 0.001$; partial ETA squared = 0.837, and Reward Condition - $F(1, 19) = 7.39$, $p = 0.014$; partial ETA squared = 0.280, with greater pupil size in the High Reward Condition ($M = 16.91$, $SD = 5.95$) than the Low Reward Condition ($M = 15.99$, $SD = 5.89$). There was also a significant interaction between Trial Type and Reward Condition - $F(2, 38) = 4.72$, $p = 0.015$; partial ETA squared = 0.199.

To investigate the effect of Reward Condition on pupil area within the Pre-Stimulus Epoch, a one-way repeated measures ANOVA was carried out on the average pupil size with Reward Condition (Low, High) as the factor. There was a significant effect of Reward Condition - $F(1, 19) = 7.47$, $p = 0.013$; partial ETA squared = 0.282, with greater pupil area in the High Reward Condition ($M = 15.78$, $SD = 5.72$) than the Low Reward Condition ($M = 14.96$, $SD = 5.61$). These results indicate that the pupil was generally more dilated in the High Reward Condition at baseline.

To investigate the interaction between Trial Type and Epoch, the two baseline Epochs were compared using a repeated measures ANOVA on the average pupil size with two factors: Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Post-Stimulus Baseline). There was a main effect of Epoch - $F(1, 19) = 12.03$, $p = 0.003$; partial ETA squared = 0.388 but not Trial Type - $F(2, 38) = 2.31$, $p = 0.113$; partial ETA squared = 0.108. There was a significant interaction between Trial Type and Epoch - $F(2, 38) = 12.64$, $p < 0.001$; partial ETA squared = 0.399. As in Experiment 2, this resulted from a contrast in a larger pupil for the Oddball Trial Type than the Deviant and Standard for the Pre-Stimulus Epoch and Post-Stimulus Baseline Epoch, and the opposite pattern of a larger pupil for the Standard Trial Type than the Deviant and Oddball for the Pre-Stimulus Baseline Epoch.

To investigate the interaction between Trial Type, Reward Condition and Epoch, three repeated measures ANOVAs (one for each Epoch) were carried out on the average pupil size with two factors: Trial Type (Oddball, Deviant, Standard) and Reward Condition (Low, High). There was a significant effect of Trial Type - $F(2, 38) = 7.74$, $p = 0.002$; partial ETA squared = 0.289 and Reward Condition - $F(1, 19) = 7.47$, $p = 0.013$; partial ETA squared = 0.282 on pupil

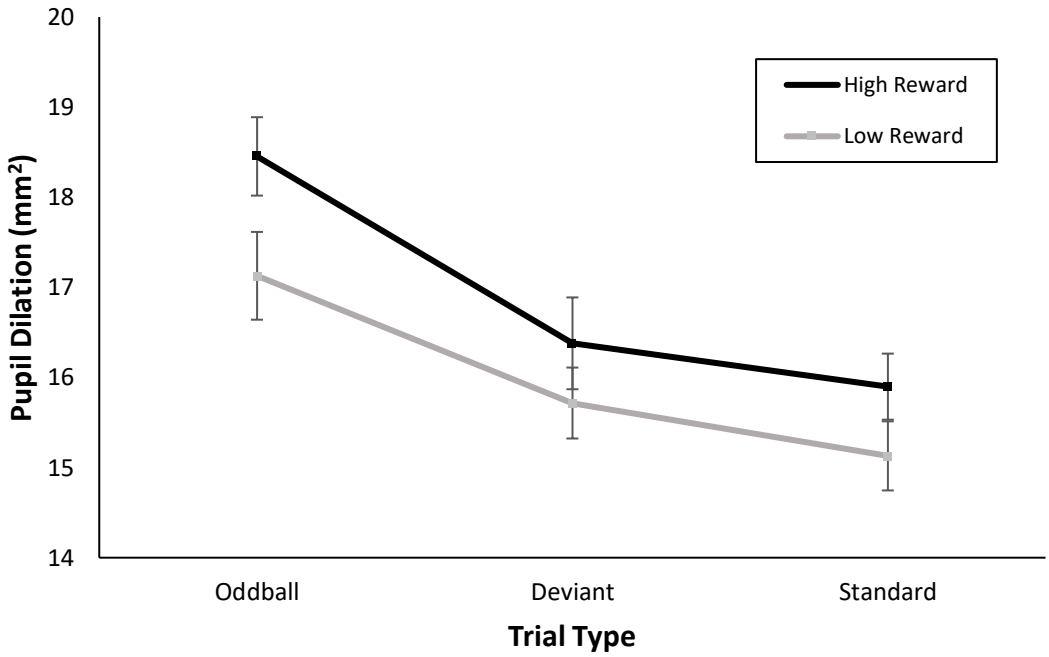
size in the Pre-Stimulus Epoch, but no significant interaction between Trial Type and Reward Condition - $F(2, 38) = 0.61$, $p = 0.548$; partial ETA squared = 0.031. In the Stimulus Response Epoch, there was a significant effect of Trial Type - $F(2, 38) = 97.78$, $p < 0.001$; partial ETA squared = 0.837 and Reward Condition - $F(1, 19) = 7.39$, $p = 0.014$; partial ETA squared = 0.280 and a significant interaction between Trial Type and Reward Condition - $F(2, 38) = 4.72$, $p = 0.015$; partial ETA squared = 0.199. In the Post-Stimulus Epoch, there was a significant effect of Trial Type - $F(2, 38) = 7.17$, $p = 0.002$; partial ETA squared = 0.274 and Reward Condition - $F(1, 19) = 6.80$, $p = 0.017$; partial ETA squared = 0.263 but no significant interaction between Trial Type and Reward Condition - $F(2, 38) = 0.51$, $p = 0.603$; partial ETA squared = 0.026.

To investigate whether Reward Condition had a phasic effect on pupil size, three repeated measures ANOVAs were carried out on the average pupil size with two factors: Trial Type and Reward Condition (Low, High). For each ANOVA, I compared two levels of Trial Type, rather than all three, to allow me to home in on any significant interactions. First, the Oddball and Deviant Trial Types were investigated: there was a significant effect of Trial Type - $F(1, 19) = 89.51$, $p < 0.001$; partial ETA squared = 0.825, Reward Condition - $F(1, 19) = 7.54$, $p = 0.013$; partial ETA squared = 0.284 and a significant interaction between Trial Type and Reward Condition - $F(1, 19) = 5.79$, $p = 0.026$; partial ETA squared = 0.233. Next, the Oddball and Standard Trial Types were investigated: there was a significant effect of Trial Type- $F(1, 19) = 142.84$, $p < 0.001$; partial ETA squared = 0.883, Reward Condition - $F(1, 19) = 9.80$, $p = 0.006$; partial ETA squared = 0.340 and a significant interaction between Trial Type and Reward Condition - $F(1, 19) = 12.42$, $p = 0.002$; partial ETA squared = 0.395. Finally, the Deviant and Standard Trial Types were investigated: there was a significant effect of Trial Type - $F(1, 19) = 16.99$, $p = 0.001$; partial ETA squared = 0.472 and Reward Condition - $F(1, 19) = 4.52$, $p = 0.047$; partial ETA squared = 0.192, but no significant interaction between Trial Type and Reward Condition - $F(1, 19) = 0.19$, $p = 0.671$; partial ETA squared = 0.010. These findings suggest that there was an effect of Reward Condition that was dependent on the Oddball Trial Type. Figure 2.7 shows a larger difference between the High and Low Reward Condition in the Oddball Trial Type than the other Trial Types for the Stimulus Response Epoch. This is indicative of a phasic effect of incentive salience on the pupil, as it

suggests that there is an additional effect of incentive salience that stems from the presentation of the Oddball Trial Type.

Figure 2.7

Mean Pupil Area in the Stimulus Response Epoch for each Trial Type sorted into Reward Conditions



Note. The error bars are 95% confidence intervals.

Manual responses

I also recorded reaction times to oddball stimuli. To investigate whether Reward Condition had an effect on these reaction times, a one-way repeated measures ANOVA was carried out on average reaction times, with Reward Condition (Low, High) as the factor. There was no significant effect of Reward Condition on reaction time -$F(1, 19) = 1.58$, $p = 0.225$; partial ETA squared = 0.077, indicating that there was a mismatch between the effect of Reward Condition on pupil size and the effect of Reward Condition on reaction time.

Discussion

My results demonstrate an amplification of the phasic effect of top-down salience on the pupil by the High Reward Condition, as well as a tonic effect of incentive salience. The tonic effect of incentive salience on the pupil is clearly seen when comparing the greater pupil area in the High Reward Condition with the smaller pupil area in the Low Reward Condition, and it is evident across all Trial Types (see Figure 2.6). The effect of Reward Condition is present before and after stimulus presentation, in the Pre-Stimulus Baseline and Post-Stimulus Baseline Epoch. Therefore, the effect of incentive salience is both phasic and tonic.

This effect of reward on pupil size must be related to incentive salience or extrinsic motivation rather than the actual rewarding effect, as reward delivery occurred after task completion. In the previous experiments, I showed that the pupil is sensitive to top-down (behavioural) and bottom-up salience. Evidence suggests that extrinsic motivation can influence top-down (incentive) salience to bias attentional selection in favour of reward incentives (Hickey et al., 2010). Therefore, the phasic and tonic effect of incentive salience on the pupil signify an effect of top-down salience on the pupil driven by extrinsic motivation. The phasic incentive salience effect is stimulus-locked and represents a combined effect of two types of top-down salience, resulting in a magnified pupil response compared with the pupil response in the Low Reward Condition, which was evoked by behavioural salience alone. This suggests that behavioural and incentive salience can combine to amplify the pupil response further so that as target stimuli produce an even larger response if they are linked to higher monetary reward. In contrast, the tonic effect of incentive salience on the pupil reflects a block effect of Reward Condition that is not

stimulus-locked, and therefore constitutes a sustained effect of incentive salience or extrinsic motivation in the absence of task relevance.

The mismatch in the effect of Reward Condition on pupil size with no effect on reaction times is surprising because it suggests that the processes driving the cognitive and behavioural effect of Reward Condition are distinct. Reaction times reflect an overt, executed action, whereas the pupil response reflects an effect of incentive salience, which is a covert, attentional process. Whilst it would seem to make sense for reward to both capture attention and influence behaviour, the underlying processes are distinct. This is in line with the results of Experiment 2, which showed that pupil responses to salient events do not relate to motor preparation nor execution. Alternatively, reaction time data has high variability, which could have masked the reward effect, especially as manual responses were only made for the Oddball Trial Type. For example, there were only about 10 Oddball Stimuli presented for each block (Oddball Trial Types had an 8% probability and there were 125 trials per block), so approximately 20 Oddballs were presented in the High Reward Condition and 20 in the Low Reward Condition. The low presentation frequency of the Oddball stimulus resulted in little reaction time data, which is typically quite noisy and therefore could have obscured the reward effect.

General discussion

The experiments presented in this chapter were carried out to explore human LC function, which can be inferred from phasic pupil responses and changes in baseline pupil size. In Experiment 1, I wanted to see whether the phasic pupil response (and therefore phasic LC activity) would differ between three Trial Types, which varied in bottom-up (stimulus probability) and top-down, behavioural salience. There was a significant main effect of Trial Type, and further exploration revealed a significant difference in the mean pupil size between all levels of Trial Type in the Stimulus Response Epoch, with the largest dilation in response to the low probability, target (oddball) stimulus, a smaller but clear dilation in response to the low probability, distractor (deviant) stimulus, and no response to the high probability, distractor (standard) stimulus. These results demonstrate that the pupil is sensitive to low probability, or bottom-up salience; however, I conducted a further

investigation to determine whether the pupil response to the oddball stimulus reflected a sensitivity to behavioural salience, or motor activity related to the behavioural response. There was no significant effect of Response Type (Counting versus Manual Response Type), indicating that the pupil response to the oddball stimulus was not driven by motor planning nor execution, but by behavioural salience. Finally, I investigated the effect of incentive salience on pupil size and found significantly greater tonic and phasic pupil dilations in the High Reward Condition than the Low Reward Condition. This suggests that reward incentives can amplify the effect of top-down salience by task relevance on the pupil response.

My results show that the pupil is sensitive to characteristics that influence both bottom-up and behavioural salience, which were manipulated by modifying stimulus probability and task relevance, respectively. Furthermore, I demonstrated that stimulus probability and task relevance can interact to magnify the effect of salience on the pupil. There is very little evidence of how top-down and bottom-up salience interact to influence attentional control, especially in auditory tasks. My findings suggest that both types of salience can interact to strengthen the overall effect of salience. This is true for the combined effect of behavioural and bottom-up salience, as well as that of behavioural and incentive salience. As pupil responses are a marker of LC activity, my findings also suggest that the LC is sensitive to these effects of salience. As mentioned, several research groups have stressed the importance of behavioural salience for task-evoked, phasic LC responses, and theories of LC function postulate that this, rather than bottom-up salience, drives the phasic LC response (Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Sales et al., 2019). This is mostly because phasic LC responses to bottom-up salience, such as unexpectedness, sometimes habituate, whereas responses to behavioural salience do not, so they are thought to be more important for the phasic LC response (Aston-Jones & Bloom, 1981; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005).

As mentioned in Chapter 1, theories of LC function propose that tonic and phasic LC firing drive changes in control state, triggered by the onset of a behavioural relevant event (Aston-Jones & Cohen, 2005; Bouret & Sara, 2005). My findings do not oppose this, as I found a phasic pupil response to the behaviourally relevant oddball stimulus. However, I

also observed a phasic pupil response related to the deviant stimulus, which was not behaviourally relevant. I would argue that the effect of behavioural relevance and low stimulus probability on the pupil infers a role for the LC related to both bottom-up and top-down salience in a manner analogous to attentional control, a system that allows for the selection of internal and external goals, as well as physically salient events (Awh et al., 2012; Corbetta & Shulman, 2002; de Wit & Dickinson, 2009; Fecteau & Munoz, 2006; Melloni et al., 2012). This role would provide a functional explanation as to why the phasic LC response habituates with repeated presentations of a stimulus-driven event: physically salient stimuli automatically capture attention but should be discarded if they do not constitute a threat or possess behavioural significance, so as to allow for goal-directed behaviour. Perhaps then, bursts of LC firing evoked by bottom-up salience eventually habituate to facilitate appropriate control and goal-directed behaviour, which is important for normal functioning in the absence of stress or threat (Aston-Jones & Bloom, 1981). This notion is supported by a recent animal study, which reported that attentional control is modulated by two distinct projections originating in the LC, innervating the prefrontal cortex (Bari et al., 2020).

I found an effect of incentive salience on the pupil, with a larger phasic pupil response to the £20 reward incentive than the 20p reward incentive. As mentioned, this effect of incentive salience on the pupil likely reflects a greater effect of top-down salience on the phasic pupil response that was driven by extrinsic motivation in the High Reward Condition. In contrast, the phasic pupil response in the Low Reward Condition is a result of behavioural salience driven by intrinsic motivation. It has been argued that behavioural goals and other top-down influences, such as reward incentives, should be viewed as distinct categories of control, because they produce conflicting selection biases (Awh et al., 2012). Therefore, in my task, the engagement of extrinsic motivational resources rather than intrinsic motivational resources could constitute a change in control state towards a reward-seeking, goal-directed mode, instead of a task-relevant, goal-directed mode. Indeed, according to the adaptive gain theory, a change in control state coincides with a change in tonic LC activity or baseline pupil size. Furthermore, this suggests that the reward incentive captured attention and drove motivational and behavioural resources over task-relevant information (although this did not affect behavioural performance measured by reaction time). This is in line with evidence in the literature which suggests that stimuli associated with high reward

capture attention over equally salient target stimuli associated with low reward, even when the reward incentive distracts participants from the task and disrupts behavioural performance (Hickey et al., 2010).

Therefore, attentional resources are drawn to reward when reward incentives and behaviourally relevant information compete. However, in the present task, reward and task contingencies were not in direct competition, as the task-relevant information and reward incentive were both signalled by the Oddball Trial Type. Perhaps, if the target stimulus and reward incentive were presented as separate stimuli, I would have found a greater effect of behavioural salience on pupil size than incentive salience. Furthermore, I cannot say whether the bottom-up salience aspect of the deviant stimulus would have interacted with incentive salience to amplify or change the overall effect of salience on the pupil, as in this experiment, the reward incentive was never associated with the Deviant Trial Type. There has been little investigation of how different types of top-down and bottom-up salience interact. Therefore, this could be investigated in future studies by associating reward incentives with distractor stimuli, which compete with target stimuli that are not associated with reward incentives.

I have shown that top-down and bottom-up salience reliably elicit changes in pupil size. Events with top-down salience include task-relevant stimuli and reward incentives, which should be thought of as separate entities. These top-down and bottom-up effects can interact to further dilate the pupil in a transient, task-evoked manner. The top-down salience effect of the reward incentive is a marker of extrinsic motivation. In addition to a phasic effect of incentive salience, reward incentives stimulated a tonic increase in baseline pupil size, which was sustained and unrelated to task relevance. Since phasic and tonic pupil responses reflect LC activity, these findings have implications for human LC function and therefore suggest that the LC functions in the underlying processes of top-down and bottom-up salience. In the next Chapter, I implement the same oddball task with a reward manipulation and concurrent pupillometry on healthy human volunteers to further investigate the underlying mechanisms of these effects of salience.

Chapter 3

Introduction

In the previous chapter, I reported effects of top-down and bottom-up salience on pupil size. This chapter reports a study that implements the same oddball task with a reward manipulation and concurrent pupillometry. The main aim was to uncover the underlying mechanisms of these effects by disrupting certain signalling systems and seeing whether this disruption altered the effects of salience on pupil size. I was particularly interested in the effects of behavioural and incentive salience on the pupil, which reflect intrinsic and extrinsic motivational processes. In addition, the overall effect of top-down and bottom-up salience on the pupil could indicate the active mode of control. For example, if there is a greater effect of bottom-up than top-down salience on the pupil, this could indicate a stimulus-driven state with a suppression of top-down salience effects on the pupil. Therefore, capturing the holistic effect of salience on the pupil and disrupting certain signalling systems provides an indication for the underlying mechanisms of attentional control.

Literature review

As mentioned in Chapter 1, evidence suggests that the allocation of internal resources is supported by two distinct systems: a top-down and bottom-up attentional control mechanism, which selects stimuli based on internal or external goals and physical characteristics, respectively (Awh et al., 2012; Corbetta & Shulman, 2002; de Wit & Dickinson, 2009; Fecteau & Munoz, 2006; Melloni et al., 2012). In a manner analogous to attentional capture, the LC responds to important environmental events, including those that are physically and behaviourally salient (Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Corbetta et al, 2008). Converging evidence suggests that the LC is also sensitive to incentive salience, which is similar to behavioural salience in that it captures attention in a top-down manner (e.g., Bouret & Richmond, 2015). Whilst behavioural goals and reward incentives generally recruit disparate brain regions, and are separate cognitive constructs, since they both influence attentional control, it seems likely that they also activate common brain regions, such as the LC (Awh et al., 2012).

A link between reward and LC activity was first reported decades ago by addiction studies, which showed that NE is necessary for the conditioned place preference of cocaine, amphetamines and opiates, and the stress-induced reinstatement of various drugs of abuse (Weinshenker & Schroeder, 2007). Despite this evidence, the contribution of the LC-NE system to reward function has historically been overlooked in favour of the dopaminergic system, which dominates this literature. Recent studies have reported a close association between the LC-NE system and extrinsic motivation, rather than the rewarding effect that occurs with reward delivery, which is linked to the dopaminergic system. These studies have suggested that the LC mobilises energy resources for goal-directed actions, such as reward-seeking behaviours (Bouret & Richmond, 2015).

In contrast, my findings presented in previous chapters indicate that the phasic pupil response (and therefore the phasic LC response) is sensitive to top-down salience even in the absence of motor execution. This suggests that phasic activity in the LC does is not directly associated with energy mobilisation for the execution of behaviour. Other research groups have suggested that the LC functions in related processes of attention and motivation, such as saliency attribution, which facilitates the attentional selection of important environmental stimuli and the according allocation of internal resources. For example, one study reported increased prefrontal NE in response to rewarding and aversive stimuli in mice (Ventura et al., 2008). Following this, the authors proposed a functional role for the NE system in the attribution of salience to highly salient stimuli, such as highly rewarding and aversive stimuli, suggesting a less specific role for the LC-NE system in reward, and a more general role in the attribution of top-down salience for attentional selection. This would not directly result in the mobilisation of energy resources for goal-directed behaviour, but it would facilitate it. Alternatively, the LC could be involved in mobilising energy resources for goal-directed actions, even if they are not always executed. This would explain why I found a phasic pupil response in the absence of overt behaviour.

The idea that the LC functions in goal-directed behaviour, whether directly or indirectly, ties in with several reports that NE regulates drug-seeking behaviours, which represent a maladaptive goal-directed behavioural state. As mentioned in Chapter 1, pharmacological inhibition of NE attenuates the stress-induced reinstatement of alcohol-, cocaine-, heroin-

and methamphetamine-seeking behaviours in rodents, and there is some evidence for this in humans (Davis et al., 1975; Erb et al., 2000; Lê et al., 2011; Leri et al., 2002; Mantsch et al., 2010; Shaham et al., 2000; Shepard et al., 2004). Furthermore, this evidence suggests that the reinstating effect of NE on drug-seeking is dependent on stress, which therefore implicates the stress system as well as NE in the underlying pathophysiology of drug abuse relapse.

The two main structures supporting central NE and the stress system—the LC and HPA axis—are highly interconnected, which backs the idea that they might have collaborative functions. Indeed, as mentioned in Chapter 1, evidence from a human study indicates that together, NE and cortisol shift behaviour from a goal-directed to a stimulus-driven, habit-based state in stress, whereas alone, neither cortisol nor NE shift behaviour (Schwabe et al., 2010; 2012). This implicates NE, and cortisol, the main product of the HPA axis, in the modulation of control state, especially in stress; in addiction pathophysiology, this mechanism could become maladaptive and promote drug-seeking**.**

Interestingly, apathy is prevalent in neurological conditions with stress-related aetiology or pathology (e.g., Chase, 2011; Söndergaard et al., 2012; Strassman et al., 1956; Tiemensma et al., 2014). Whilst the underlying mechanisms of apathy are unclear, the above evidence implicates the LC-NE system and cortisol, which work synergistically to alter goal-directed behaviour in stress. If this system is disrupted, the result could be an amotivated presentation, in which individuals are not drawn to goals nor driven to work towards them. In support of this notion, patients diagnosed with primary adrenal insufficiency, who display aberrant cortisol dynamics, commonly present with apathy, indicating a link between cortisol dysregulation and amotivation. Furthermore, when this disease pathology is unmasked by removing cortisol replacement therapy, patients with primary adrenal insufficiency lose their sympathetic response to stressors, demonstrating an association between cortisol and NE (Kaye & Lightman, 2006). Therefore, these patients provide evidence for a new mechanism of apathy, in which the collaborative function of cortisol and NE on control state and goal-directed behaviour is disrupted.

One way to investigate this mechanism would be to disrupt cortisol and NE signalling in healthy human volunteers undergoing a measure of apathy. If this disruption resulted in apathy symptoms compared with placebo, this would implicate cortisol and/or NE signalling in apathy. However, as mentioned in Chapter 1, measures of apathy consist of self-reports, which lack clinical validity (Carrozzino, 2019). Therefore, some investigations have implemented alternative measures, such as by examining reward sensitivity (Muhammed et al., 2016; Rochat et al., 2013). The rationale for examining reward sensitivity comes from reports describing reward insensitivity in patients with apathy (Adam et al., 2013; Martínez-Horta et al., 2014; Muhammed et al., 2016; Rochat et al., 2013). However, it is important to keep in mind that whilst reward sensitivity is a marker of extrinsic motivation, and this can be assessed as a measure of apathy, intrinsic motivation could also be disrupted in apathy. Therefore, I implemented my oddball task with a reward manipulation and concurrent pupillometry to assess the effects of behavioural and incentive salience on pupil size in healthy human volunteers. These effects of salience are linked to motivation and will therefore provide an indication of the levels of intrinsic and extrinsic motivation in my participants.

To investigate the influence of cortisol and/or NE signalling on these effects of salience, I disrupted cortisol and NE signalling with cortisol and NE receptor antagonists. The effects of these antagonist treatments on salience compared with placebo indicates whether cortisol and/or NE function in intrinsic and extrinsic motivation, and therefore whether such mechanisms could become disrupted in apathy to impair motivation. Cortisol signals via two main receptors: the MR and GR, so cortisol effects on salience (and motivation) could occur via either or both of these receptors (Timmermans et al., 2019). Therefore, I administered both MR and GR antagonists. If I were to administer an MR antagonist without a GR antagonist (or vice versa) and found no effect of the treatment on salience, I could not rule out cortisol effects on salience via the other receptor. Spironolactone and mifepristone are MR and GR antagonists available on prescription that have been used previously in human research. In addition, research teams have reported specific dosing regiments to block cortisol activity that consider the fluctuating levels of cortisol due to its circadian and ultradian rhythms (see Method section; Gaillard et al., 1984; Young et al., 1998; 2016).

Therefore, I used spironolactone and mifepristone to block MR and GR signalling in the present study.

NE binds to alpha and beta adrenoceptors, so to disrupt NE signalling, I could block one or both of these receptors (Ramos & Arnsten, 2007). The problem with alpha adrenoceptor antagonists is that they do not readily cross the blood brain barrier (Guo et al., 1991; Nikolić et al., 2013; Prys-Roberts & Farndon, 2002). Therefore, if I were to administer an alpha adrenoceptor antagonist, I would risk more peripheral than central effects. However, I wanted to investigate the effects of NE signalling disruption on salience and motivation, which are cognition functions, so I needed a NE receptor antagonist that crosses the blood brain barrier into the brain. Propranolol, which is a beta adrenoceptor antagonist, is highly lipophilic and readily crosses the blood brain barrier (Neil-Dwyer et al., 1981; Pintor, 2009). In addition, it is a common treatment for high blood pressure and anxiety that is well tolerated in humans. Therefore, to disrupt NE signalling, I used propranolol.

In this study, I investigated a prospective mechanism of apathy by implementing my previously developed oddball task with a reward manipulation on healthy human volunteers undergoing pupillometry. In addition, NE and cortisol signalling were pharmacologically disrupted with propranolol, mifepristone and spironolactone to see whether these treatments influenced the effect of salience on pupil size (and therefore LC activity), especially the effects of behavioural and incentive salience, which indicate underlying intrinsic and extrinsic motivation. The study design was a randomised, single-blind, placebo-controlled, four-arm, crossover study, in which participants underwent all four treatment arms (mifepristone, spironolactone, propranolol and placebo), with a >10 day wash out period between each arm (see Figure 3.1). In this way, each participant acted as their own control, so that salience effects on the pupil between mifepristone or spironolactone or propranolol treatment arms and placebo were compared in each participant. A difference in the effects of incentive salience on the pupil in one of the treatment arms compared with placebo would indicate that cortisol or NE function in extrinsic motivation via the LC, as discussed. Furthermore, if this mechanism were disrupted, it could result in the development of apathy. Therefore, these data have implications for the treatment of apathy.

Method

Participants

There were 24 healthy volunteers aged 18-45 (14 females), with no history of psychiatric disease. All participants had normal or corrected-to-normal vision and were paid for participation. Informed participant consent was obtained, and this study was approved by the Faculty of Health Sciences Research Ethics Committee, University of Bristol, UK. Participants were recruited from the University of Bristol community via poster advertisements, webpage advertisements and email lists.

Materials

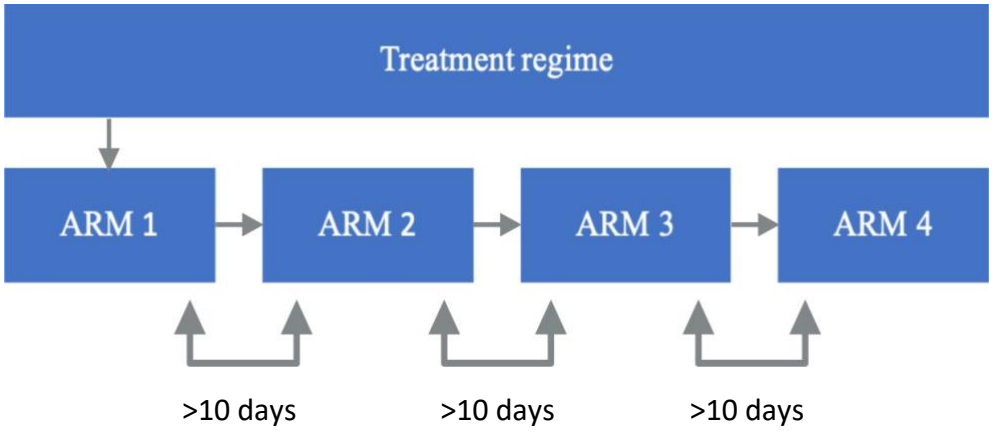
During testing, participants were seated in front of a computer screen. The computer display was 22.5 inches and had a resolution of 1920 x 1200 pixels; viewing distance was 60 cm. Pupil area (left pupil) and eye position were recorded at a sampling rate of 500 Hz by a tower-mounted Eyelink 1000 eye tracker (SR Research, Canada), which has a typical operating spatial resolution of 0.5^{o} or better. Head movement was minimised using a chin and forehead rest. Manual responses were recorded via a computer keyboard.

Study design

I implemented a randomised, single-blind, four-arm, crossover study design (see Figure 3.1). The four treatment arms (mifepristone, propranolol, spironolactone and placebo) were randomised across participants by the Pharmacy Trials Unit (University Hospitals Bristol, UK) and separated by a >10-day washout period. For each treatment arm, participants completed two visits: a drug dispensing visit, in which participants received the tablet treatment, and a testing visit, in which participants completed the oddball task with a reward manipulation and pupillometry. At the drug dispensing visit, a research nurse dispensed the allocated treatment and instructed participants when to take the tablets so that they could self-administer at home. Participants were instructed to take the dispensed tablets on the day of testing (see Table 3.1 for dosing regimen), and the testing visit always took place between 9AM and 10AM. Prior to study commencement, participants attended a screening session, during which they underwent task training to control for task practice effects.

Figure 3.1

Study Design



Note. This figure shows the study design, which is a randomised, placebo-controlled, four-arm, crossover study. Participants completed all four treatment arms in a randomised order. Each treatment arm was separated by at least 10 days to allow for drug washout. This figure was created with BioRender.com.

Dosing regimen

The dosing times for mifepristone and spironolactone shown in Table 3.1 were established by reviewing the previous use of these drugs in research and by considering the drug pharmacodynamics and pharmacokinetics (Young et al., 2016). For instance, in healthy humans, the amplitude of ultradian pulses of cortisol increase in the early morning, just before waking. Therefore, administering mifepristone or spironolactone at 12AM will prevent the genomic mechanisms of these nocturnal pulses as well as their effects on behaviours measured in the morning. However, one study found that a 12AM dose of mifepristone (4.5 mg/kg and 6 mg/kg) reduced feedback inhibition on the HPA axis and therefore actually increased plasma cortisol between 8AM and 12PM (Gaillard et al., 1984). In addition, another study reported that two 400mg doses of spironolactone, administered 5 hours apart, increased plasma cortisol when tested 30 minutes to 4 hours later (Young et al., 1998). Thus, in order to maintain blockade of cortisol signalling over the morning period, an additional morning dose of mifepristone or spironolactone is required. This should prevent the fast and slow effects of cortisol via MR or GR on cognition and behaviour during testing if testing is carried out in the morning. For example, although the plasma half-life of

mifepristone is only 1-2 hours, studies have found that mifepristone prevents the GR-mediated release of heat-shock protein and the translocation of the receptor complex for 24 hours after administration (Raux-Demay et al., 1990). In contrast to mifepristone, spironolactone has a much longer half-life of 28 hours, and the active metabolites of spironolactone with anti-mineralocorticoid effects have a half-life of 14 hours (Gardiner et al., 1989). From this evidence, two doses of mifepristone (400 mg) or spironolactone (400 mg) were administered, one at 12AM and the other at 7AM (see Table 3.1) to maintain MR and GR blockade until after testing, which took place between 9AM and 10AM.

The decision to use mifepristone and spironolactone was partly due to their prescriptive status, thus their side effects are well known. For example, mifepristone is a medication available on prescription for the termination of pregnancy in combination with another medication (typically, the prostaglandin misoprostol). Reported side effects for mifepristone relate to its use with misoprostol in pregnant women, rather its lone use and two one-off doses in healthy volunteers, such as in the present experiment. Reported common side effects for mifepristone include abdominal cramps, diarrhoea, infection, nausea, pelvic inflammatory disease, uterine disorders, vaginal haemorrhage and vomiting (Mifepristone, n.d.).

Spironolactone is a diuretic medication available on prescription for the treatment of oedema (fluid retention) in heart failure, cirrhosis and kidney disease. Reported side effects for spironolactone relate to its chronic administration, rather than two one-off doses, such as in the present experiment. The National Institute for Health and Care Excellence does not report any common side effects for spironolactone, only side effects with an unknown frequency, including acute renal failure, alopecia, benign breast tumour, breast pain, changes in libido, confusion, dizziness, drowsiness, electrolyte disturbances and gastro-intestinal disturbances. As spironolactone is a diuretic, the most probable side effect for my participants was increased urination overnight, potentially leading to tiredness and fatigue during testing (see Spironolactone, n.d., for a full list of side effects).

I administered propranolol to reduce NE signalling via the beta adrenoceptor, a G-protein coupled membrane receptor that responds rapidly to changes in NE, unlike the slower

genomic responses to cortisol. Propranolol has a plasma half-life of 3-6 hours. Therefore, one 80 mg dose administered at 7AM is sufficient to block endogenous NE throughout the testing period (9-10AM). Again, the rationale for blocking the beta adrenoceptor rather than the alpha adrenoceptor subtype was mostly due to evidence that propranolol readily crosses the blood brain barrier, whereas most adrenoceptor antagonists do not (Neil-Dwyer et al., 1981; Pintor, 2009). In addition, propranolol is safe and well tolerated in humans. Reported side effects relate to the chronic administration of propranolol, rather than a one-off dose, such as in the present experiment. The most commonly reported side effects include abdominal discomfort, bradycardia, confusion, depression, dizziness, diarrhoea and fatigue (see Propranolol hydrochloride, n.d., for a full list of side effects).

Although side effects of mifepristone, spironolactone or propranolol were not expected, other than increased urination with spironolactone, it was ensured that participants were feeling well immediately prior to testing. The only complaints included feeling unwell due to a common cold infection and some tiredness. Any specific drug-related side effects were not relayed to me but to a research nurse, as I was blinded from the drug randomisation.

Table 3.1

Drug dose regimen

	Treatment arm			
	Mifepristone	Spironolactone	Propranolol	Placebo
12AM dose (mg)	400	400	—	Placebo
7AM dose (mg)	400	400	80	Placebo

Note. *A 12AM dose of placebo was not administered in the Propranolol Treatment Condition as participants were not blind to the treatment randomisation.*

Task design

The implemented oddball task was the same as Experiment 3 of Chapter 2. It was written using Psychophysics Toolbox v3 (Brainard, 1997) running under Matlab 2015a (The Mathworks Inc., Natick). This task was implemented during testing visits, as well as during task training at screening.

During the task, participants were played a randomised sequence of short auditory tones. They were required to listen for, and respond to, a rarely occurring tone (the oddball target), which occurred alongside another rarely occurring tone (the deviant stimulus) and a frequently occurring tone (the standard stimulus), neither of which required responses. The standard stimulus had an auditory frequency of 1,400 Hz, and the oddball and deviant were either 1,200 or 1,600 Hz (counterbalanced between participants). For each participant, the auditory frequency and assignment of these tones was maintained across the whole study (including the screening and each testing visit). All tones had a duration of 100 ms, and the interstimulus interval was randomly varied between 1.2 and 1.7 s. In each block, 8% of the trials were deviant and oddball tones and 84% were standard tones. Responses to oddball stimuli were made via a keyboard press.

The task consisted of 1 practice block of 25 auditory trials, followed by 4 experimental blocks of 125 auditory trials. In two of the experimental blocks, the oddball stimulus was associated with 20p; in the other two it was associated with £20. The participants were told to respond quickly to the oddball stimulus if they wanted to win the associated reward. They were provided with on-screen feedback, which informed them whether they had responded in time (within 0.7 s).

At the end of the task, one of the oddball trials from the whole task was selected at random, and if the participant had responded within 0.7 s to the selected trial, they received the associated monetary reward in cash. High and low reward blocks were alternated within participants, and the block order was counterbalanced between participants. Half of the participants were told to respond to the low tone for the whole experiment (1,200 Hz), and the other half were told to respond to the high tone (1,600 Hz).

Procedure

Participants self-administered the dispensed treatment as instructed, with slightly different dosing times across treatment arms (see Table 3.1 for dosing regimen). Testing took place on the same day as drug administration.

At the testing site, participants were given printed task instructions, so that they could remind themselves of the task rules, which they learnt during task training at screening. During testing, participants were seated in front of a computer monitor and a tower-mounted eye-tracking system. They were instructed to place their chin on the chinrest and fixate on a central fixation box throughout each block, responding to the oddball stimulus by pressing any key on the computer keyboard in front of them. Once participants were seated comfortably, the lights were switched off, and a short practice block was initiated prior to task initiation.

After the practice block, pupil calibration and validation measures were carried out (these preceded each experimental block), and if they were met, the first experimental block was initiated. At the start of each block, instructions appeared on the screen to inform participants of the amount of monetary reward associated with the oddball stimulus (£20 or 20p). All text was then removed from the screen and the central fixation box appeared. Throughout the task, visual stimuli appeared within the fixation box to provide participants with feedback as to whether they had responded to the oddball stimulus in time (within 0.7 s of stimulus presentation). Between each block, participants were allowed a one-minute break. After completing all four experimental blocks, a lottery incentive reward system was executed, and if the participants won a monetary reward, they received the cash immediately.

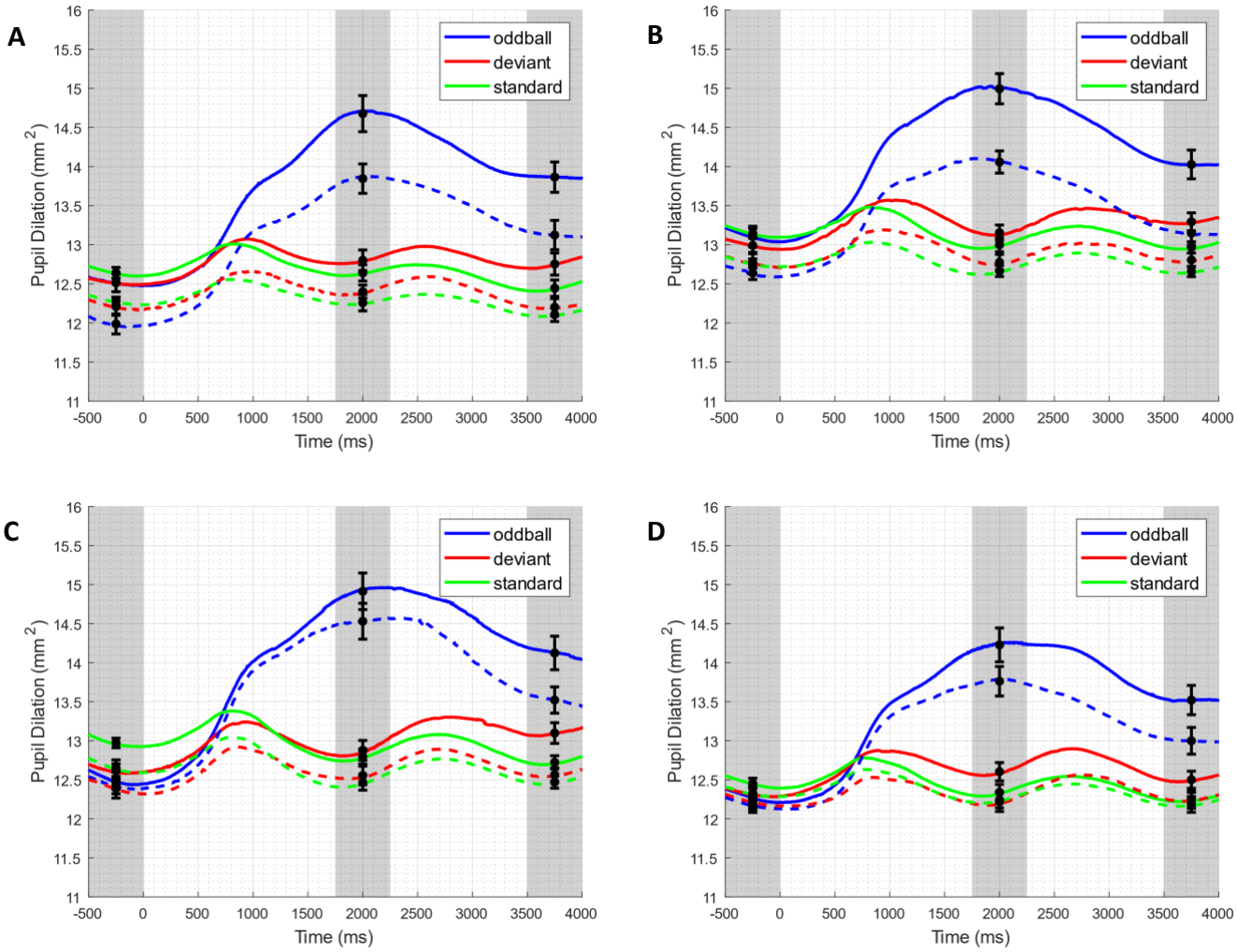
Participants were booked in for the next treatment arm after a 10-day washout period, completing each of the subsequent 3 treatment arms, all of which followed the same procedure.

Results

Grand average pupil responses for the 3 Trial Types and 2 Reward Conditions in each of the 4 Treatment Conditions are plotted in Figures 3.2A-D. Figure 3.2A shows the pupil responses in the Placebo Treatment Condition, which acts as a comparison for the other treatments. In comparison with the Placebo, each of the Treatment Conditions (other than Propranolol in Figure 3.2B) show a smaller difference between Reward Conditions, perhaps suggesting a blunted effect of incentive salience on the pupil. Each figure shows a clear pupil response to the oddball stimulus across all four Treatment Conditions, with a greater pupil response in the High Reward Condition than the Low Reward Condition. The peak of the pupil responses is around 2,000 ms, so the Stimulus Response Epoch was defined as 1,750-2,250 ms. In all four figures, the Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey, and the mean pupil response for each Epoch and Trial Type is plotted in black, for both Reward Conditions.

Figure 3.2

Grand Average Pupil Responses to the Oddball, Deviant and Standard Stimuli for Both Reward Conditions in all Treatment Conditions



Note. **(A) Grand average pupil responses in the Placebo Treatment Condition. (B) Propranolol Treatment Condition. (C) Spironolactone Treatment Condition. (D) Mifepristone Treatment Condition. The High Reward Condition is represented by the solid line, the Low Reward Condition is represented by the dashed line. The Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey. The mean pupil area for each Epoch is plotted in black with error bars to show the range.**

The primary objective of the study was to investigate whether the Mifepristone, Spironolactone and Propranolol Treatment Conditions showed different phasic and tonic incentive salience effects on the pupil compared with the Placebo Treatment Condition. Secondary outcomes included examining differences in the phasic pupil response by bottom-up and top-down salience effects of Trial Type (Oddball, Deviant and Standard) between the three drug Treatment Conditions and Placebo. To investigate this, three repeated measures ANOVAs were carried out on the mean pupil size (one for each drug treatment) with the following factors: Treatment Condition (Mifepristone or Spironolactone or Propranolol versus Placebo), Reward Condition (Low, High), Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline).

Mifepristone

First, a four-way repeated measures ANOVA was carried out with the Mifepristone and Placebo levels of Treatment Condition. There was a significant main effect of Epoch – $F(2, 46) = 65.260$, $p < 0.001$; partial ETA squared = 0.739; Trial Type – $F(2, 46) = 38.109$, $p < 0.001$; partial ETA squared = 0.624, and Reward Condition – $F(1, 23) = 5.231$, $p = 0.032$; partial ETA squared = 0.185. There was no significant main effect of Treatment Condition – $F(1, 23) = 3.789$, $p = 0.767$; partial ETA squared = 0.004. There were significant interactions between Treatment Condition and Trial Type – $F(2, 46) = 6.171$, $p = 0.004$; partial ETA squared = 0.212; Epoch and Trial Type – $F(4, 92) = 35.788$, $p < 0.001$; partial ETA squared = 0.609; Treatment Condition, Epoch and Trial Type – $F(4. 92) = 10.387$, $p < 0.001$; partial ETA squared = 0.311; Epoch and Reward Condition – $F(2, 46) = 6.294$, $p = 0.004$; partial ETA squared = 0.215; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 4.046$, $p = 0.024$; partial ETA squared = 0.150; Trial Type and Reward Condition – $F(2, 46) = 23.022$, $p < 0.001$; partial ETA squared = 0.500; Treatment Condition, Trial Type and Reward Condition – $F(2, 46) = 15.190$, $p < 0.001$; partial ETA squared = 0.398; Epoch, Trial Type and Reward Condition – $F(4, 92) = 18.280$, $p < 0.001$; partial ETA squared = 0.443; and Treatment Condition, Epoch, Trial Type and Reward Condition – $F(4, 92) = 25.795$, $p < 0.001$; partial ETA squared = 0.529.

The most important finding from this analysis is that there was no main effect of Treatment Condition nor was there a significant interaction between Treatment Condition and Reward

Condition. This suggests that there was not a tonic effect of Treatment Condition on the pupil—that is, there was no change in baseline pupil size by Treatment Condition nor by the effect of Treatment Condition on Reward Condition. However, a phasic effect of Treatment Condition on the pupil has not been ruled out by this analysis. In fact, the interaction effects between Treatment Condition and the other factors suggests that there was a phasic effect of Treatment Condition on the pupil. Phasic pupil responses are transient and stimulus-locked, so phasic effects on the pupil can be investigated by focusing on the effects of Trial Type. Therefore, three repeated measures ANOVAs were carried out on the average pupil size with four factors: Treatment Condition (Mifepristone, Placebo), Reward Condition (Low, High), Trial Type (Oddball and Deviant, Oddball and Standard or Deviant and Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). For each ANOVA, two levels of Trial Type were compared, rather than all three, to home in on any significant interactions.

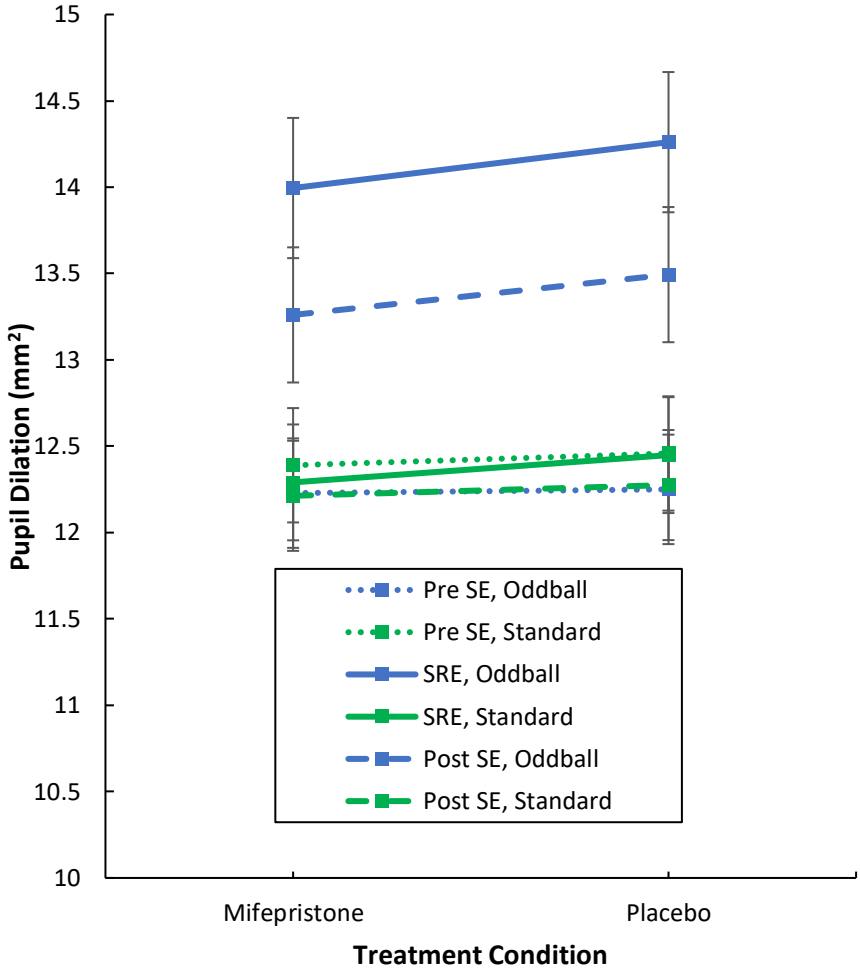
First, the Oddball and Deviant Trial Types were investigated: there was no significant effect of Treatment Condition – $F(1, 23) = 0.103$, $p = 0.747$; partial ETA squared = 0.005; nor were there any interactions between Treatment Condition and any other factor (Treatment Condition and Epoch – $F(2, 46) = 1.344$, $p = 0.271$; partial ETA squared = 0.055; Treatment Condition and Trial Type – $F(1, 23) = 0.144$, $p = 0.708$; partial ETA squared = 0.006; Treatment Condition, Epoch and Trial Type – $F(2, 46) = 0.613$, $p = 0.546$; partial ETA squared = 0.026; Treatment Condition and Reward Condition – $F(1, 23) = 2.870$, $p = 0.104$; partial ETA squared = 0.111; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 0.529$, $p = 0.593$; partial ETA squared = 0.022; Treatment Condition, Trial Type and Reward Condition – $F(1, 23) = 0.654$, $p = 0.427$; partial ETA squared = 0.028; and Treatment Condition, Epoch, Trial Type and Reward Condition – $F(2, 46) = 1.638$, $p = 0.205$; partial ETA squared = 0.066).

Next, I investigated the Oddball and Standard Trial Types, and found significant interactions between Treatment Condition, Epoch and Trial Type – $F(2, 46) = 26.062$, $p < 0.001$; partial ETA squared = 0.531; Treatment Condition and Reward Condition – $F(1, 23) = 14.080$, $p = 0.001$; partial ETA squared = 0.380; and Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 28.0178$, $p < 0.001$; partial ETA squared = 0.550.

Figure 3.3 shows the relationship between Treatment Condition, Epoch and Trial Type (Oddball, Standard). As you can see, the pupil is the most dilated for the Oddball Trial Type in the Stimulus Response Epoch followed by the Oddball Trial Type in the Post-Stimulus Baseline Epoch. In contrast, the extent of pupil dilation is similar across all other combinations of factors. Overall, the pupil was more dilated in the Placebo Treatment Condition than the Mifepristone Treatment Condition, especially for the Oddball Trial Type in the Stimulus Response and Post-Stimulus Baseline Epochs. In addition, the difference in pupil size between the Oddball and Standard Trial Types was greater in the Placebo Treatment Condition than the Mifepristone Treatment Condition for the Stimulus Response Epoch (Mifepristone: 1.70 mm^2; Placebo: 1.81 mm^2) and the Post-Stimulus Baseline Epoch (Mifepristone: 1.05 mm^2; Placebo: 1.22 mm^2), indicating a blunted phasic effect of the Oddball Trial Type or behavioural salience on the pupil in the Mifepristone Treatment Condition compared with Placebo.

Figure 3.3

Line Chart showing Pupil Dilation for the Oddball and Standard Trial Types across the Pre-Stimulus Baseline (Pre SE), Stimulus Response (SRE) and Post-Stimulus Baseline Epoch (Post SE) in the Mifepristone and Placebo Treatment Conditions

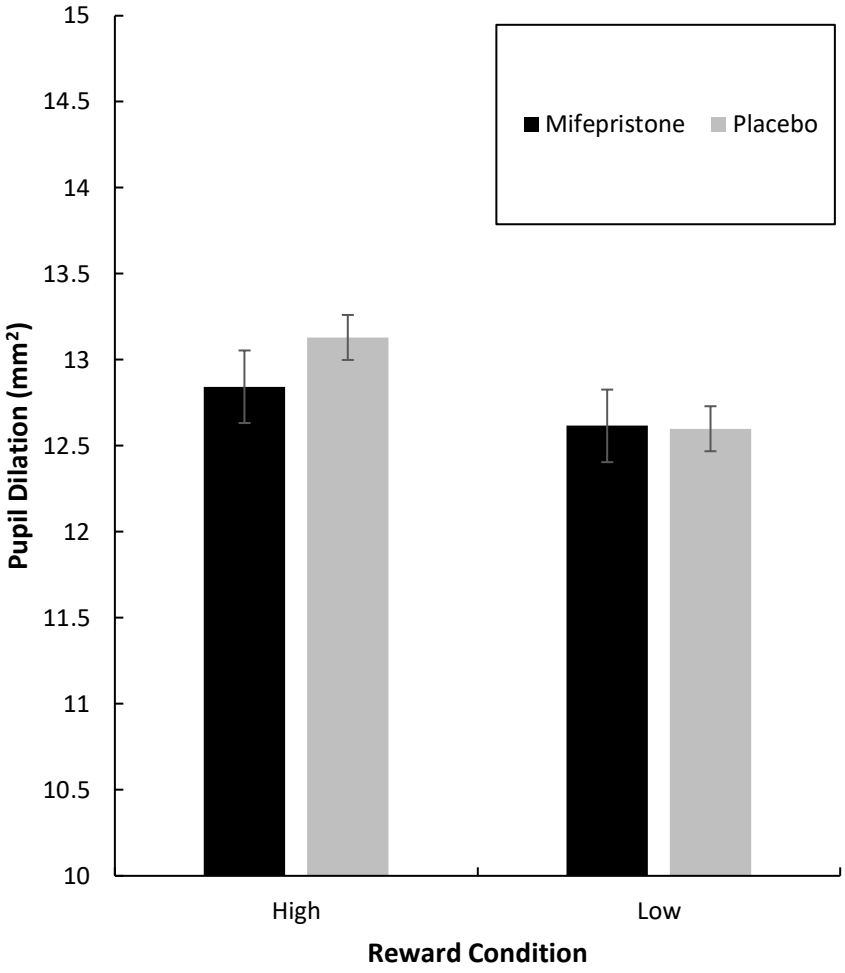


Note. **Error bars are standard error of the mean.**

Figure 3.4 depicts the relationship between Treatment Condition and Reward Condition. The interaction between these two factors appears to stem from a more dilated pupil in the High Reward Condition than the Low Reward Condition across both Treatment Conditions but a blunted effect of incentive salience by the Mifepristone Treatment Condition compared with Placebo (because there is a smaller difference in pupil size between the Reward Conditions in the Mifepristone Treatment Condition).

Figure 3.4

Bar Chart showing Pupil Dilation in the High and Low Reward Condition in the Mifepristone and Placebo Treatment Condition



Note. **Error bars are standard error of the mean.**

Similarly, exploration of the interaction between Treatment Condition, Epoch and Reward Condition revealed a smaller effect of incentive salience on the pupil by Mifepristone compared with Placebo. The added effect of Epoch came from a more dilated pupil in the Stimulus Response Epoch than the Post-Stimulus Baseline Epoch and Pre-Stimulus Baseline Epoch, respectively. This effect of Epoch was maintained across both Reward Conditions and Treatment Conditions.

Next, the Deviant and Standard Trial Types were investigated: there was no significant effect of Treatment Condition – $F(1, 23) = 0.069$, $p = 0.795$; partial ETA squared = 0.003; nor were there any interactions between Treatment Condition and any other factor (Treatment Condition and Epoch – $F(2, 46) = 0.882$, $p = 0.421$; partial ETA squared = 0.037; Treatment Condition and Trial Type – $F(1, 23) = 0.121$, $p = 0.731$; partial ETA squared = 0.005; Treatment Condition, Epoch and Trial Type – $F(2, 46) = 0.015$, $p = 0.985$; partial ETA squared = 0.001; Treatment Condition and Reward Condition – $F(1, 23) = 1.938$, $p = 0.177$; partial ETA squared = 0.078; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 1.870$, $p = 0.166$; partial ETA squared = 0.075; Treatment Condition, Trial Type and Reward Condition – $F(1, 23) = 0.400$, $p = 0.533$; partial ETA squared = 0.017; and Treatment Condition, Epoch, Trial Type and Reward Condition – $F(2, 46) = 1.6574$, $p = 0.218$; partial ETA squared = 0.064).

Spironolactone

I carried out a repeated measures ANOVA on the average pupil size with four factors: Treatment Condition (Spironolactone, Placebo), Reward Condition (Low, High), Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). There was a significant main effect of Epoch – $F(2, 46) = 83.932$, $p < 0.001$; partial ETA squared = 0.785; Trial Type – $F(2, 46) = 48.138$, $p < 0.001$; partial ETA squared = 0.677; and Reward Condition – $F(1, 23) = 6.683$, $p = 0.017$; partial ETA squared = 0.225. There was no significant main effect of Treatment Condition – $F(1, 23) = 0.959$, $p = 0.338$; partial ETA squared = 0.040. There were significant interactions between Treatment Condition and Trial Type – $F(2, 46) = 7.165$, $p = 0.002$; partial ETA squared = 0.238; Epoch and Trial Type – $F(4, 92) = 39.715$, $p < 0.001$; partial ETA squared = 0.633; Treatment Condition, Epoch and Trial Type – $F(4, 92) = 19.571$, $p < 0.001$; partial ETA squared = 0.460; Epoch and Reward Condition – $F(2, 46) = 9.602$, $p < 0.001$; partial ETA squared = 0.295; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 7.125$, $p = 0.002$; partial ETA squared = 0.237; Trial Type and Reward Condition – $F(2, 46) = 18.062$, $p < 0.001$; partial ETA squared = 0.440; Treatment Condition, Trial Type and Reward Condition – $F(2, 46) = 19.228$, $p < 0.001$; partial ETA squared = 0.455; Epoch, Trial Type and Reward Condition – $F(4, 92) = 20.391$, $p < 0.001$; partial ETA squared = 0.470; Treatment Condition, Epoch, Trial Type and Reward Condition – $F(4, 92) = 23.859$, $p < 0.001$; partial ETA squared = 0.509.

Again, there was no main effect of Treatment Condition nor was there a significant interaction between Treatment Condition and Reward Condition, suggesting that there was no tonic effect of Treatment Condition on the pupil. However, the various interaction effects between Treatment Condition, Reward Condition, Trial Type and Epoch suggest that there is a phasic effect of Treatment Condition on the pupil. The phasic effects of Spironolactone on pupil size were investigated in the same way as for the Mifepristone Treatment Condition—by carrying out three repeated measures ANOVAs on the average pupil size with four factors: Treatment Condition (Spironolactone, Placebo), Reward Condition (Low, High), Trial Type (Oddball and Deviant, Oddball and Standard or Deviant and Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline).

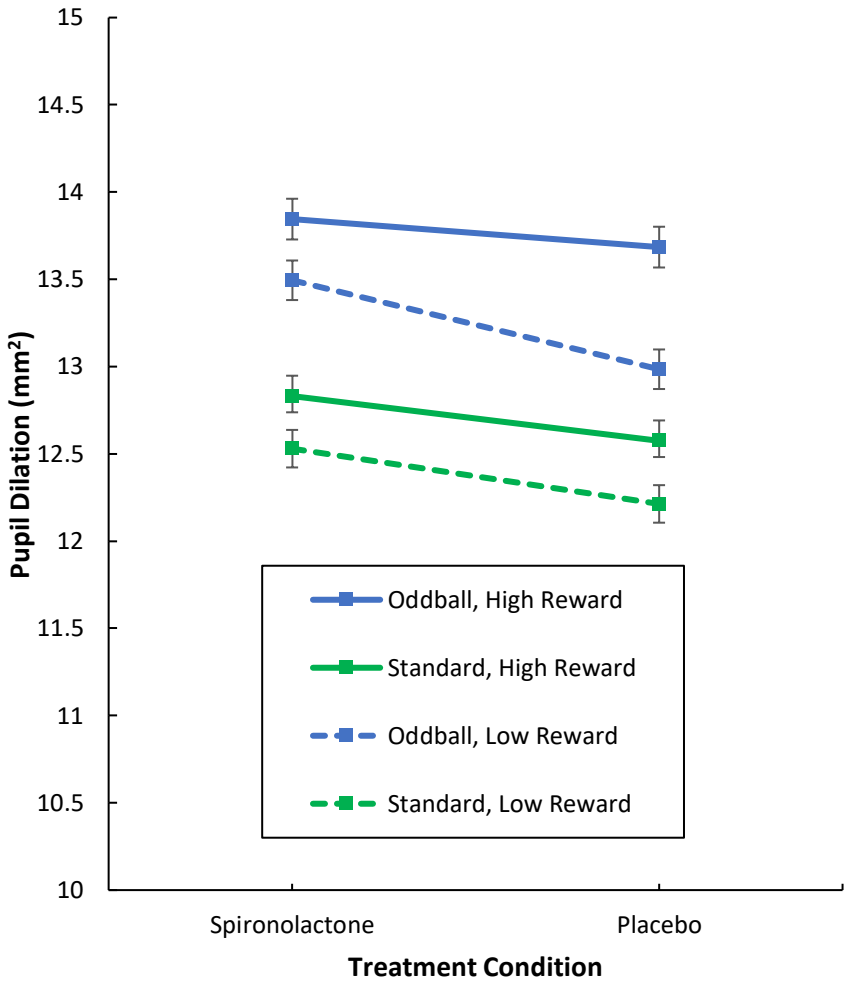
First, the Oddball and Deviant Trial Types were investigated: there was no significant effect of Treatment Condition – $F(1, 23) = 0.883$, $p = 0.357$; partial ETA squared = 0.037; nor were there any interactions between Treatment Condition and any other factor (Treatment Condition and Epoch – $F(2, 46) = 1.656$, $p = 0.202$; partial ETA squared = 0.067; Treatment Condition and Trial Type – $F(1, 23) = 0.615$, $p = 0.283$; partial ETA squared = 0.050; Treatment Condition, Epoch and Trial Type – $F(2, 46) = 1.013$, $p = 0.371$; partial ETA squared = 0.042; Treatment Condition and Reward Condition – $F(1, 23) = 1.468$, $p = 0.238$; partial ETA squared = 0.060; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 2.580$, $p = 0.087$; partial ETA squared = 0.101; Treatment Condition, Trial Type and Reward Condition – $F(1, 23) = 2.194$, $p = 0.152$; partial ETA squared = 0.087; Treatment Condition, Epoch, Trial Type and Reward Condition – $F(2, 46) = 1.169$, $p = 0.320$; partial ETA squared = 0.048).

Next, I investigated the Oddball and Standard Trial Types, and found a significant interaction between Treatment Condition, Trial Type and Reward Condition – $F(1, 23) = 4.919$, $p = 0.037$; partial ETA squared = 0.176. This interaction resulted from a more dilated pupil in the High Reward Condition than the Low Reward Condition, and although the pupil was more dilated in the Spironolactone Treatment Condition, Figure 3.5 shows that there was a smaller difference between the two Reward Conditions in Spironolactone Treatment Condition compared with Placebo. Therefore, the phasic response to incentive salience,

which is reflected in the difference in pupil size between Reward Conditions, is smaller in the Spironolactone Treatment Condition compared with Placebo.

Figure 3.5

Line Chart showing Pupil Dilation for the Oddball and Standard Trial Types across both Reward Conditions in the Mifepristone and Placebo Treatment Conditions



Note. **Error bars are standard error of the mean.**

Next, I investigated the Deviant and Standard Trial Types and found a significant interaction between Treatment Condition, Epoch and Trial Type – $F(2, 46) = 4.607$, $p = 0.015$; partial ETA squared = 0.167. This interaction appeared to come from a more dilated pupil in the Spironolactone Treatment Condition compared with Placebo but a similar pattern across the two Treatment Conditions of greater pupil size in the Deviant Trial Type than the Standard Trial Type in the Stimulus Response and Post-Stimulus Baseline Epoch, and the contrasting pattern of greater pupil size in the Standard Trial Type than the Deviant Trial Type in the Pre-Stimulus Baseline Epoch.

Propranolol

I carried out a repeated measures ANOVA on the average pupil size with four factors: Treatment Condition (Propranolol, Placebo), Reward Condition (Low, High), Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). There was a significant main effect of Epoch – $F(2, 46) = 63.057$, $p < 0.001$; partial ETA squared = 0.733; Trial Type – $F(2, 46) = 44.959$, $p < 0.001$; partial ETA squared = 0.662; and Reward Condition – $F(1, 23) = 6.760$, $p = 0.016$; partial ETA squared = 0.227. There was no significant main effect of Treatment Condition – $F(1, 23) = 1.321$, $p = 0.262$; partial ETA squared = 0.054. There was a significant interaction between Treatment Condition and Epoch – $F(2, 46) = 5.761$, $p = 0.006$; partial ETA squared = 0.200; Treatment Condition and Trial Type – $F(2, 46) = 4.209$, $p = 0.021$; partial ETA squared = 0.155; Epoch and Trial Type – $F(4, 92) = 38.841$, $p < 0.001$; partial ETA squared = 0.628; Treatment Condition, Epoch and Trial Type – $F(4, 92) = 15.076$, $p < 0.001$; partial ETA squared = 0.396; Epoch and Reward Condition – $F(2, 46) = 5.329$, $p = 0.008$; partial ETA squared = 0.188; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 5.927$, $p = 0.005$; partial ETA squared = 0.205; Trial Type and Reward Condition – $F(2, 46) = 29.608$, $p < 0.001$; partial ETA squared = 0.563; Treatment Condition, Trial Type and Reward Condition – $F(2, 46) = 8.894$, $p = 0.001$; partial ETA squared = 0.279; Epoch, Trial Type and Reward Condition – $F(4, 92) = 20.028$, $p < 0.001$; partial ETA squared = 0.465; and Treatment Condition, Epoch, Trial Type and Reward Condition – $F(4, 92) = 23.772$, $p < 0.001$; partial ETA squared = 0.508.

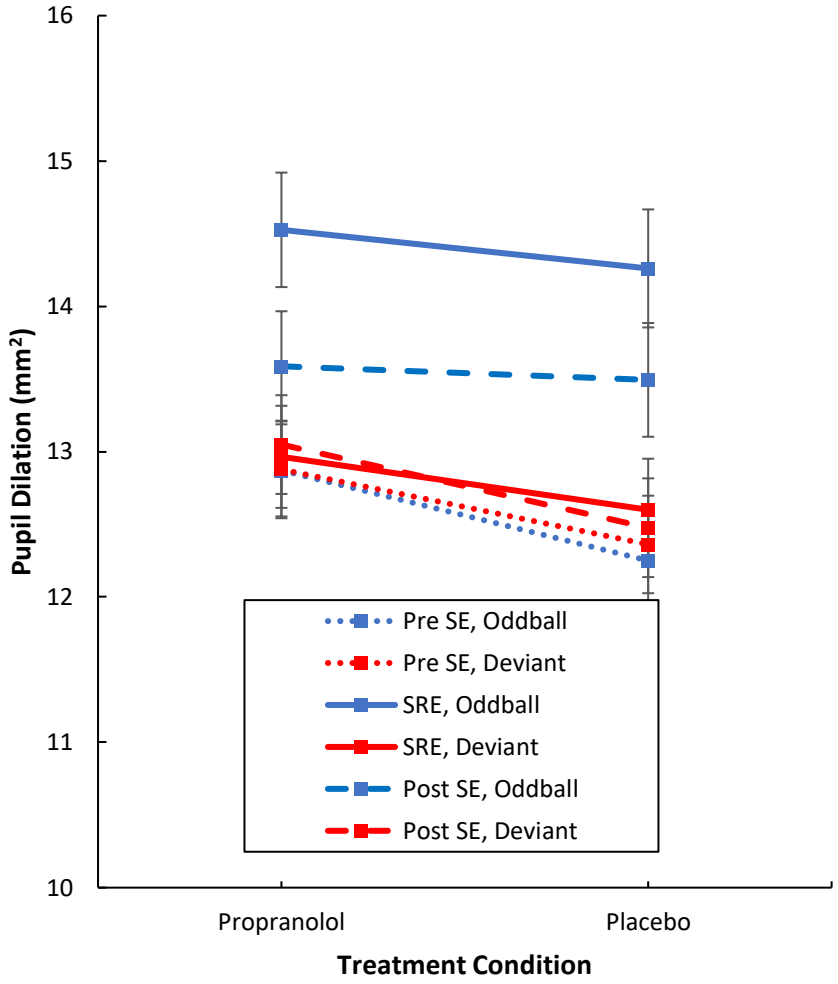
Again, there was no main effect of Treatment Condition nor was there a significant interaction between Treatment Condition and Reward Condition. Therefore, there was no

tonic effect of Treatment Condition on the pupil, nor was there a tonic effect of incentive salience on the pupil by Treatment Condition. However, there is some evidence for a phasic effect of Treatment Condition on the pupil in the various interaction effects with Treatment Condition. To investigate the phasic effect, three repeated measures ANOVAs were carried out on the average pupil size with four factors: Treatment Condition (Propranolol, Placebo), Reward Condition (Low, High), Trial Type (Oddball and Deviant, Oddball and Standard or Deviant and Standard) and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). Again, for each ANOVA, two levels of Trial Type were compared, rather than all three, to home in on any significant interactions.

First, I investigated the Oddball and Deviant Trial Types and found a significant interaction between Treatment Condition and Epoch – $F(2, 46) = 5.587$, $p = 0.007$; partial ETA squared = 0.195; and Treatment Condition, Epoch and Trial Type – $F(2, 46) = 3.521$, $p = 0.038$; partial ETA squared = 0.133. Figure 3.6 shows the relationship between Treatment Condition, Trial Type and Epoch. This figure shows that, although the pupil was more dilated in the Propranolol Treatment Condition, the same pattern of a more dilated pupil for Oddball Trial Type in the Stimulus Response Epoch followed by the Oddball Trial Type in the Post-Stimulus Baseline Epoch was conserved across Treatment Conditions. In addition, the difference in pupil size between the Oddball and Deviant Trial Types was greater in the Placebo Treatment Condition than the Propranolol Treatment Condition for the Stimulus Response Epoch (Propranolol: 1.56 mm^2; Placebo: 1.66 mm^2) and the Post-Stimulus Baseline Epoch (Propranolol: 0.54 mm^2; Placebo: 1.02 mm^2). This data suggests that the phasic response to the oddball stimulus (i.e., behavioural salience) was suppressed in the Propranolol Treatment Condition compared with Placebo.

Figure 3.6

Line Chart showing Pupil Dilation for the Oddball and Deviant Trial Types across the Pre-Stimulus (Pre SE), Stimulus Response (SRE) and Post-Stimulus Baseline Epoch (Post SE) in the Propranolol and Placebo Treatment Conditions



Note. **Error bars are standard error of the mean.**

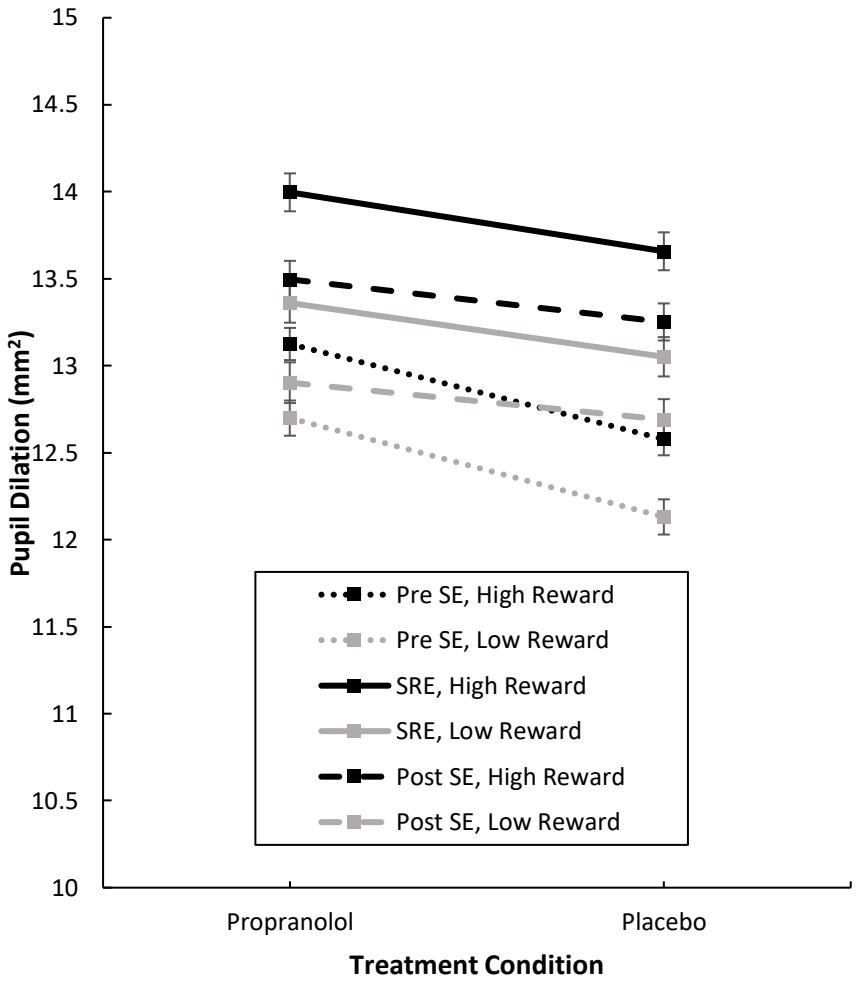
Next, I investigated the Oddball and Standard Trial Types and found significant interactions between Treatment Condition and Epoch – $F(2, 46) = 6.087$, $p = 0.005$; partial ETA squared = 0.209; Treatment Condition, Epoch and Trial Type – $F(2, 46) = 35.918$, $p < 0.001$; partial ETA squared = 0.610; and Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 26.824$, $p < 0.001$; partial ETA squared = 0.538. The interaction between Treatment Condition, Epoch and Trial Type is similar to that of the previous ANOVA (see Figure 3.6), with a more dilated

pupil in the Propranolol Treatment Condition than Placebo, but a similar pattern of a more dilated pupil at the level of the Oddball Trial Type in the Stimulus Response Epoch followed by the Oddball Trial Type in the Post-Stimulus Baseline Epoch, then similar pupil size between other combinations of factors. Again, the difference in pupil size between the Oddball and Standard Trial Types was greater in the Placebo Treatment Condition than the Propranolol Treatment Condition for the Stimulus Response Epoch (Propranolol: 1.70 mm^2; Placebo: 1.81 mm^2) and the Post-Stimulus Baseline Epoch (Propranolol: 0.78 mm^2; Placebo: 1.22 mm^2), indicating a blunted phasic effect of the oddball stimulus (i.e., behavioural salience) on pupil dilation in the Propranolol Treatment Condition compared with Placebo.

Figure 3.7 shows the relationship between Treatment Condition, Epoch and Reward Condition, with a more dilated pupil in the Propranolol Treatment Condition compared with Placebo and the High Reward Condition compared with the Low Reward Condition across both Treatment Conditions. The difference in pupil dilation between Reward Conditions was greater in the Propranolol Treatment Condition than Placebo for the Stimulus Response Epoch (Propranolol: 0.64 mm^2; Placebo: 0.61 mm^2) and the Post-Stimulus Baseline Epoch (Propranolol: 0.59 mm^2; Placebo: 0.56 mm^2), suggesting a greater phasic effect of incentive salience on the pupil in the Propranolol Treatment Condition.

Figure 3.7

Line Chart showing Pupil Dilation in the High and Low Reward Conditions across the Pre-Stimulus Baseline (Pre SE), Stimulus Response (SRE) and Post-Stimulus Baseline Epoch (Post SE)in the Propranolol and Placebo Treatment Conditions



Note. **Error bars are standard error of the mean.**

Finally, the Deviant and Standard Trial Types were investigated: there was no significant effect of Treatment Condition – $F(1, 23) = 1.662$, $p = 0.210$; partial ETA squared = 0.067, nor were there significant interactions between Treatment Condition and any other factor (Treatment Condition and Epoch – $F(2, 46) = 2.616$, $p = 0.084$; partial ETA squared = 0.102; Treatment Condition and Trial Type – $F(1, 23) = 0.042$, $p = 0.840$; partial ETA squared = 0.002; Treatment Condition, Epoch and Trial Type – $F(2, 46) = 0.408$, $p = 0.667$; partial ETA squared = 0.017; Treatment Condition and Reward Condition – $F(1, 23) = 0.060$, p 0.809;

partial ETA squared = 0.003; Treatment Condition, Epoch and Reward Condition – $F(2, 46) = 0.030$, $p = 0.970$; partial ETA squared = 0.001; Treatment Condition, Trial Type and Reward Condition – $F(1, 23) = 0.076$, $p = 0.785$; partial ETA squared = 0.003; Treatment Condition, Epoch, Trial Type and Reward Condition – $F(2, 46) = 0.264$, $p = 0.769$; partial ETA squared = 0.011).

Manual responses

In addition to pupillometry data, the manual response times of keyboard presses made to oddball stimulus presentations were recorded. The effects of Reward Condition and Treatment Condition on these manual responses were investigated by carrying out a repeated measures ANOVA on the average reaction time with two factors: Treatment Condition (Mifepristone, Spironolactone, Propranolol, Placebo), Reward Condition (Low, High). There was a significant main effect of Reward Condition – $F(1, 23) = 5.775$, $p = 0.025$; partial ETA squared = 0.201; but not Treatment Condition – $F(3, 69) = 0.321$, $p = 0.810$; partial ETA squared = 0.014, with faster reaction times in the High Reward Condition (M = 0.64 ms) than the Low Reward Condition (M = 0.65 ms; SD = 0.40 ms). There was no significant interaction between Treatment Condition and Reward Condition – $F(3, 69) = 0.454$, $p = 0.715$; partial ETA squared = 0.019.

Discussion

Whilst there was no tonic effect on pupil dilation by Mifepristone, Spironolactone nor Propranolol, there were significant phasic effects on the pupil by these drug Treatment Conditions compared with Placebo. Administration of Mifepristone, a GR antagonist, and Spironolactone, an MR antagonist, suppressed the phasic effect of incentive salience on pupil dilation. Mifepristone also blunted the phasic effect of behavioural salience on the pupil. The beta blocker Propranolol amplified the phasic effect of incentive salience on the pupil but suppressed the phasic effect of behavioural salience on the pupil compared with Placebo. Interestingly, although manual response times to oddball stimuli showed a reward effect with faster reaction times in the High Reward Condition than the Low Reward Condition, there was no interaction between Treatment Condition and Reward Condition on the average reaction time. Therefore, the effects of Treatment Condition on incentive

salience were only reflected in pupil sensitivity and were locked to the task stimuli of the oddball task.

The suppressed phasic effect of incentive salience by Mifepristone and Spironolactone is consistent with my proposal that cortisol signalling via MR or GR is part of the brain circuitry underlying extrinsic motivation. Mifepristone and Spironolactone are GR and MR antagonists, and therefore inhibit the effects of cortisol signalling. Thus, if cortisol functions in extrinsic motivation, a blunted effect of incentive salience and reduced reward sensitivity by Mifepristone and/or Spironolactone would be expected. It is important to note that although the pupil appears generally more dilated in the Spironolactone Treatment Condition than Placebo (see Figure 3.2A and C), there is a smaller difference between Reward Conditions with Spironolactone (i.e., a blunted phasic effect of incentive salience), and this small difference is similar to that of the Mifepristone Treatment Condition.

In addition to the smaller phasic effect of incentive salience, administration of Mifepristone caused a suppressed phasic effect of behavioural salience on the pupil compared with Placebo. This phasic effect was measured by comparing the difference in the peak of the pupil response to the Oddball with the peak of the pupil response to the Deviant or Standard Trial Types. Therefore, a reduced phasic effect of behavioural salience by Mifepristone could reflect a reduced effect of behavioural salience by the Oddball Trial Type or an enhanced effect of bottom-up salience by the Deviant or Standard Trial Type (or both). However, the Standard Trial Type should not have stood out from the other Trial Types as it was not task relevant (i.e., behaviourally salient) nor did it possess low stimulus probability (i.e., bottom-up salience). Therefore, I propose that this suppressed phasic effect is related to a reduced effect of behavioural salience rather than an increase in the bottom-up salience effect, as this effect was only present in an ANOVA investigating the Oddball and Standard levels of Trial Type. The suppression of both top-down salience effects (behavioural and incentive salience) by Mifepristone suggests that Mifepristone inhibited top-down or goal-directed processes. According to theories of action control, goal-directed and stimulus-driven modes compete for dominance (de Wit & Dickinson, 2009). This suggests that if Mifepristone suppressed behavioural salience and incentive salience, which are both related to goal-directed processes, it could have enhanced stimulus-driven

processes, marked by increased bottom-up salience. However, the pupil response to the Deviant Trial Type, which had bottom-up salience, was not elevated in the Mifepristone Treatment Condition compared with Placebo. Thus, it seems that the effect of Mifepristone was an overall dampening of top-down, goal-directed processes.

On the other hand, the suppressive effects of Spironolactone on the phasic pupil response were targeted to incentive salience and not behavioural salience, as the difference in pupil size between Reward Conditions was smaller in the Spironolactone Treatment Condition compared with Placebo, but otherwise the difference between Trial Types was similar between the Spironolactone Treatment Condition and Placebo. Altogether, this suggests that the blockade of MR by Spironolactone impeded reward sensitivity and therefore the incentive salience effect by extrinsic motivation, whereas blockade of GR by Mifepristone impeded general top-down salience effects related to both intrinsic and extrinsic motivation. Therefore, perhaps cortisol signalling via MR is a better target for future investigations of cortisol function in motivation and reward. However, it is important to point out that the inhibitory effects of these drugs on extrinsic motivation did not translate to behaviour, as there was no effect of Treatment Condition on reaction times. Thus, although my data provides evidence for a role of cortisol in extrinsic motivation—a novel finding in this field—it does not provide evidence for an effect on behaviour. This suggests that cortisol functions in an attentional process underlying extrinsic motivation that does not necessarily translate to behaviour. Having said this, reaction time data is quite variable, and so it is possible that this variability obscured the effects of Treatment Condition on reaction time. As mentioned in Chapter 2, there were only about 10 Oddball Stimuli presented for each block (Oddball Trial Types had an 8% probability and there were 125 trials per block), so approximately 20 Oddballs were presented in the High Reward Condition and 20 in the Low Reward Condition. The low presentation frequency of the Oddball stimulus resulted in little reaction time data, which is typically quite noisy and therefore could have obscured the a significant effect of Treatment Condition on reaction time.

The literature is divided on whether cortisol enhances or reduces reward sensitivity. As mentioned, the direction of cortisol's effects on the reward system seem to depend on

whether stress or ultradian levels of plasma cortisol are present, and whether genomic or non-genomic mechanisms are involved. However, the drug dosing regimens for Spironolactone and Mifepristone in this study were designed to prevent both the fast (non-genomic) and slow (genomic) effects of cortisol by administering a midnight dose, which blocked the effects of long-acting genomic responses overnight, plus a morning dose, which antagonised more rapid non-genomic responses during testing. My study was not designed to test the effects of these agents on the response to stressors, and I avoided any stressful components in my relatively straight-forward task, thus maintaining plasma cortisol at non-stress levels. In this non-stress state, the literature suggests that a goal-directed mode is favoured over a stimulus-driven mode, especially within a task setting with a reward manipulation (Bogdan & Pizzagalli, 2006; Braunstein-Bercovitz et al., 2001; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017; Schwabe & Wolf, 2009; 2011). This would suggest that, in my study, blockade of MR and GR with Spironolactone and Mifepristone prevented the normal function of cortisol in facilitating a goal-directed, rewarding seeking mode and therefore suppressed the phasic effect of incentive salience on the pupil. As mentioned, the effect of Spironolactone on top-down salience is specific to incentive salience and not behavioural salience, whereas Mifepristone exerted an overall dampening influence on top-down salience. Therefore, perhaps cortisol signalling via GR, which is blocked by Mifepristone, is required for a goal-directed state in favour of internal goals, whereas cortisol signalling via either MR or GR is sufficient for goal-directed, reward-seeking states. In other words, the effects of cortisol on incentive salience are not specific to MR or GR.

In contrast to the Spironolactone and Mifepristone Treatment Conditions, Propranolol enhanced the phasic effect of Reward Condition on the pupil compared with Placebo. This finding is more difficult to interpret as although Propranolol is a beta adrenoceptor antagonist and thus inhibits the effects of NE on the pupil, the NE control of pupil dilation and constriction involves a delicate balance between alpha and beta adrenoceptor activation. For example, the iris sphincter muscle, which constricts the pupil, expresses inhibitory beta adrenoceptors that dilate the pupil, and excitatory alpha adrenoceptors that constrict the pupil. In contrast, the iris dilator muscle, which dilates the pupil, has more excitatory alpha adrenoceptors that dilate the pupil than beta adrenoceptors that constrict the pupil (Pintor, 2009; Yoshitomi et al., 1985). Blockade of either of these receptor

subtypes disrupts the balance between excitatory input and inhibitory input to both muscles and leads to an increase in the activation of the other subtype. Therefore, administering Propranolol could dilate the pupil by causing an increase in NE signalling via the alpha adrenoceptor. However, Propranolol did not cause an overall more dilated pupil compared with Placebo, as its effects were phasic, and stimulus locked. This suggests that Propranolol enhanced the phasic effect of incentive salience on the pupil, perhaps by increasing NE signalling at the alpha adrenoceptor. Interestingly, blockade of the alpha adrenoceptor with yohimbine abolishes pupil responses evoked by electrical stimulation of the LC in rats (Y. Liu et al., 2017). This suggests that the pathway connecting the LC to the pupil expresses alpha adrenoceptors, and perhaps the effects of alpha adrenoceptor blockade on salience measured via the pupil should be investigated in future.

I found evidence for an inhibited phasic response to behavioural salience by the Oddball Trial Type in the Propranolol Treatment Condition compared with Placebo. This indicates that the phasic effect of behavioural salience on the pupil was suppressed by beta adrenoceptor blockade and therefore likely involves NE signalling via beta adrenoceptors, although the response was not completely abolished so it could also involve activation of the alpha adrenoceptor subtype. The enhanced phasic effect of incentive salience by Propranolol compared with the suppressed phasic effect of behavioural salience by Propranolol suggests that the processes driving these two types of top-down salience are distinct and comprise different pathways. As proposed by Awh et al. (2012), events related to internal or external goals should be thought of as distinct phenomena. Therefore, it is possible for a given control state to facilitate goal-directed, reward-biased behaviours whilst suppressing other types of goal-directed behaviour, and vice versa. This brings into question dual-system theories of control state, which propose that attentional control is biased towards top-down or bottom-up events. In contrast, there was evidence of an increased effect by one top-down event but not another. In terms of my expectation that the LC-NE system functions in intrinsic and extrinsic motivation, this evidence suggests that central NE is involved in mediating processes related to motivation, but that distinct intrinsic and extrinsic motivational processes require the activation of different receptor subtypes.

There is one main limitation of the present study in that it is only possible to draw indirect inferences about underlying mechanisms from pupillometry data. For example, although it is clear that there is an effect of incentive salience on the pupil, it is unclear which extrinsic motivational process is driving this effect on the pupil, and so which process is influenced by Treatment Condition. For example, this process could be saliency attribution, the detection of salience, the mobilisation of energy resources for reward-seeking behaviours, and so on. These limitations extend to the previous experiments documented in this thesis, but they are particularly important to keep in mind when drawing conclusions from the present data, which was collected to test a novel mechanism of apathy. This study cannot confirm whether a mechanism involving a dysfunctional LC and HPA axis in apathy exists, but the findings do suggest that cortisol and NE influence incentive salience and reward sensitivity, which become disrupted in apathy. In addition, this evidence suggests the mechanism involved is more closely linked to extrinsic motivation than the rewarding effect, as the phasic pupil response was evoked by reward incentives and not reward delivery. Future investigations should examine the effects of disrupting cortisol and NE signalling on different parts of the reward processes in an attempt to establish the precise contribution of these mechanisms to motivation and reward function.

I have shown that, regardless of the precise underlying mechanisms, blockade of cortisol signalling via MR and GR suppresses a phasic effect of incentive salience on the pupil (and therefore LC activity), demonstrating cortisol involvement in a reward sensitivity process by the LC that is closely associated with extrinsic motivation. Additionally, blockade of NE signalling via the beta adrenoceptor enhanced the phasic effect of incentive salience on the pupil, possibly by enhancing NE signalling via the alpha adrenoceptor. In contrast, beta adrenoceptor blockade suppressed the phasic effect of behavioural salience on the pupil, suggesting an involvement of beta adrenoceptor signalling in top-down salience that was not present in the incentive salience effect by propranolol. Notably, although the pupil is innervated by NE input, blocking NE signalling via the beta adrenoceptor did not lead to a significantly overall more dilated pupil in the Propranolol Treatment Condition compared with Placebo. The differences in pupil dilation were related to stimulus presentation. Therefore, the effects of Propranolol were on salience and cognition rather than directly on the mechanics of the pupil. Whilst the directionality of these findings was not entirely

expected, the literature suggests a regulatory effect of these systems on extrinsic motivation, which indicates that effects could be bidirectional depending on the context. Future investigations questioning the precise role of cortisol and NE signalling in mechanisms of motivation and reward could be important for elucidating the underlying mechanisms of apathy. This study demonstrates how pupillometry techniques might act as a useful tool for indicating novel avenues for the research of human cognition and behaviour.

In the next chapter, I explore the mechanisms driving these effects of salience on the pupil further by administering the same oddball task with a reward manipulation and concurrent pupillometry on healthy human volunteers exposed to a stress dose of hydrocortisone. Given the distinct fast and slow effects of cortisol, I was interested to see whether these effects of cortisol on salience vary over time.

Chapter 4

Introduction

In this chapter, I report a study investigating the fast and slow effects of a stress dose of cortisol on bottom-up and top-down salience effects on the pupil. The rationale for carrying out this study comes from the lack of research investigating the fast and slow effects of cortisol in humans, the effects of cortisol on reward and the link between the HPA axis and the LC-NE system. This study was exploratory, carried out in collaboration with another Ph.D. student.

Literature review

The glucocorticoid stress hormone cortisol is the main downstream effector of the HPA axis in humans. Cortisol is a very dynamic hormone with circadian rhythmicity and a superimposed ultradian rhythm (Windle et al., 1998; Young et al., 2004). Glucocorticoids synchronise physiological changes through their widely distributed receptors: the high affinity MR and the low affinity GR (Smith and Vale, 2006). In addition to the slow effects, which are achieved by ligand-receptor binding, nuclear translocation and transcription, glucocorticoids exert fast effects via non-genomic mechanisms and membrane-bound receptors, such as membrane-bound MR (Groeneweg et al. 2011). The fast effects occur within seconds to minutes of glucocorticoid exposure (Wiegert et al., 2005).

A few human studies have investigated the fast effects of glucocorticoids, focusing on the rapid effects of cortisol on brain connectivity, cognition and behaviour in stress (Henckens et al., 2010; 2012; Hermans et al., 2011; 2014; Lovallo et al., 2010; van Marle et al., 2009; 2010) . It is thought that, in the early stages of the stress response, energy resources are redistributed throughout the brain from the executive control network, which comprises frontal brain regions, toward the salience network, which includes the dorsal anterior cingulate cortex and the anterior insula, and functions in detecting and filtering salient stimuli for attentional selection (Hermans et al., 2014; Schwabe, 2017). In addition, evidence suggests that there is a rapid increase in connectivity between the LC and the amygdala, which mediates a surge in vigilance, whilst also facilitating saliency detection (Hermans et al., 2014; van Marle et al., 2009; 2010). These fast changes in brain activation

underlie a rapid increase in emotional distractibility and vigilance (Braunstein-Bercovitz et al., 2001; Skosnik et al., 2000; van Marle et al., 2009; 2010). In addition, there is a shift in control state from a goal-directed mode to a habit based and stimulus-driven mode (Braunstein-Bercovitz et al., 2001; Henckens et al., 2010; 2012; Hermans et al., 2014; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017; Schwabe & Wolf, 2009; 2011; van Marle et al., 2009; 2010). Overall, this leads to the development of a highly aroused behavioural state in which individuals are alert and readily drawn to salient events within their environment and quick to react. This is a highly adaptive response to threat detection, which promotes survival (Dallman, 2005; Henckens et al., 2010; Hermans et al., 2014).

In contrast, prolonged changes can be maladaptive, so it is important to revert to homeostasis. This could explain why genomic mechanisms of cortisol result in opposing brain activity patterns compared to non-genomic mechanisms. Evidence suggests that the genomic mechanisms redirect resources back from the salience network towards the executive control network, facilitating higher-order cognitive processes, and reinstating a less vigilant and distractable arousal state (Henckens et al., 2012; Hermans et al., 2014). Behavioural studies have reported results that compliment these brain activation findings: compared with enhanced vigilance, distractibility and stimulus-driven and habit-based behaviour, which is seen in the early stages of the stress response, the slower genomic mechanisms of cortisol facilitate executive function and goal-directed behaviour (Henckens et al., 2012; Hermans et al., 2014). In sum, it seems that non-genomic mechanisms serve to mediate cortisol's short-term effects on cognition and behaviour, whereas the genomic mechanisms reinstate and maintain homeostasis.

Some of the brain regions mentioned above overlap with networks of motivation and reward. For example, the anterior cingulate cortex and the LC, which are both involved in saliency detection, are interconnected with brain regions that function in reward, such as the ventral tegmental area (Cole et al., 2013; Hermans et al., 2014; Seeley et al., 2007). This link between networks of saliency and reward makes sense, given the functional relationship between the two phenomena. For instance, like behavioural goals, reward incentives possess a sort of top-down salience, which captures attention and motivates goal-directed behaviour (Awh et al., 2012; Fecteau & Munoz, 2006; Gong & Liu, 2018). In

addition, as described above, stress causes a shift in control state, promoting bottom-up processing as well as habit-based and stimulus-driven behavioural states rather than top-down processing and goal-directed behaviour for internal and external goals (Braunstein-Bercovitz et al., 2001; Henckens et al., 2010; 2012; Hermans et al., 2014; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017; Schwabe & Wolf, 2009; 2011; van Marle et al., 2009; 2010). This suggests there is a negative relationship between stress and goal-directed, reward-seeking control states. Indeed, although the literature is limited, evidence suggests that reward sensitivity is altered in stress (Bogdan & Pizzagalli, 2006; Kinner et al., 2016; Montoya et al., 2014).

Whilst there is some evidence of altered reward functioning and goal-directed behaviour in stress, there is no evidence of the real-time effects of cortisol on intrinsic and extrinsic motivation. As mentioned in Chapter 3, a state of intrinsic or extrinsic goal-directed mode or a stimulus-driven mode can be inferred from the effect of salience on the pupil. For example, if there is a greater effect of incentive salience on the pupil than behavioural and bottom-up salience, this would suggest a goal-directed control state for external rewards and not internal goals. In contrast, if there is a greater effect of behavioural salience on the pupil than incentive and bottom-up salience, this would suggest a goal-directed state for internal goals and not external rewards. Finally, if there is a greater effect of bottom-up salience on the pupil than top-down salience, this would be indicative of a stimulus-driven control state.

To investigate how these effects of salience on the pupil change over time in stress, I can compare the effects of salience on the pupil at different stages of a stress pulse of cortisol. My research group has previously trialled a subcutaneous pump for the infusion of hydrocortisone into the abdominal tissue of healthy human volunteers and patients with primary adrenal insufficiency (The Pulses Study, 2014). They developed a dosing regimen, which included the successful suppression of endogenous cortisol with metyrapone and the replacement of endogenous cortisol with hydrocortisone (Russell et al., 2014). Whilst it might be easier to administer oral hydrocortisone, this method does not control for endogenous cortisol, which varies throughout the day within and between individuals due to its circadian and ultradian rhythmicity. In addition, the timing of hydrocortisone

absorption into the systemic circulation from oral administration is slow and varied. By using the subcutaneous infusion pump with metyrapone, I can control the timing of ultradian and stress pulses of synthetic cortisol or hydrocortisone, as well as the levels of endogenous cortisol.

Therefore, the effects of top-down and bottom-up salience on the pupil were investigated over the course of a single hydrocortisone stress pulse in healthy human volunteers, using a subcutaneous infusion pump. The oddball task with a reward manipulation was administered in combination with pupillometry on both the rising and falling phases of the stress pulse. This allowed me to compare the pupil sensitivity of healthy human volunteers to incentive salience between the rising and falling phases of the stress pulse. Any differences in incentive salience effects on the pupil between these phases indicated a change in reward sensitivity and therefore extrinsic motivation over time. In addition, pupil sensitivity to behavioural salience was examined (an indicator of intrinsic motivation) and bottom-up salience. Any difference between phases indicated a change in intrinsic motivation or bottom-up processes over time. These findings have implications for the fast and slow effects of cortisol on salience and motivation, as well as the stress function of the human LC in control state. As mentioned, this investigation was part of a large research project that I collaborated on with another Ph.D. student.

Method

Participants

Participants were primarily recruited for another Ph.D. project by Jamini Thakrar using the subcutaneous infusion pump, which was attached to participants for seven days. As part of Jamini's recruitment, I advertised a small pupillometry experiment that would take place on 1 of the 7 days. However, as this was not my research project, participants were screened for eligibility on Jamini's project, which required healthy male volunteers, and did not include pupillometry screening. Thus, some recruited participants were unsuitable for pupillometry. Eight participants were excluded from my analysis due to missing pupil data and issues with attaching the subcutaneous infusion pump. Therefore, the participants were 10 right-handed, healthy male volunteers aged 18-26, with no history of psychiatric disease.

All participants had normal or corrected-to-normal vision. Informed consent was obtained from each participant prior to testing, and all participants were paid for participation. The study was approved by the Faculty of Health Sciences Research Ethics Committee, University of Bristol, UK. Participants were recruited from the University of Bristol community via poster advertisements, webpage advertisements and email lists.

Materials

During testing, participants were seated in front of a computer screen. The computer display was 22.5 inches and had a resolution of 1920 x 1200 pixels; viewing distance was 60 cm. Pupil area (left pupil) and eye position were recorded at a sampling rate of 500 Hz by a tower-mounted Eyelink 1000 eye tracker (SR Research, Canada), which has a typical operating spatial resolution of 0.5^{o} or better. Head movement was minimised using a chin and forehead rest. Manual responses were recorded via a computer keyboard.

Crono-P© pump and infusion set

A portable infusion pump called the Crono-P pump (see Figure 4.1) was attached to each participant via a subcutaneous infusion line, which was implanted into abdominal tissue. Participants were given a waist-fitted sports belt, with a pocket in it, which fitted the Crono-P Pump, so that they could carry the pump around with them hands-free.

Figure 4.1

Crono-P Pump (Applied Medical Technology, n.d.)

Study design

As mentioned, this experiment was part of a larger, seven-day study. On study day 1, participants attended a clinical facility for Crono-P pump attachment, endogenous cortisol suppression and task training. Cortisol suppression was achieved with the metyrapone dosing regimen displayed in Table 4.1, which included oral tablets and a gradual increase in dosage. Participants were instructed to self-administer metyrapone at home and show evidence of conforming to the dosing regimen by attending several tablet counts. The selected metyrapone dosing regimen was determined to be efficacious by a previous study (Russell et al., 2014).

Table 4.1

Metyrapone Tablet Dosing Regimen

Day	1	2	3	4	5	6	7	8
Breakfast dose (mg)	—	250	500	750	750	750	750	750
Lunch dose (mg)	250	500	500	750	750	750	750	—
Evening dose (mg)	250	500	750	750	750	750	750	—

Note. Tablets were taken at mealtimes with food to prevent nausea

The Crono-P pump replaced suppressed endogenous cortisol with hydrocortisone (Efcortisol), in a physiological three-hour pattern (see Table 4.2 for dosing regimen). The pump allowed us to control the timing of cortisol pulses, and to deliver stress-level doses (15 mg) of cortisol on data collection days. This high-level, stress dose was found to achieve the desired plasma cortisol level in a pilot study carried out by Jamini Thakrar. The pilot study acutely suppressed endogenous cortisol with dexamethasone and administered different subcutaneous doses of hydrocortisone.

Table 4.2

Hydrocortisone dosing regimen as administered via Crono-P pump

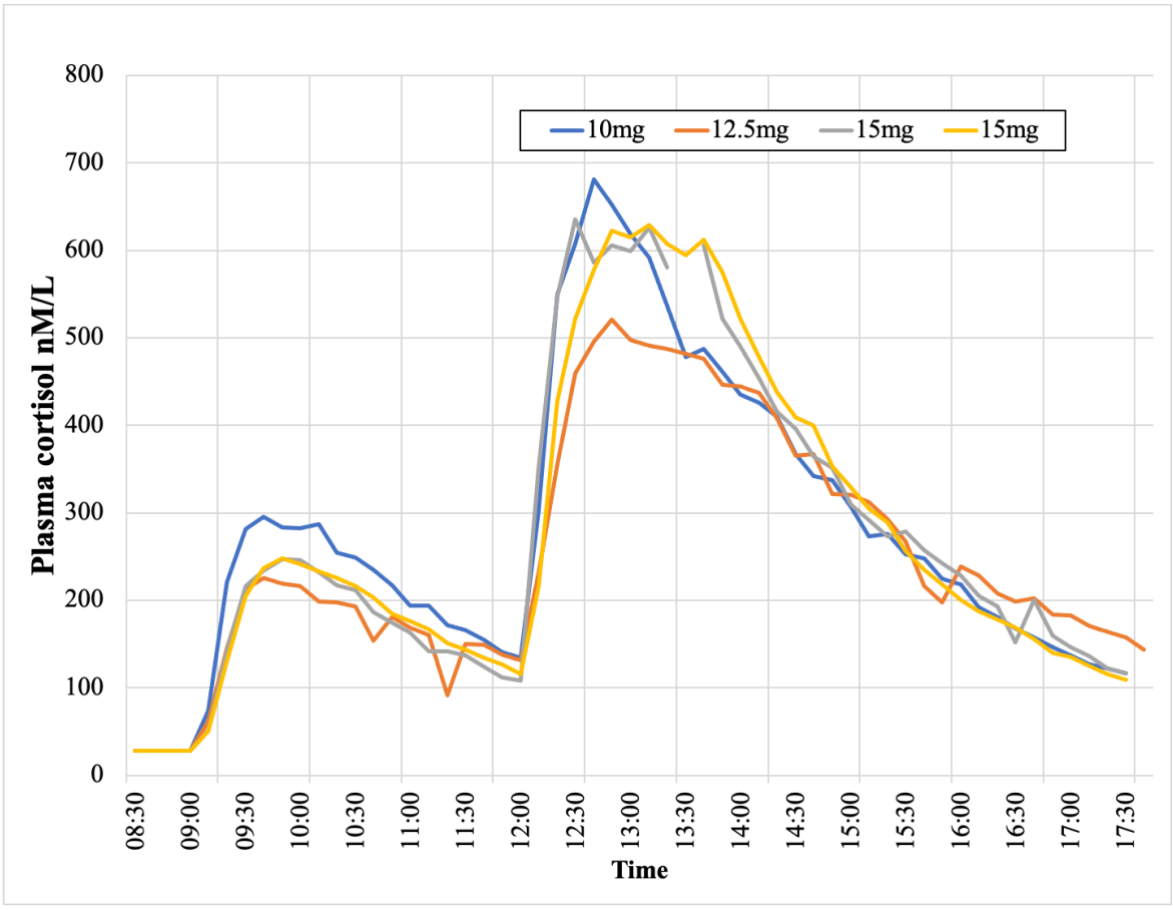
Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
09:00		4.0 mg	4.0 mg	4.0 mg	4.0 mg	15.0 mg	15.0 mg	Saline
12:00		2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	
15:00	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	
18:00	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	2.3 mg	
21:00	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	
00:00	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	
03:00	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	
06:00	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	4.0 mg	

Pilot study

Four healthy volunteers were acutely supressed with dexamethasone and administered 4 mg subcutaneous hydrocortisone at 9AM, with a subsequent elevated dose of 10, 12.5 or 15 mg at 12PM. As can be seen from Figure 4.2, after trialling 10, 12.5 and 15 mg doses, it was found that 15 mg gave the desired sustained plasma cortisol level of 600 nM/L. Therefore, Jamini decided to use this 15 mg dose as the elevated study dose for her study.

Figure 4.2

Plasma Cortisol Profiles after Infusion of Two Pulses in Four Human Volunteers



Note. **The legend key displays the infused hydrocortisone doses. One anomalous sample at 13:30 for the 15 mg dose has been excluded due to possible contamination. This graph was produced by Jamini Thakrar.**

Participants wore the pump from day 1 for a duration of 7 days in total for Jamini's study. I collected data on study day 6, during which participants received the 15 mg stress-level dose of hydrocortisone.

Task design

On study day 6, participants completed the oddball task with a reward manipulation and concurrent pupillometry. The task design is the same as Chapter 3 and Experiment 3 of Chapter 2. It was written using Psychophysics Toolbox v3 (Brainard, 1997) running under Matlab 2015a (The Mathworks Inc., Natick). During the task, participants were played a randomised sequence of short auditory tones. They were required to listen for, and respond

to, a rarely occurring tone (the oddball target), which occurred alongside another rarely occurring tone (deviant tone) and a frequently occurring tone (standard tone), neither of which required responses. The standard tone had an auditory frequency of 1,400 Hz, and the oddball and deviant were either 1,200 or 1,600 Hz (counterbalanced between participants). All tones had a duration of 100 ms, and the interstimulus interval was randomly varied between 1.2 and 1.7 s. In each block, 8% of the trials were deviant and oddball tones and 84% were standard tones.

The task consisted of 1 practice block of 25 auditory trials, followed by 4 experimental blocks of 125 auditory trials. In 2 of the experimental blocks, the oddball stimulus was associated with 20p; in the other 2 it was associated with £20. The participants were told to make quick manual responses to the oddball stimulus if they wanted to win the associated reward. They were provided with on-screen feedback, which informed them whether they had responded in time to the oddball stimulus (within 0.7 s).

At the end of the task, one of the oddball trials from the whole task was selected at random, and if the participant had responded within 0.7 s to the selected trial, they received the associated monetary reward in cash. High and low reward blocks were alternated within participants, and the block order was counterbalanced between participants.

Procedure

At 8AM on the sixth day of the study, participants attended a clinical facility, where they were given a new Crono-P pump, programmed to deliver the stress-level dose of hydrocortisone at 9AM on the same day. The old pump was detached from the infusion line, the line was changed, and the new pump was attached to the new infusion line, then participants were escorted to another facility for testing.

At the testing facility, participants were given breakfast, and at 9AM, during pulse delivery (see Table 4.2 for dosing regimen), the infusion line was checked to ensure that there had been no leakage of the stress dose. Participants were given printed task instructions, so that they could remind themselves of the task rules, which they learnt during task training on study day 1. At 9:20AM, during the rising phase of the stress pulse (as determined by the

pilot study—see Figure 4.2), testing began. Half of the participants were told to respond to the low tone (1,200 Hz), and the other half were told to respond to the high tone (1,600 Hz) for the whole experiment.

During testing, participants were seated in front of a computer monitor and a tower-mounted eye-tracking system. They were instructed to place their chin on the chinrest and fixate on a central fixation box throughout each block, responding to the oddball stimulus by pressing any key on the computer keyboard in front of them. Once participants were seated comfortably, the lights were switched off, and a short practice block was initiated prior to task administration.

After the practice block, pupil calibration and validation measures were carried out (these preceded each experimental block), and if they were met, the first experimental block was initiated. At the start of each block, instructions appeared on the screen to remind participants the amount of monetary reward associated with the oddball stimulus for that block (£20 or 20p). All text was then removed from the screen and the central fixation box appeared. Throughout the task, visual stimuli appeared within the fixation box to provide participants with feedback as to whether they had responded to the oddball stimulus in time (within 0.7 s). Between each block, participants were allowed a one-minute break. After completing all four experimental blocks, a lottery incentive reward system was executed, and if the participants won a monetary reward, they received it in cash immediately.

Participants then completed a ten minute N-back task as part of Jamini's study. This was followed by a long break (1-2 hours) before the same procedure was repeated and participants completed the oddball task with a reward manipulation and concurrent pupillometry again, during the falling phase of the pulse (at 11:20AM).

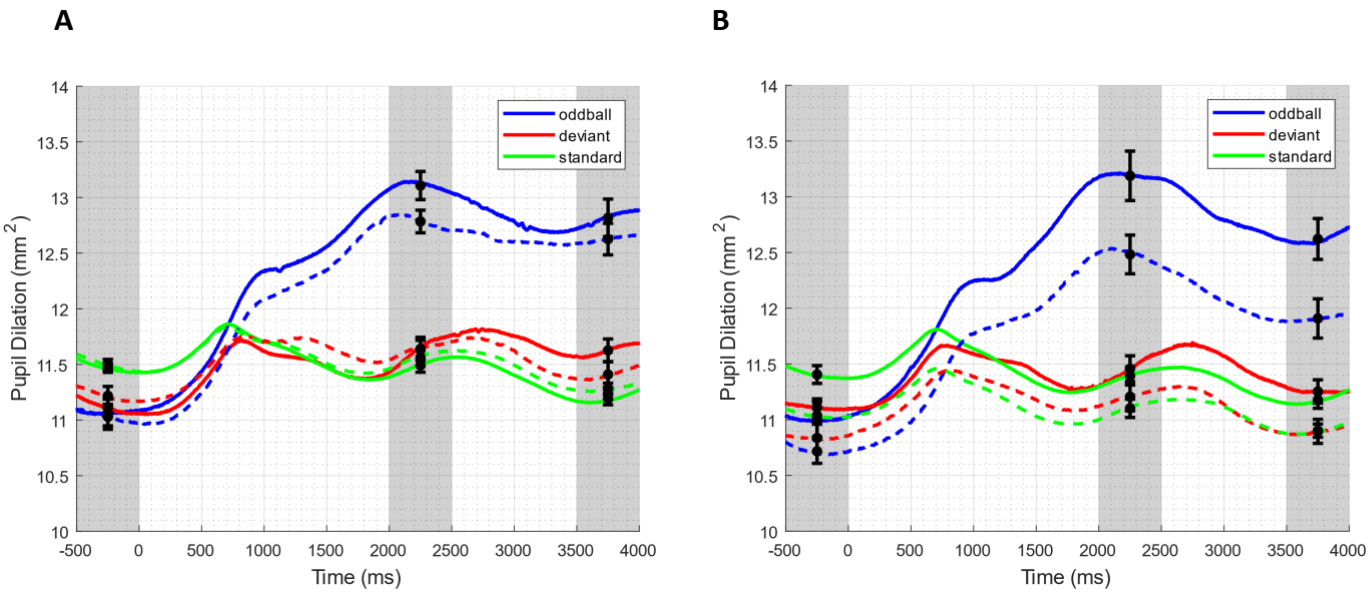
Results

Grand average pupil responses for the 3 Trial Types and 2 Reward Conditions over the Rising Phase are plotted in Figure 4.3A. Figure 4.3B depicts the equivalent for the Falling Phase. The figures show a clear pupil response to the oddball stimulus in both Reward Conditions,

with a greater pupil response to the oddball stimulus in the High Reward Condition than the Low Reward Condition during the Falling Phase in Figure 4.3B. This is also true for the Rising Phase, although there is less of a difference in pupil response to the oddball stimulus between the High Reward Condition than the Low Reward Condition for this Phase (see Figure 4.3A). The peak of the pupil responses is around 2,250 ms, so the Stimulus Response Epoch was defined as 2,000-2,500 ms. In both figures, the Pre-Stimulus Baseline, Stimulus Response and Post-Stimulus Baseline Epochs are shaded in grey, and the mean pupil response for each Epoch and Trial Type is plotted in black, for both Reward Conditions.

Figure 4.3

Grand Average Pupil Responses in the Rising and Falling Phases



Note. **(A) The grand average pupil response in the Rising Phase. (B) The grand average pupil response in the Falling Phase. The solid line depicts the pupil responses in the High Reward Condition and the dashed line depicts the pupil response in the Low Reward Condition. The mean pupil area for each Epoch is plotted in black with error bars to show the range.**

I carried out a repeated measures ANOVA on the average pupil size with the following factors: Phase (Rising, Falling), Reward Condition (Low, High); Trial Type (Oddball, Deviant, Standard); and Epoch (Pre-Stimulus Baseline, Stimulus Response, Post-Stimulus Baseline). There was a significant main effect of Trial Type – $F(2, 18) = 43.649$, $p = < 0.001$; partial ETA

squared = 0.829, and Epoch – $F(2, 18) = 47.151$, $p = < 0.001$; partial ETA squared = 0.840, and a significant interaction between Reward Condition and Trial Type - $F(2, 18) = 4.415$, $p = 0.028$; partial ETA squared = 0.329, and Epoch and Trial Type – $F(4, 36) = 18.133$, $p < 0.001$; partial ETA squared = 0.668. There was no significant effect of Phase – $F(1, 9) = 0.367$, $p = 0.559$; partial ETA squared = 0.039, nor Reward Condition – $F(1, 9) = 0.541$, $p = 0.481$; partial ETA squared = 0.057.

The significant interaction between Reward Condition and Trial Type indicates that Reward Condition had a phasic effect on pupil size. To investigate this, a three two-way repeated measures ANOVAs was carried out on the average pupil size in the Stimulus Response Epoch, with two factors: Trial Type and Reward Condition (Low, High). For each ANOVA, I compared two levels of Trial Type, rather than all three, to allow me to home in on any significant interactions.

First, the Oddball and Deviant Trial Types were investigated: there was a significant effect of Trial Type - $F(1, 9) = 28.725$, $p < 0.001$; partial ETA squared = 0.761, and a significant interaction between Trial Type and Reward Condition - $F(1, 9) = 5.346$, $p = 0.046$; partial ETA squared = 0.373.

Next, the Oddball and Standard Trial Types were investigated: there was a significant effect of Trial Type - $F(1, 9) = 40.245$, $p < 0.001$; partial ETA squared = 0.817, and, again, a significant interaction between Trial Type and Reward Condition - $F(1, 9) = 8.918$, $p = 0.015$; partial ETA squared = 0.498.

Finally, the Deviant and Standard Trial Types were investigated: there was no significant effect of Trial Type - $F(1, 9) = 2.400$, $p = 0.156$; partial ETA squared = 0.211, nor was there a significant interaction between Trial Type and Reward Condition - $F(1, 9) = 0.010$, $p = 0.922$; partial ETA squared = 0.001. These results suggest that, within the Trial Type factor, the Oddball Trial Type contributed to the significant interaction between Reward Condition and Trial Type, indicative of a phasic effect of incentive salience on the pupil. In contrast, there was no main effect of Reward Condition on the average pupil size, so there was no tonic effect of incentive salience. This discrepancy, marked by a phasic but no tonic effect of

incentive salience on the pupil, was not present in Experiment 3 of Chapter 2, which showed both phasic and tonic effects on the pupil evoked in the same oddball task with a reward manipulation. This suggests that the stress hydrocortisone dose in the present study disrupted the tonic but not the phasic effect of incentive salience on the pupil.

To investigate the interaction between Trial Type and Epoch, the two baseline Epochs were compared using a repeated measures ANOVA on the average pupil size with two factors: Trial Type (Oddball, Deviant, Standard) and Epoch (Pre-Stimulus Baseline, Post-Stimulus Baseline). There was a main effect of Epoch - $F(1, 9) = 31.113$, $p < 0.001$; partial ETA squared = 0.776, and Trial Type - $F(2, 18) = 23.361$, $p < 0.001$; partial ETA squared = 0.722. There was also a significant interaction between Epoch and Trial Type - $F(2, 18) = 20.059$, $p < 0.001$; partial ETA squared = 0.690. This interaction resulted from contrasting patterns of a larger pupil for the Standard *(*$M = 11.35$ mm^2; SD = 2.58 mm^2*)* than the Oddball ($M = 10.95$ mm^2; SD = 2.37 mm^2) and Deviant ($M = 11.07$ mm^2; SD = 2.36 mm^2) Trial Types in the Pre-Stimulus Baseline Epoch and a larger pupil for the Oddball ($M = 12.49$ mm^2; SD = 3.04 mm^2) and Deviant ($M = 11.30$ mm^2; SD = 2.48 mm^2) than the Standard Trial Type ($M = 11.14$; SD = 2.28 mm^2) in the Post-Stimulus Baseline Epoch.

To investigate the effect of Trial Type on pupil size within the Stimulus Response Epoch, a one-way repeated measures ANOVA was carried out on the average pupil size within this Epoch, with Trial Type (Oddball, Deviant, Standard) as the factor. There was a significant effect of Trial Type - $F(2, 18) = 33.952$, $p < 0.001$; partial ETA squared = 0.785. Post hoc testing revealed significant differences between the Oddball and Deviant, and the Oddball and Standard, but not the Deviant and Standard Trial Types (Paired Samples t-test: [Oddball - Deviant: $t(9) = 5.360$, $p < 0.001$; Oddball - Standard: $t(9) = 6.344$, $p < 0.001$; Deviant - Standard: $t(9) = 1.549$, $p = 0.156$]).

Manual responses

To investigate the effect of Phase and Reward Condition on manual response times to the oddball stimulus, a two-way repeated measures ANOVA was carried out on average reaction times, with Phase (Rising, Falling) and Reward Condition (Low, High) as the factors. There

was no significant effect of Phase - $F(1, 9) = 1.447$, $p = 0.260$; partial ETA squared = 0.138 nor Reward Condition - $F(1, 9) = 1.245$, $p = 0.293$; partial ETA squared = 0.122.

Discussion

I carried out an oddball task with a reward manipulation and concurrent pupillometry on the rising and falling phase of a stress pulse of cortisol in 10 healthy human volunteers. The main objective was to see whether there was any difference in the pupil response to behavioural salience by the Oddball Trial Type, and it's modulation by incentive salience, between Phases. A difference in pupil sensitivity to Trial Type between Phases would indicate changes in bottom-up and top-down salience, if related to the Deviant and Oddball Trial Type, respectively. A difference in pupil sensitivity to Reward Condition between phases would reflect a change in incentive salience and therefore reward sensitivity, indicative of altered extrinsic motivation. Although there appears to be more of a difference in pupil size between the High and Low Reward Condition for the Oddball Trial type in the Falling Phase (see Figure 4.3A and B), there was no significant effect of Phase, indicating that there was no difference in pupil sensitivity to Trial Type nor Reward Condition between the Rising and Falling Phase of the stress pulse. In other words, the effects of cortisol on salience did not change over time. There was a significant interaction between Trial Type and Reward Condition, and further exploration of this interaction revealed a phasic effect of Reward Condition—in other words, the effect of incentive salience was locked to the oddball stimulus. This is interesting because due to the absence of a significant main effect—that is, a tonic effect—of Reward Condition and therefore incentive salience. Additionally, in a previous experiment using the same task without hydrocortisone administration, there was both a phasic and tonic effect of Reward Condition (Experiment 3 in Chapter 2). Given that the task design was the same, the absence of a tonic effect of incentive salience in the present experiment could be down to hydrocortisone administration. This suggests that the stress dose of cortisol disrupted incentive salience and reward sensitivity in my participants.

This disrupted effect is in line with evidence suggesting a shift in control from a goal-directed to a stimulus-driven state in stress (Braunstein-Bercovitz et al., 2001; Henckens et al., 2010; 2012; Hermans et al., 2014; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017;

Schwabe & Wolf, 2009; 2011; van Marle et al., 2009; 2010). For example, in a stimulus-driven state, top-down processes are suppressed, which could explain why the top-down effect of incentive salience on the pupil was disrupted. This change in control state is advantageous in states of stress, as it facilitates threat detection by promoting environmental scanning and automatic attentional capture by bottom-up salience (Henckens et al., 2012; Hermans et al., 2014; van Marle et al., 2009; 2010). It is thought that this shift in control state is reversed later in the stress response, to restore goal-directed behaviour, which is important for the higher-order cognitive functioning required for daily tasks (Henckens et al., 2010; 2012; Hermans et al., 2014). Perhaps then, in the early stages of the stress response, I would expect to see an increase in pupil sensitivity to bottom-up salience, and decreased sensitivity to top-down salience, reflecting the promotion of a stimulus-driven control state, followed later by the opposite pattern. Whilst there was a partly disrupted effect of top-down, incentive salience on the pupil by cortisol, there was no evidence for a change in the effects of salience over time and therefore a shift in control state, as there was no significant effect of Phase. However, perhaps there was a change or reversal in control state occurring at a later time point, which was not captured by the present study. Alternatively, it is important to point out that the analysis for this study only included 10 participants, so it is possible that a significant effect of Phase and a tonic effect of Reward Condition on the pupil was lost as a result of reduced power, rather than an insignificant effect of Phase and a disrupted effect of incentive salience by the hydrocortisone dose.

It is difficult to say how the phasic and tonic effects of incentive salience on the pupil differ in terms of the internal processes that drive them. Some studies have interpreted phasic and tonic changes in pupil size using the adaptive gain theory, which associates changes in phasic and tonic LC activity and pupil responses with shifts in control state (Aston-Jones & Cohen, 2005; Gilzenrat et al., 2010). To reiterate, this theory posits that changes in LC activity regulate exploitation and exploration behaviours. Exploitation promotes task engagement and optimises behavioural performance, whereas exploration promotes task disengagement, a decline in behavioural performance and reward-seeking behaviour (Aston-Jones & Cohen, 2005; Gilzenrat et al., 2010). Phasic responses act as an attentional filter for behaviourally relevant events and occur frequently during task engagement with

intermediate levels of tonic activity. Therefore, the phasic effect of incentive salience on the pupil could reflect a reward-biased attentional filtering process and an increase in reward sensitivity by extrinsic motivation, which was not sustained over block duration. This effect of incentive salience did not translate to behaviour as there was no effect of Reward Condition on behavioural performance, as measured by manual response times to oddball stimuli, despite a phasic effect of Reward Condition on the pupil.

The disrupted tonic effect of incentive salience on the pupil is especially interesting given the small number of studies that have investigated the effects of stress or stress-level doses of cortisol on reward sensitivity in humans. One human study administered a 40 mg dose of hydrocortisone to male participants and found a downregulation in brain activity across the reward network, 50 minutes after treatment (Montoya et al., 2014). This occurred in the basolateral amygdala, striatum, caudate and nucleus accumbens, in rewarding and non-rewarding trials, indicating a widespread disruption in reward circuitry by cortisol. These findings are backed by a few other studies that have used similar doses and implemented testing at similar time delays after cortisol administration (e.g., Kinner et al., 2016).

However, some human studies have reported the complete opposite. For example, Putman et al. (2010) found that a 40 mg dose of hydrocortisone increased reward sensitivity in men completing a gambling task, which was administered 2-hours post infusion. In comparison with the placebo group, the treatment group made more risky choices, which either resulted in a large reward or an equally large loss. This suggests that the treated participants were more motivated to receive large rewards. It is possible that these conflicting results were caused by time dependent effects of cortisol, which perhaps disrupt and facilitate reward sensitivity due to engagement of the different genomic and non-genomic mechanisms. Although there was no evidence for this, the mechanisms of cortisol are complex, and may not have been fully captured here due to study limitations, especially due to the small sample size.

It is important to point out that studies across the literature have used different doses of cortisol. As we know, the plasma concentration of cortisol can determine its target effects by influencing the proportion of bound MR and GR. Therefore, I must consider the effects of

the selected dose. For example, perhaps if we had used a higher stress-level dose of cortisol, there would have been a more disrupted, or even an absent, incentive salience effect, or a change in the effects of Reward Condition and Trial Type across time, due to increased GR signalling (GR is the low affinity receptor and so would become increasingly occupied under higher plasma cortisol concentration). In addition, administering a stress dose of cortisol does not replicate a real physiological stress response. Most notably, the NE part of the stress response is absent. Therefore, perhaps the changes to control state that have been reported in the literature depend on the NE part of the stress response. Indeed, as mentioned in Chapter 1, a healthy volunteer study reported that a 20 mg stress-level dose of hydrocortisone in combination with the alpha adrenoceptor antagonist yohimbine (increases central NE), disrupted goal-directed behaviour, whilst facilitating habit-based actions. This change in behaviour was not achieved by hydrocortisone nor yohimbine alone, indicating that stress-induced changes by cortisol on control state require NE as well (Schwabe et al., 2010; 2012).

Another important consideration in this research field is the control of endogenous cortisol. It could be argued that the present study is more reliable than most, due to the suppression of endogenous cortisol, which we achieved using metyrapone. In contrast, other studies have attempted to control for the effects of endogenous cortisol by implementing their experiments at times of the day when plasma cortisol concentrations are relatively low, such as during the afternoon (e.g., Kinner et al., 2016; Montoya et al., 2014). Therefore, in these studies, the combined effect of administered and endogenous cortisol could have resulted in different receptor signalling patterns than would have been achieved if endogenous levels were pharmacologically suppressed. In this way, the present study possesses more reliable data.

However, a major caveat can be seen from my study design, which did not alternate the order of rising and falling phases across participants. Therefore, the task experience during the rising phase always affected the falling phase, and never vice versa. This is mostly problematic due to the reward delivery component of the study. For example, if participants received a £20 reward during the rising phase, it could have impacted the effect of incentive salience and reward sensitivity by increasing extrinsic motivation during the falling phase.

This limitation arose as I was only able to administer testing on participants on one morning a week, as they were mainly recruited for participation in another experiment. If I had more time with these participants, I could have administered two separate stress pulses of hydrocortisone and implemented my task on the falling phase before the rising phase in half of the participants. To investigate order effects in my collected data, I could examine how the earlier experiences of reward affect those later on, by comparing the effect of incentive salience on the pupil in the falling phase between participants who received larger reward than smaller rewards or no reward in the rising phase. However, there were only ten participants in this study—too few to carry out a meaningful analysis of this effect, especially since the groups are unbalanced for those who won 0, 20p and £20 in the rising phase.

Although studies have demonstrated that changes in receptor occupancy, genomic and non-genomic mechanisms result in varying phenotypes by cortisol over time, there was no evidence for this as there was no significant effect of Phase. Instead, I report a disrupted tonic effect of incentive salience on the pupil by a stress-level dose of cortisol. Compared with Experiment 3 of Chapter 2, this could reflect a disrupted change in control state to a reward-biased mode, with a reduced effect of reward sensitivity and extrinsic motivation.

Chapter 5

General discussion

The main aims

The main goal of the research presented in this thesis was to use pupillometry as a marker of human LC activity in order to investigate a prospective mechanism of apathy, in which cortisol and NE signalling is disrupted, causing reduced incentive salience, reward sensitivity and extrinsic motivation. Another aim was to explore how the effects cortisol on incentive salience vary over time in stress. The first experiments reported in this thesis built up towards these investigations and were carried out with the idea of developing a robust pupillometric technique for the later investigations.

First, I implemented a simple oddball task in humans, similar to those used in animal studies of LC function. Here, the main objective was to establish pupil responses to task stimuli that resemble the characteristic phasic and tonic LC responses reported by animal studies. The idea was that this would validate the use of my oddball task in combination with pupillometry as a marker of human LC activity. In addition, my findings would contribute to the small human pupillometry literature of LC function that has simply attempted to replicate the phasic and tonic LC responses found by these animal studies in humans. These animal studies reported phasic LC responses to presentations of stimuli with bottom-up or top-down salience. Therefore, another objective of my research has been to explore how bottom-up and top-down salience affects pupil size.

Summary of findings

My experiments demonstrated that the effects of top-down (behavioural and incentive salience) and bottom-up salience on the phasic and tonic pupil response are reliable: I repeatedly found a large phasic pupil response to task relevance combined with low stimulus probability with the oddball stimulus, a smaller phasic response to low stimulus probability with the deviant stimulus and no observable change in pupil size in response to high stimulus probability and task irrelevance with the standard stimulus. The addition of a reward manipulation amplified the phasic effect of top-down salience and induced an

additional sustained or tonic increase in pupil dilation in the High Reward Condition compared with the Low Reward Condition. Thus, there was an effect of extrinsic motivation that was locked to the behaviourally salient reward incentive as well as a sustained effect of incentive salience on the pupil. The literature suggests that pupil responses are a marker of LC activity. Therefore, these findings suggest that the LC functions in processes related to behavioural and incentive salience, such as intrinsic and extrinsic motivation, as well as processes related to bottom-up salience. This is in line with the LC literature. However, many human pupillometry studies of the LC have explicitly investigated hypotheses based on the adaptive gain theory of LC function and have designed task paradigms accordingly. The present research reconciles this literature by implementing an oddball task that is similar in design to the animal studies, and by reporting pupillometry data that resembles characteristic phasic LC responses to similar stimulus properties. Therefore, this thesis supports the use of pupillometry for the investigation of human LC function.

In addition, I found that the effect of incentive salience on the pupil was manipulated by disrupting cortisol and NE signalling. This suggests involvement of cortisol and NE signalling in the underlying mechanisms of incentive salience, such as extrinsic motivation, which becomes disrupted in apathy. An unexpected finding was that the effects of top-down and bottom-up salience on the pupil did not change over the course of a stress hydrocortisone pulse, although the tonic effect of incentive salience on the pupil was disrupted. However, both of these findings could have been confounded by the small sample size (10 participants), which reduced the power of the study.

Impact on the research field

Surprisingly, very few studies have investigated the effects of cortisol on reward sensitivity in humans (e.g., Kinner et al., 2016; Montoya et al., 2014; Putman et al., 2010). The present research found a suppressed phasic effect of incentive salience on the pupil by blockade of cortisol signalling. This suggests a role for cortisol in the underlying processes, such as extrinsic motivation. According to the adaptive gain theory, the phasic LC or pupil response acts as an attentional filter that is biased to behaviourally relevant events (presumably including reward incentives; Aston-Jones & Cohen, 2005). Therefore, perhaps cortisol functions in the attentional filtering or saliency attribution of reward incentives for extrinsic

motivation. If so, blocking cortisol signalling would reduce incentive salience, explaining why the phasic pupil response was blunted by administration of MR and GR antagonists. In addition, the adaptive gain theory proposes that phasic responses occur frequently during task engagement. However, the suppressed phasic effect by the MR and GR antagonists was not associated with inferior task performance, as there was no difference in reaction times between Treatment Conditions.

In a separate experiment, I found that a stress-level dose of hydrocortisone abolished a tonic effect of incentive salience on the pupil. Again, this suggests a function in extrinsic motivation, albeit through a slightly different mechanism. The adaptive gain theory (and a substantial amount of evidence from animal studies) indicates that tonic LC activity (and therefore baseline pupil size) covaries with control state and linearly with arousal (Aston-Jones & Bloom, 1981; Aston-Jones & Cohen, 2005; Foote et al., 1980; Hobson et al., 1975). According to this interpretation, in my experiment, the stress-level dose of cortisol prevented a shift in control mode to a goal-directed, reward-biased state, as cortisol prevented the increase in baseline pupil size by incentive salience that was observed in a previous study. Therefore, cortisol regulates goal-directed control states.

The effects of propranolol on the phasic pupil response to incentive salience suggest a similar role of central NE in the attentional filtering or saliency attribution of behaviourally relevant information. Although this might seem like quite a convoluted explanation for pupillometry data, a substantial amount of evidence supports the notion that NE drives large-scale network reorganisation, especially involving the salience network (Guedj et al., 2017; Hermans et al., 2011; Zerbi et al., 2019). This suggests that the LC-NE system can drive changes in control state, and perhaps saliency attribution in line with the active mode of control. Recent evidence in support of this comes from Zerbi et al. (2019) who explored changes in local and global brain networks induced by LC activation. The authors achieved selective LC activation in transgenic mice by using a viral vector to deliver a modified receptor to LC neurons that expressed codon-improved recombinase under the dopamine-beta-hydroxylase promotor. The modified receptors were designed to become activated by clozapine. Clozapine injection followed by resting state fMRI revealed a rapid activation of the salience network in transgenic mice compared with controls. In addition, these changes

in brain activity were accompanied by the development of symptoms of anxiety with reduced locomotion and exploration. Together, this suggests that the LC-NE system drives rapid changes in functional connectivity in the salience network, with corresponding changes in behaviour and cognition. In other words, these findings show that the LC is able to drive changes in attentional control and therefore they support previous reports that propose a role for the LC in related processes such as saliency attribution. Interestingly, Zerbi et al. (2019) also found that, within one minute of clozapine injection, the transgenic mice exhibited a more dilated pupil compared with control mice. Therefore, this evidence also provides support for the use of pupillometry in investigations of LC function.

Such descriptions of the LC-NE system driving large scale changes in network activity are strikingly similar to the stress-induced change in control state by cortisol described in Chapter 4. To recap, evidence suggests that the fast effects of cortisol redistribute energy resources towards the salience network in states of stress (Hermans et al., 2014; Schwabe, 2017). This coincides with a shift in control state towards a habit-based and stimulus-driven mode (Braunstein-Bercovitz et al., 2001; Henckens et al., 2010; 2012; Hermans et al., 2014; Sänger et al., 2014; Schwabe et al., 2010; 2012; 2017; Schwabe & Wolf, 2009; van Marle et al., 2009). Since the LC is activated by PVN neurons of the HPA axis and by cortisol, and the HPA axis is stimulated by the LC, it is likely that the changes in network activity described by both of these literatures involve both of these systems (Valentino, 1988; Valentino & van Bockstaele, 2008; Ward et al., 1976). Indeed, as mentioned in Chapter 1, Schwabe et al (2010; 2012) described a shift in control state to a habit-based mode that was dependent on cortisol and NE. In contrast, blockade of NE with propranolol inhibited a stress-induced shift in control state from a goal-directed to a habit-based mode (Schwabe et al. 2011). This suggests that the combined effect of cortisol and NE drives the stimulus-driven control state in stress.

A disrupted ability to shift between control states could lead to a number of conditions, including addiction and apathy. In addiction, the reinstatement of drug-seeking behaviours is often triggered by stress, which stimulates the expression of adaptive behaviours promoting survival rather than goal-directed behaviours in the healthy population (Mantsch et al., 2016; Schwabe & Wolfe, 2009; 2011). However, in addiction, this shift in behaviour

during stress is not only inhibited, but the goal-directed or reward-seeking state becomes hypersensitised (Robinson & Berridge, 1993; 2008). The HPA axis is highly plastic and vulnerable to maladaptive changes (de Kloet et al., 2005). Therefore, perhaps the pathology of addiction includes maladaptive plastic changes to the HPA axis, which subsequently drives an abnormal shift in attentional control on exposure to stress, rather than a physiological shift that facilitates adaptive stress behaviours. These maladaptive changes could include connections with the LC.

Apathy is characterised by an opposing presentation compared with addiction, as rather than displaying an extremely biased motivational state for drugs of abuse, patients are profoundly unmotivated. Therefore, in apathy, maladaptive plastic changes to the stress system could prevent the adaptive shift in control state in the opposing direction. In line with this idea, Klaasen et al. (2017) reported an association between symptoms of apathy and inhibited cognitive set shifting. Cognitive flexibility is required for shifting between control states. Therefore, the findings reported by Klaasen et al. (2017) could suggest that apathy is associated with an impaired ability to shift internal resources to a goal-directed control state. Unlike addiction, apathy is not typically triggered by an episode of stress, but it is common to numerous neurological diseases that have stress-related aetiology and/or pathology (e.g., Chase, 2011; Söndergaard et al., 2012; Strassman et al., 1956; Tiemensma et al., 2014). This suggests that, although a single episode of stress does not trigger the presentation of apathy, the stress system is involved in the underlying mechanisms. Altogether, my findings with the evidence presented here implicates the endogenous stress system, including the LC-NE system, in the underlying mechanism of apathy. In addition, it supports the research of NE and cortisol in motivation and reward processes, rather than focusing on the dopaminergic system alone.

Limitations in the field

Through my literature searches, I identified several problems within the research field, which I addressed and partly reconciled by carrying out the experiments reported in this thesis. For example, salience has been poorly defined in the literature, particularly in biomedical research, thus effects that have been induced by top-down or bottom-up salience, or both, are often attributed to overarching *stimulus salience* (e.g., Eschenko et al.,

2017). For example, the salience network is often described as a network that supports the detection of behaviourally relevant events (Uddin, 2015). However, behaviourally relevant events are not intrinsically salient—salience is defined as "the bottom-up, distinctiveness of an object" (Fecteau & Munoz, 2006, p. 382). Therefore, the salience network is also described as a network for detecting stimulus-driven salience rather than behavioural relevance (Menon & Uddin, 2010). Such ambiguity in the literature can lead to confusing or inaccurate interpretations. The same is true for animal studies of the LC that report phasic responses to salient events. These *salient* events can have top-down or bottom-up salience or both but are generally referred to as being *salient*, without a clear definition. For example, numerous reports have stated that the phasic LC response is sensitive to *salience*; however, most of these statements are actually referring to top-down salience rather than bottom-up salience, because, as mentioned, the consensus is that behaviour salience drives the phasic response (e.g., Sales et al., 2019; Vazey et al., 2018). In this thesis, I have distinguished between bottom-up and top-down salience. In addition, I have shown that the pupil—and therefore the LC—is sensitive to both.

The distinction between top-down and bottom-up control processes is important because they involve different underlying mechanisms. For example, bottom-up attentional control activates the temporoparietal lobe, and detects events based on physical features or stimulus-driven characteristics, such as physical salience, novelty and unexpectedness (Marois et al., 2000). In contrast, top-down control activates the intraparietal and superior frontal lobes, and selects stimuli based on certain cognitive factors, such as goals, motivation and expectations (Hopfinger et al., 2000). As mentioned, similarly, behavioural goals and external rewards are classified under the top-down or goal-directed category by dual-system theories (Awh et al., 2012; de Wit & Dickinson, 2009; Fecteau & Munoz, 2006). Although this conceptualisation can be useful in guiding research, it is important to keep in mind that behavioural goals and rewards represent disparate cognitive constructs when interpreting data and reporting research to prevent the dissemination of overgeneralised conclusions (Awh et al., 2012).

Aside from the biomedical literature, perhaps the most pervasive problem in human pupillometry studies of the LC is the implementation of inconsistent stimulus characteristics,

such as the interstimulus interval, stimulus duration and task duration (Furukawa et al., 2014; Gilzenrat et al., 2010; Hong et al., 2014; Liao et al., 2016; Murphy et al., 2011). Such discrepancies are problematic because slight differences in task design can modify subjective bottom-up salience and reduce the validity of any conclusions drawn from the data. For example, the onset of stimulus presentation is more unexpected and therefore more salient with a randomly varied rather than stable interstimulus interval. In addition, if the interstimulus interval is too short pupil responses accumulate and cause a larger phasic pupil response than would have been recorded if the presentation of consecutive stimuli was more spread out (Liao et al, 2016). However, studies across this field have used very different interstimulus intervals, such as Liao et al. (2016) and Gilzenrat et al. (2010) who presented auditory tones, with an interstimulus interval of 300ms and 4.2s, respectively. Similarly, inconsistent perceptions of bottom-up salience could have resulted from discrepancies in the auditory frequency of oddball tones implemented between studies, with some presenting white noise, laser gun sounds and 1,000 Hz tones as oddball stimuli (Furukawa et al., 2014; Hong et al., 2014; Murphy et al., 2011). The problem is that if a stimulus is more intense, such as greater in pitch or volume, it could be perceived as more salient and thus evoke a larger pupil response in one study compared with another. Again, similar problems arise for differences in stimulus probabilities, which also manipulate bottom-up salience and have varied between studies (e.g., Gilzenrat et al., 2010; Liao et al., 2016). Finally, varied task duration is likely to affect results by influencing arousal, and therefore the tonic LC and pupil response (Hou et al., 2005). To reconcile this literature, future studies should try to maintain consistency in basic task parameters, unless imperative to their research—for example, if they are specifically investigating the effect of stimulus probability on pupil size. It is especially important to draw comparisons between the implemented task paradigm and those implemented by earlier animal studies that this human research is based on, as the pupil response is a marker of LC activity, and by no means a direct measure.

Future directions for the research field

I have pointed out a significant gap in the pupillometry literature of the LC, with few human studies replicating similar oddball paradigms compared with electrophysiological animal studies and attempting to replicate and explore the phasic LC response. Although the

research presented in this thesis reduces this gap, there is still a need for pupillometry studies to implement more simplistic task paradigms, especially to establish reliable pupil responses before measuring the effect of adding more complex task manipulations on the pupil. Otherwise, we cannot be so sure about the neural underpinnings of the evoked pupil response. In addition, there has not been much interest in the relationship between reward sensitivity or extrinsic motivation and the HPA axis, nor the temporal variation in this relationship. Perhaps this is partly due to the focus on dopaminergic systems in reward processes. However, there is a strong link between a diseased HPA axis and reward-related symptomology, especially in psychiatric disease (Stokes & Sikes, 1991). Therefore, research in this field could have important clinical implications and so it is essential that future studies investigate this relationship. The same is true for the relationship between the LC-NE system and extrinsic motivation, although it is starting to receive more attention (e.g., Bouret & Richmond, 2015; Jahn et al., 2018). Finally, there have been very few studies investigating the link between the LC-NE system and the HPA axis, especially in relation to disease pathology. In part, this could be down to the challenges of investigating the LC in humans. However, pupillometry and improving fMRI technology, such as neuromelanin-sensitive imaging, have enabled the investigation of the LC in humans (Joshi et al., 2016; Sasaki et al., 2006).

Future directions for my research

The next step for my research would involve patient studies. Although my findings suggest that the mechanisms of extrinsic motivation involve cortisol and NE signalling, they do not provide direct evidence for these mechanisms becoming disrupted in apathy. To resolve this, I could implement my oddball task with a reward manipulation and concurrent pupillometry on patients with apathy compared with a group of healthy human volunteers. In my previous experiments, I have only conducted within-subject analyses on the average pupil size as this type of data is quite variable. Therefore, for a between-subject patient study I would need to recruit a much larger sample to allow for a reliable comparison between groups. Another problem is that apathy is a syndrome, with different profiles of motivational deficits in goal-directed behaviour, cognition and/or emotion (Robert et al., 2009; 2018). In addition, apathy is present in a wide variety of diseases, and even the healthy population experience reduced motivation. Therefore, as part of this patient study it

would be essential to establish the presence of any amotivation symptoms, as well as whether the presenting symptoms fall under categories of impaired goal-directed behaviour, cognition or emotion. This research would answer whether there is a deficit in extrinsic motivation in apathy compared with healthy controls that can be identified by examining the pupil response. A blunted phasic or tonic pupil response incentive salience would indicate reduced reward sensitivity and therefore extrinsic motivation by the LC-NE system, which drives the psychosensory pupil response.

During my literature search, I found reports indicating that addiction pathology could be related to an impaired stress system, including the LC, which drives abnormal extrinsic motivation and reward-seeking behaviour (Davis et al., 1975; Erb et al., 2000; Schwabe et al., 2010; 2012). Therefore, it would be interesting to carry out a similar patient study to see whether drug-dependent patients exhibit abnormal pupil responses to incentive salience. Ideally, recruited patients would include those that had and had not experienced a stress-induced reinstatement of drug-seeking behaviour to allow for a comparison between these groups, which could show distinct pathology. In contrast to apathy, I would expect reward incentives to evoke a larger phasic pupil response and/or change in baseline pupil size in drug-dependent patients compared with healthy volunteers.

Clinical implications

Ultimately, this research field aims to uncover new treatment strategies for diseases marked by abnormal motivation. This is particularly important for apathy, which does not have a standard prescriptive therapy. However, in addition to this, pupillometry might have some value in clinical diagnostics or tracking disease progression. For example, some studies have investigated the use of pupillometry in uncovering pre-symptomatic changes in Alzheimer's disease, as well as monitoring neocortical plaque accumulation by measuring the pupillary light reflex (Frost et al., 2013). In addition, investigations in patients with Parkinson's disease have reported that a blunted pupil response prior to motor execution is an indicator of impaired movement preparation compared with controls (Wang et al., 2016). Therefore, pupillometry could be used to monitor the progression of symptoms related to action control and potentially other cognitive impairments in Parkinson's disease.

However, it seems that not everyone is a good candidate for pupillometry. My experience of this led me to screen prospective participants for pupillometry during task training. For some, the eye tracker could not identify the location of the pupil and so would not complete the calibration procedure. I had to exclude these participants as I was unable to measure their pupil size. This was particularly the case for individuals with dark eyelashes, although sometimes I could not determine the reason for unsuccessful calibration. The use of various eye trackers, such as those that are more mobile and attach directly to the head could prove to have more frequently successful calibration procedures by allowing improved adjustment of the recording angle. For example, the eye tracker I used had an adjustable chinrest and a headrest, so in my experiments, participants were only able to move the chinrest vertically (see Figure 2.1). Perhaps if I had been able to tilt the headrest to adjust the recording angle, the eye tracker would have located the pupil for all participants and I would not have had to exclude as many due to unsuccessful calibration procedures. This problem of being unable to successfully calibrate everyone limits the clinical use of pupillometry.

However, if this problem is mitigated, pupillometry is a desirable diagnostic tool because it is inexpensive and non-invasive. Therefore, in the future, pupillometry in combination with a reward task could be used to uncover impaired extrinsic motivation that is specifically caused by a disrupted link between the stress system and the LC. Since apathy has a variety of presentations, with deficits in goal-directed behaviour, cognition and emotion, its pathology might differ across the patient population. Thus, it is important to identify those patients that are eligible for treatments targeted to the stress or LC-NE system. Pupillometry could be used to identify these patients from other patients with apathy and a more intact stress or LC-NE system that does not drive the apathetic presentation. Furthermore, pupillometry could be used to track disease progression or improvement with therapy.

Conclusion

This thesis provides new insights into the effects of top-down and bottom-up salience on the phasic and tonic pupil response, which were recorded as an index of LC activity. These effects are reliable. The effects of incentive salience on the pupil can be manipulated by blockade of cortisol signalling via the MR and GR, and by blockade of NE signalling via the beta adrenoceptor. Further research will be required to fully understand how these

signalling pathways contribute to the processes underlying incentive salience and extrinsic motivation. However, especially given the lack of research in this field, the experiments reported in this thesis provide an important step forward.

References

Adam, R., Leff, A., Sinha, N., Turner, C., Bays, P., Draganski, B. & Husain, M. (2013). Dopamine reverses reward insensitivity in apathy following globus pallidus lesions. *Cortex*, 49(5), 1292-1303.

Aguilera, G., Young, W., Kiss, A. & Bathia, A. (1995). Direct regulation of hypothalamic corticotropin-releasing-hormone neurons by angiotensin II. *Neuroendocrinology*, 61(4), 437-444.

Akaike, T. (1982). Periodic bursting activities of locus coeruleus neurons in the rat. *Brain Research*, 239(2), 629-633.

Akaoka, H. & Aston-Jones, G. (1991). Opiate withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input. *Journal of Neuroscience*, 11(12), 3830-3839.

Anderson, B. (2013). A value-driven mechanism of attentional selection. *Journal of Vision*, 13(3), 7-7.

Aston-Jones, G. & Bloom, F. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *Journal of Neuroscience*, 1, 876-886.

Aston-Jones, G. & Cohen, J. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience*, 28, 403-450.

Aston-Jones, G., Rajkowski, J. & Cohen, J. (1999). Role of locus coeruleus in attention and behavioral flexibility. *Biological Psychiatry*, 46(9), 1309-1320.

Aston-Jones, G., Rajkowski, J. & Kubiak, P. (1997). Conditioned responses of monkey locus coeruleus neurons anticipate acquisition of discriminative behavior in a vigilance task. *Neuroscience*, 80, 697-715.

Aston-Jones, G., Rajkowski, J., Kubiak, P. & Alexinsky, T. (1994). Locus coeruleus neurons in monkey are selectively activated by attended cues in vigilance task. *The Journal of Neuroscience*, 14(7), 4467-4480.

Avanzino, G., Ermirio, R., Cogo, C., Ruggeri, P. & Molinari, C. (1987). Effects of corticosterone on neurones of the locus coeruleus, in the rat. *Neuroscience Letters*, 80(1), 85-88.

Awh, E., Belopolsky, A. & Theeuwes, J. (2012). Top-down versus bottom-up attentional control: A failed theoretical dichotomy. *Trends in Cognitive Sciences*, 16(8), 437-443.

Bao, A. & Swaab, D. (2019). The human hypothalamus in mood disorders: the HPA axis in the center. *IBRO Reports*, 6, 45-53.

Bari, A., Xu, S., Pignatelli, M., Takeuchi, D., Feng, J., Li, Y. & Tonegawa, S. (2020). Differential attentional control mechanisms by two distinct noradrenergic coeruleo-frontal cortical pathways. *Proceedings of the National Academy of Sciences*, 117(46), 29080-29089.

Beatty, J. (1982). Task-evoked pupillary responses, processing load, and the structure of processing resources. *Psychological Bulletin*, 91(2), 276.

Berridge, K. & Robinson, T. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist*, 71(8), 670.

Berridge, C. & Waterhouse, B. (2003). The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*, 42(1), 33-84.

Bogdan, R. & Pizzagalli, D. (2006). Acute stress reduces reward responsiveness: implications for depression. *Biological Psychiatry*, 60(10), 1147-1154.

Bouret, S. & Richmond, B. (2015). Sensitivity of locus coeruleus neurons to reward value for goal-directed actions. *The Journal of Neuroscience*, 35(9), 4005-4014.

Bouret, S. & Sara, S. (2004). Reward expectation, orientation of attention and locus coeruleus-medial frontal cortex interplay during learning. *European Journal of Neuroscience*, 20(3), 791-802.

Bouret, S. & Sara, S. (2005). Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends in Neurosciences*, 28(11), 574-582.

Bradley, A. & Dinan, T. (2010). A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *Journal of Psychopharmacology*, 24, 91-118.

Brainard, D. (1997). The psychophysics toolbox. *Spatial Vision*, 10, 433-436.

Braunstein-Bercovitz, H., Dimentman-Ashkenazi, I. & Lubow, R. (2001). Stress affects the selection of relevant from irrelevant stimuli. *Emotion*, 1(2), 182.

Bunce, S., Harris, J., Bixler, E., Taylor, M., Muelly, E., Deneke, E., ... & Meyer, R. (2015). Possible evidence for re-regulation of HPA axis and brain reward systems over time in treatment in prescription opioid-dependent patients. *Journal of Addiction Medicine*, 9(1), 53-60.

Butler, W. & Taube, J. S. (2015). The nucleus prepositus hypoglossi contributes to head direction cell stability in rats. *Journal of Neuroscience*, 35(6), 2547-2558.

Cane Crono P Pump. (n.d.). Applied Medical Technology. https://applied-medical.co.uk/project/crono-p/.

Carroll, B., Cassidy, F., Naftolowitz, D., Tatham, N., Wilson, W., Iranmanesh, A., ... & Veldhuis, J. (2007). Pathophysiology of hypercortisolism in depression. *Acta Psychiatrica Scandinavica*, 115, 90-103.

Carrozzino, D. (2019). Clinimetric approach to rating scales for the assessment of apathy in Parkinson's disease: a systematic review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 94. doi: 10.1016/j.pnpbp.2019.109641.

Chase, T. (2011). Apathy in neuropsychiatric disease: diagnosis, pathophysiology, and treatment. *Neurotoxicity Research*, 19(2), 266-278.

Chiew, K. & Braver, T. (2013). Temporal dynamics of motivation-cognitive control interactions revealed by high-resolution pupillometry. *Frontiers in Psychology*, 4. doi: 10.3389/fpsyg.2013.00015.

Chiew, K. & Braver, T. (2014). Dissociable influences of reward motivation and positive emotion on cognitive control. *Cognitive, Affective, & Behavioral Neuroscience*, 14(2), 509-529.

Clayton, E., Rajkowski, J., Cohen, J. & Aston-Jones, G. (2004). Phasic activation of monkey locus ceruleus neurons by simple decisions in a forced-choice task. *Journal of Neuroscience*, 24(44), 9914-9920.

Cleghorn, R. (1951). Adrenal cortical insufficiency. *Canadian Medical Association Journal*, 65(5), 449-454.

Cole, D., Oei, N., Soeter, R., Both, S., van Gerven, J., Rombouts, S. & Beckmann, C. (2013). Dopamine-dependent architecture of cortico-subcortical network connectivity. *Cerebral Cortex*, 23(7), 1509-1516.

Corbetta, M., Patel, G. & Shulman, G. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron*, 58(3), 306-324.

Corbetta, M. & Shulman, G. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, 3(3), 201-215.

Cunningham Jr, E, Bohn, M. & Sawchenko, P. (1990). Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *Journal of Comparative Neurology*, 292(4), 651-667.

Dajani, D. & Uddin, L. (2015). Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience. *Trends in Neurosciences*, 38(9), 571-578.

Dallman, M. (2005). Fast glucocorticoid actions on brain: back to the future. *Frontiers in Neuroendocrinology*, 26, 103-108.

Datson, N., van der Perk, J., de Kloet, E. & Vreugdenhil, E. (2001). Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *European Journal of Neuroscience*, 14, 675-689.

Davis, W., Smith, S. & Khalsa, J. (1975). Noradrenergic role in the self-administration of morphine or amphetamine. *Pharmacology Biochemistry and Behavior*, 3(3), 477-484.

Dayan, P. & Yu, A. (2006). Phasic norepinephrine: a neural interrupt signal for unexpected events. *Network: Computation in Neural Systems*, 17(4), 335-350.

de Gee, J., Colizoli, O., Kloosterman, N., Knapen, T., Nieuwenhuis, S., & Donner, T. (2017). Dynamic modulation of decision biases by brainstem arousal systems. *Elife*, 6. doi: 10.7554/eLife.23232.

de Gee, J., Knapen, T. & Donner, T. (2014). Decision-related pupil dilation reflects upcoming choice and individual bias. *Proceedings of the National Academy of Sciences*, 111(5), E618-E625.

de Kloet, E., Joels, M. & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, 6, 463-475.

den Brok, M., van Dalen, J., van Gool, W., Moll van Charante, E., de Bie, R. & Richard, E. (2015). Apathy in Parkinson's disease: a systematic review and meta-analysis. *Movement Disorders*, 30(6), 759-769.

Depue, R. & Collins, P. (1999). Neurobiology of the structure of personality: Dopamine, facilitation of incentive motivation, and extraversion. *Behavioral and Brain Sciences*, 22(3), 491-517.

de Wit, S. & Dickinson, A. (2009). Associative theories of goal-directed behaviour: a case for animal–human translational models. *Psychological Research PRPF*, 73(4), 463-476.

Dix, A. & Li, S. (2020). Incentive motivation improves numerosity discrimination: Insights from pupillometry combined with drift-diffusion modelling. *Scientific Reports*, 10(1), 1-11.

Dong, H., Petrovich, G. & Swanson, L. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Research Reviews*, 38(1-2), 192-246.

Drouin, C., Darracq, L., Trovero, F., Blanc, G., Glowinski, J., Cotecchia, S. & Tassin, J. (2002). α1b-Adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *Journal of Neuroscience*, 22(7), 2873-2884.

Dworkin, S., Guerin, G., Co, C., Goeders, N. & Smith, J. (1988). Lack of an effect of 6-hydroxydopamine lesions of the nucleus accumbens on intravenous morphine self-administration. *Pharmacology Biochemistry and Behavior*, 30(4), 1051-1057.

Einhäuser, W., Stout, J., Koch, C. & Carter, O. (2008). Pupil dilation reflects perceptual selection and predicts subsequent stability in perceptual rivalry. *Proceedings of the National Academy of Sciences*, 105(5), 1704-1709.

Elam, M., Svensson, T. & Thoren, P. (1985). Differentiated cardiovascular afferent regulation of locus coeruleus neurons and sympathetic nerves. *Brain Research*, 358(1-2), 77-84.

Erb, S., Hitchcott, P., Rajabi, H., Mueller, D., Shaham, Y. & Stewart, J. (2000). Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. *Neuropsychopharmacology*, 23(2), 138-150.

Eschenko, O., Mello-Carpes, P. & Hansen, N. (2017). New Insights into the Role of the Locus Coeruleus-Noradrenergic System in Memory and Perception Dysfunction. *Neural Plasticity*, 2017. doi: 10.1155/2017/4624171.

Evans, D., Stempel, A., Vale, R. & Branco, T. (2019). Cognitive control of escape behaviour. *Trends in Cognitive Sciences*, 23(4), 334-348.

Farah, J., Lauand, C., Chequi, L., Fortunato, E., Pasqualino, F., Bignotto, L., ... & Aprahamian, I. (2015). Severe psychotic disorder as the main manifestation of adrenal insufficiency. *Case Reports in Psychiatry*, 2015. doi: 10.1155/2015/512430.

Fauth-Bühler, M. (2019). Similarities and differences between gambling disorder and other addiction-like behaviors. In A. Heinz, N. Romanczuk-Seiferth & M. Potenza (Eds). *Gambling Disorder* (pp. 235-246). Springer.

Fecteau, J. & Munoz, D. (2006). Salience, relevance, and firing: a priority map for target selection. *Trends in Cognitive Sciences*, 10(8), 382-390.

Fernandes, P., Regala, J., Correia, F. & Gonçalves-Ferreira, A. (2012). The human locus coeruleus 3-D stereotactic anatomy. *Surgical and Radiologic Anatomy*, 34(10), 879-885.

Fernández-Gil, M., Palacios-Bote, R., Leo-Barahona, M. & Mora-Encinas, J. (2010). Anatomy of the brainstem: a gaze into the stem of life. *Seminars in Ultrasound, CT and MRI*, 31(3), 196-219.

Fitzsimons, C., Van Hooijdonk, L., Schouten, M., Zalachoras, I., Brinks, V., Zheng, T., ... & Vreugdenhil, E. (2013). Knockdown of the glucocorticoid receptor alters functional

integration of newborn neurons in the adult hippocampus and impairs fear-motivated behavior. *Molecular Psychiatry*, 18(9), 993-1005.

Foote, S., Aston-Jones, G. & Bloom, F. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proceedings of the National Academy of Sciences*, 77**,** 3033-3037.

Frost, S., Kanagasingam, Y., Sohrabi, H., Bourgeat, P., Villemagne, V., Rowe, C., ... & AIBL Research Group. (2013). Pupil response biomarkers for early detection and monitoring of Alzheimer's disease. *Current Alzheimer Research*, 10(9), 931-939.

Furukawa, S., Liao, H-I., Kidani, S., Yoneya, M. & Kashino. M. (2014, September). *Evaluating the salience of auditory events through eyes* [Paper presentation]. Forum Acusticum, Krakow. http://www.kecl.ntt.co.jp/people/liao.hsini/paper/Furukawa_etal_14.pdf.

Gabay, S., Pertzov, Y. & Henik, A. (2011). Orienting of attention, pupil size, and the norepinephrine system. *Attention, Perception, & Psychophysics*, 73(1), 123-129.

Gaillard, R., Riondel, A., Muller, A., Herrmann, W. & Baulieu, E. (1984). RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day. *Proceedings of the National Academy of Sciences*, 81(12), 3879-3882.

Gardiner, P., Schrode, K., Quinlan, D., Martin, B., Boreham, D., Rogers, M., ... & Karim, A. (1989). Spironolactone metabolism: steady-state serum levels of the sulfur-containing metabolites. *The Journal of Clinical Pharmacology*, 29(4), 342-347.

Gilzenrat, M., Nieuwenhuis, S., Jepma, M. & Cohen, J. (2010). Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. *Cognitive, Affective & Behavioural Neuroscience*, 10, 252-269.

Gong, M. & Liu, T. (2018). Reward differentially interacts with physical salience in feature-based attention. *Journal of Vision*, 18(11), 12-12.

Goschke, T. & Bolte, A. (2014). Emotional modulation of control dilemmas: The role of positive affect, reward, and dopamine in cognitive stability and flexibility. *Neuropsychologia*, 62, 403-423.

Grant, S., Aston-Jones, G. & Redmond Jr, D. (1988). Responses of primate locus coeruleus neurons to simple and complex sensory stimuli. *Brain Research Bulletin*, 21(3), 401-410.

Groeneweg, F., Karst, H., de Kloet, E. & Joëls, M. (2011). Rapid non-genomic effects of corticosteroids through the membrane-associated MR and GR and their role in the central stress. *Journal of Endocrinology*, 209(2), 153-167.

Guedj, C., Monfardini, E., Reynaud, A., Farne, A., Meunier, M. & Hadj-Bouziane, F. (2017). Boosting norepinephrine reconfiguration of brain networks at rest. *Cerebral Cortex*, 27(10), 4691-4700.

Guo, T., Tinkienberg, J., Oliker, R. & Maze, M. (1991). Central α1-adrenoceptor stimulation functionally antagonizes the hypnotic response to dexmedetomidine, an α2-adrenoceptor agonist. *The Journal of the American Society of Anesthesiologists*, 75(2), 252-256.

Harvey, A., Pattinson, K., Brooks, J., Mayhew, S., Jenkinson, M., & Wise, R. (2008). Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine*, 28(6), 1337-1344.

Hatfield, C., Herbert, J., Van Someren, E., Hodges, J. & Hastings, M. (2004). Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. *Brain*, 127(5), 1061-1074.

Henckens, M., van Wingen, G., Joels, M. & Fernandez, G. (2010). Time-dependent effects of corticosteroids on human amygdala processing. *Journal of Neuroscience*, 30, 12725-12732.

Henckens, M., van Wingen, G., Joëls, M. & Fernández, G. (2012). Time-dependent effects of cortisol on selective attention and emotional interference: a functional MRI study. *Frontiers in Integrative Neuroscience*, 6. doi: 10.3389/fnint.2012.00066.

Hermans, E., Henckens, M., Joëls, M. & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences*, 37(6), 304-314.

Hermans, E., van Marle, H., Ossewaarde, L., Henckens, M. & Qin, S. (2011). Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science*, 334(6059), 1151-1153.

Heron, C., Apps, M. & Husain, M. (2018). The anatomy of apathy: A neurocognitive framework for amotivated behaviour. *Neuropsychologia*, 118, 54-67.

Herrero, M., Estrada, C., Maatouk, L. & Vyas, S. (2015). Inflammation in Parkinson's disease: role of glucocorticoids. *Frontiers in neuroanatomy*, 9. doi: 10.3389/fnana.2015.00032.

Hickey, C., Chelazzi, L. & Theeuwes, J. (2010). Reward changes salience in human vision via the anterior cingulate. *Journal of Neuroscience*, 30(33), 11096-11103.

Hinz, B. & Hirschelmann, R. (2000). Rapid non-genomic feedback effects of glucocorticoids on CRF-induced ACTH secretion in rats. *Pharmaceutical Research*, 17, 1273-1277.

Hirata, H. & Aston-Jones, G. (1994). A novel long-latency response of locus coeruleus neurons to noxious stimuli: mediation by peripheral C-fibers. *Journal of Neurophysiology*, 71(5), 1752-1761.

Hobson, J., McCarley, R. & Wyzinski, P. (1975). Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*, 189(4196), 55-58.

Hong, L., Walz, J. & Sajda, P. (2014). Your eyes give you away: prestimulus changes in pupil diameter correlate with poststimulus task-related EEG dynamics. *PLos One*, 9(3). doi: 10.1371/journal.pone.0091321.

Hopfinger, J., Buonocore, M. & Mangun, G. (2000). The neural mechanisms of top-down attentional control. *Nature Neuroscience*, 3(3), 284-291.

Hou, R., Freeman, C., Langley, R., Szabadi, E. & Bradshaw, C. (2005). Does modafinil activate the locus coeruleus in man? Comparison of modafinil and clonidine on arousal and autonomic functions in human volunteers. *Psychopharmacology*, 181(3), 537-549.

Husain, M. & Roiser, J. (2018). Neuroscience of apathy and anhedonia: a transdiagnostic approach. *Nature Reviews Neuroscience*, 19(8), 470-484.

Ishizaki, J. & Mimura, M. (2011). Dysthymia and apathy: diagnosis and treatment. *Depression Research and Treatment*, 2011. doi: 10.1155/2011/893905.

Itthipuripat, S., Cha, K., Byers, A. & Serences, J. (2017). Two different mechanisms support selective attention at different phases of training. *PLoS Biology*, 15(6). doi: 10.1371/journal.pbio.2001724.

Jahn, C., Gilardeau, S., Varazzani, C., Blain, B., Sallet, J., Walton, M. & Bouret, S. (2018). Dual contributions of noradrenaline to behavioural flexibility and motivation. *Psychopharmacology*, 235(9), 2687-2702.

Jones, B. & Yang, T. (1985). The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. *Journal of Comparative Neurology*, 242(1), 56-92.

Joshi, S., Li, Y., Kalwani, R. & Gold, J. (2016). Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. *Neuron*, 89(1), 221-234.

Kadmiel, M. & Cidlowski, J. (2013). Glucocorticoid receptor signaling in health and disease. *Trends in Pharmacological Sciences*, 34(9), 518-530.

Kalwani, R., Joshi, S. & Gold, J. (2014). Phasic activation of individual neurons in the locus ceruleus/subceruleus complex of monkeys reflects rewarded decisions to go but not stop. *Journal of Neuroscience*, 34(41), 13656-13669.

Kant, R., Duffy, J. & Pivovarnik, A. (1998). Prevalence of apathy following head injury. *Brain Injury*, 12(1), 87-92.

Karssen, A., Meijer, O., Van Der Sandt, I., Lucassen, P., De Lange, E., De Boer, A. & De Kloet, E. (2001). Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology*, 142(6), 2686-2694.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schütz, G. & Joëls, M. (2005). Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proceedings of the National Academy of Sciences*, 102(52), 19204-19207.

Kaye, J. & Lightman, S. (2006). Glucocorticoids and the cardiovascular response to stress: a pilot study of the 35% CO2 challenge in Addison's disease. *Clinical Endocrinology*, 65(3), 282-286.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G. & Schatzberg, A. (2017). HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. *Molecular Psychiatry*, 22(4), 527-536.

Keren, N., Lozar, C., Harris, K., Morgan, P. & Eckert, M. (2009). In vivo mapping of the human locus coeruleus. *Neuroimage*, 47(4), 1261-1267.

Kiang, M., Christensen, B., Remington, G. & Kapur, S. (2003). Apathy in schizophrenia: clinical correlates and association with functional outcome. *Schizophrenia Research*, 63(1-2), 79-88.

Kinner, V., Wolf, O. & Merz, C. (2016). Cortisol alters reward processing in the human brain. *Hormones and Behavior*, 84, 75-83.

Kitchen, A., O'Leary, D. & Scislo, T. (2006). Sympathetic and parasympathetic component of bradycardia triggered by stimulation of NTS P2X receptors. *American Journal of Physiology-Heart and Circulatory Physiology*, 290(2), H807-H812.

Klaasen, N., Kos, C., Aleman, A. & Opmeer, E. (2017). Apathy is related to reduced activation in cognitive control regions during set-shifting. *Human Brain Mapping*, 38(5), 2722-2733.

Kolbe, I., Dumbell, R. & Oster, H. (2015). Circadian clocks and the interaction between stress axis and adipose function. *International Journal of Endocrinology*, 2015. doi: 10.1155/2015/693204.

Koob, G. & Kreek, M. (2007). Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *American Journal of Psychiatry*, 164(8), 1149-1159.

Krieger, D., Allen, W., Rizzo, F. & Krieger, H. (1971). Characterization of the Normal Temporal Pattern of Plasma Corticosteroid Levels. *The Journal of Clinical Endocrinology and Metabolism*, 32(2), 266-284.

Kwon, O., Kim, M., Lee, H., Sung, K. & Lee, S. (2015). The cortisol awakening response in patients with poststroke depression is blunted and negatively correlated with depressive mood. *BioMed Research International*, 2015. doi: 10.1155/2015/709230.

Lê, A., Funk, D., Juzytsch, W., Coen, K., Navarre, B., Cifani, C. & Shaham, Y. (2011). Effect of prazosin and guanfacine on stress-induced reinstatement of alcohol and food seeking in rats. *Psychopharmacology*, 218(1), 89-99.

Leri, F., Flores, J., Rodaros, D. & Stewart, J. (2002). Blockade of stress-induced but not cocaine-induced reinstatement by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. *Journal of Neuroscience*, 22(13), 5713-5718.

Lewis, D. & Coote, J. (1990). Excitation and inhibition of rat sympathetic preganglionic neurones by catecholamines. *Brain Research*, 530(2), 229-234.

Liao, H-I., Yoneya, M., Kidani, S., Kashino, M. & Furukawa, S. (2016). Human pupillary dilation response to deviant auditory stimuli: effects of stimulus properties and voluntary attention. *Frontiers in Neuroscience*, 10(43). doi: 10.3389/fnins.2016.00043.

Liu, K., Marijatta, F., Hämmerer, D., Acosta-Cabronero, J., Düzel, E. & Howard, R. (2017). Magnetic resonance imaging of the human locus coeruleus: a systematic review. *Neuroscience & Biobehavioral Reviews*, 83, 325-355.

Liu, Y., Rodenkirch, C., Moskowitz, N., Schriver, B. & Wang, Q. (2017). Dynamic lateralization of pupil dilation evoked by locus coeruleus activation results from sympathetic, not parasympathetic, contributions. *Cell Reports*, 20(13), 3099-3112.

Lovallo, W. (2006). The hypothalamic–pituitary–adrenocortical axis in addiction. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 59(3), 193.

Lovallo, W., Robinson, J., Glahn. & Fox P. (2010). Acute effects of hydrocortisone on the human brain: an fMRI study. *Psychoneuroendocrinology*, 35, 15-20.

Makino, S., Smith, M. & Gold, P. (2002). Regulatory role of glucocorticoids and glucocorticoid receptor mRNA levels on tyrosine hydroxylase gene expression in the locus coeruleus during repeated immobilization stress. *Brain Research*, 943(2), 216-223.

Maldonado, R. & Koob, G. (1993). Destruction of the locus coeruleus decreases physical signs of opiate withdrawal. *Brain Research*, 605(1), 128-138.

Mano, T. & Iwase, S. (2003). Sympathetic nerve activity in hypotension and orthostatic intolerance. *Acta Physiologica Scandinavica*, 177(3), 359-365.

Manohar, S. & Husain, M. (2015). Reduced pupillary reward sensitivity in Parkinson's disease. *NPJ Parkinson's disease*, 1(1). doi: 10.1038/npjparkd.2015.26.

Mantsch, J., Baker, D., Funk, D. Lê, A. & Shaham, Y. (2016). Stress-Induced Reinstatement of Drug Seeking: 20 Years of Progress. *Neuropsychopharmacology*, 41(1), 335-356.

Mantsch, J., Weyer, A., Vranjkovic, O., Beyer, C., Baker, D. & Caretta, H. (2010). Involvement of noradrenergic neurotransmission in the stress-but not cocaine-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: role for β-2 adrenergic receptors. *Neuropsychopharmacology*, 35(11), 2165-2178.

Marin, R. (1991). Apathy – a neuropsychiatric syndrome. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 3, 243-254.

Martínez-Horta, S., Riba, J., de Bobadilla, R., Pagonabarraga, J., Pascual-Sedano, B., Antonijoan, R. M., ... & Kulisevsky, J. (2014). Apathy in Parkinson's disease: neurophysiological evidence of impaired incentive processing. *Journal of Neuroscience*, 34(17), 5918-5926.

Marois, R., Leung, H. & Gore, J. (2000). A stimulus-driven approach to object identity and location processing in the human brain. *Neuron*, 25(3), 717-728.

Mather, M., Huang, R., Clewett, D., Nielsen, S., Velasco, R., Tu, K., ... & Kennedy, B. (2020). Isometric exercise facilitates attention to salient events in women via the noradrenergic system. *Neuroimage*, 210. doi: 10.1016/j.neuroimage.2020.116560.

Matheson, G., Branch, B. & Taylor, A. (1971). Effects of amygdaloid stimulation on pituitary-adrenal activity in conscious cats. *Brain Research*, 32(1), 151-167.

McCall, J., Al-Hasani, R., Siuda, E., Hong, D., Norris, A, Ford, C. & Bruchas, M. (2015). CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. *Neuron*, 87(3), 605-620.

McDougal, D. & Gamlin, P. (2015). Autonomic control of the eye. *Comprehensive Physiology*, 5(1), 439-473.

McMaster, A., Jangani, M., Sommer, P., Han, N., Brass, A., Beesley, S., ... & Ray, D. (2011). Ultradian cortisol pulsatility encodes a distinct, biologically important signal. *PLoS One*, 6(1). doi: 10.1371/journal.pone.0015766.

Melloni, L., van Leeuwen, S., Alink, A. & Müller, N. (2012). Interaction between bottom-up saliency and top-down control: how saliency maps are created in the human brain. *Cerebral Cortex*, 22(12), 2943-2952.

Menon, V. & Uddin, L. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function*, 214(5-6), 655-667.

Mifepristone. (n.d.). National Institute for Health and Care Excellence. https://bnf.nice.org.uk/drug/mifepristone.html#sideEffects.

Mifsud, K. & Reul, J. (2016). Acute stress enhances heterodimerization and binding of corticosteroid receptors at glucocorticoid target genes in the hippocampus. *Proceedings of the National Academy of Sciences*, 113(40), 11336-11341.

Mikl, M., Mareček, R., Hluštík, P., Pavlicová, M., Drastich, A., Chlebus, P., ... & Krupa, P. (2008). Effects of spatial smoothing on fMRI group inferences. *Magnetic resonance imaging*, 26(4), 490-503.

Montoya, E., Bos, P., Terburg, D., Rosenberger, L. & van Honk, J. (2014). Cortisol administration induces global down-regulation of the brain's reward circuitry. *Psychoneuroendocrinology*, 47, 31-42.

Morris, L, McCall, J., Charney, D., & Murrough, J. (2020). The role of the locus coeruleus in the generation of pathological anxiety. *Brain and Neuroscience Advances*, 4. doi: 10.1177/2398212820930321.

Muhammed, K., Manohar, S., Ben Yehuda, M., Chong, T., Tofaris, G., Lennox, G., ... & Husain, M. (2016). Reward sensitivity deficits modulated by dopamine are associated with apathy in Parkinson's disease. *Brain*, 139(10), 2706-2721.

Murphy, P., O'Connell, R., O'Sullivan, M., Robertson, I. & Balsters, J. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Human Brain Mapping*, 35(8), 4140-4154.

Murphy, P., Robertson, I., Balsters, J. & O'Connell, R. (2011). Pupillometry and P3 index the locus coeruleus–noradrenergic arousal function in humans. *Psychophysiology*, 48(11), 1532-1543.

Neil-Dwyer, G., Bartlett, J., McAinsh, J. & Cruickshank, J. (1981). Beta-adrenoceptor blockers and the blood-brian barrier. *British Journal of Clinical Pharmacology*, 11(6), 549-553.

Nieuwenhuis, S. & Jepma, M. (2010). Investigating the role of the noradrenergic system in human cognition. In T. Robbins, M. Delgado, & E. Phelps (Eds.), Decision making. Attention & Performance, Vol. XXIII, (pp. 367-370). Oxford University Press.

Nicolaides, N., Charmandari, E., Chrousos, G. & Kino, T. (2014). Circadian endocrine rhythms: the hypothalamic–pituitary–adrenal axis and its actions. *Annals of the new York Academy of Sciences*, 1318, 71-80.

Nikolić, K., Filipić, S., Smolinski, A., Kaliszan, R. & Agbaba, D. (2013). Partial least square and hierarchical clustering in ADMET modeling: prediction of blood-brain barrier permeation of alpha-adrenergic and imidazoline receptor ligands. *Journal of Pharmacy and Pharmaceutical Sciences*, 16(4), 622-647.

Nobis, L. & Husain, M. (2018). Apathy in Alzheimer's disease. *Current Opinion in Behavioral Sciences*, 22, 7-13.

Nobukawa, S., Shirama, A., Takahashi, T., Takeda, T., Ohta, H., Kikuchi, M., ... & Toda, S. (2021). Pupillometric Complexity and Symmetricity Follow Inverted-U Curves Against Baseline Diameter Due to Crossed Locus Coeruleus Projections to the Edinger-Westphal Nucleus. *Frontiers in Physiology*, 12. doi: 10.3389/fphys.2021.614479

O'Bryan, S. & Scolari, M. (2021). Phasic pupillary responses modulate object-based attentional prioritization. *Attention, Perception, & Psychophysics*. doi: 10.3758/s13414-020-02232-7.

Olsson, T., Marklund, N., Gustafson, Y. & Näsman, B. (1992). Abnormalities at different levels of the hypothalamic-pituitary-adrenocortical axis early after stroke. *Stroke*, 23(11), 1573-1576.

Petrovich, G. & Swanson, L. (1997). Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. *Brain Research*, 763(2), 247-254.

Pettit, H., Ettenberg, A., Bloom, F. & Koob, G. (1984). Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology*, 84(2), 167-173.

Pintor, J. (2009). Autonomic nervous system: ophthalmic control. In L. Squire (Ed.), *The Encyclopedia of Neuroscience* (pp. 967-974). Academic press.

Pomara, N., Greenberg, W., Branford, M. & Doraiswamy, P. (2003). Therapeutic implications of HPA axis abnormalities in Alzheimer's disease: review and update. *Psychopharmacology Bulletin*, 37(2), 120-134.

Propranolol Hydrochloride. (n.d.). National Institute for Health and Care Excellence. https://bnf.nice.org.uk/drug/propranolol-hydrochloride.html#sideEffects.

Prys-Roberts, C. & Farndon, J. (2002). Efficacy and safety of doxazosin for perioperative management of patients with pheochromocytoma. *World Journal of Surgery*, 26(8), 1037-1042.

Putman, P., Antypa, N., Crysovergi, P. & van der Does, W. (2010). Exogenous cortisol acutely influences motivated decision making in healthy young men. *Psychopharmacology*, 208(2), 257-263.

Qiyuan, J., Richer, F., Wagoner, B. & Beatty J. (1985). The pupil and stimulus probability. *Psychophysiology*, 22, 530-534.

Radley, J., Morilak, D., Viau, V. & Campeau, S. (2015). Chronic stress and brain plasticity: mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. *Neuroscience & Biobehavioral Reviews*, 58, 79-91.

Rajkowski, J., Kubiak, P. & Aston-Jones, G. (1993). Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Society for Neuroscience Abstracts*, 19, 974.

Rajkowski, J., Kubiak, P. & Aston-Jones, G. (1994). Locus coeruleus activity in monkey: phasic and tonic changes are associated with altered vigilance. *Brain Research Bulletin*, 35, 607-616.

Rajkowski, J., Majczynski, H., Clayton, E. & Aston-Jones, G. (2004). Activation of monkey locus coeruleus neurons varies with difficulty and performance in a target detection task. *Journal of Neurophysiology*, 92(1), 361-371.

Rajkowski, J., Majczynski, H., Clayton, E., Cohen, J. & Aston-Jones, G. (2002). Phasic activation of monkey locus coeruleus (LC) neurons with recognition of motivationally relevant stimuli. *Society for Neuroscience*, 86(10), 280.

Ramos, B. & Arnsten, A. (2007). Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacology & Therapeutics*, 113(3), 523-536.

Rasmussen, K., Morilak, D. & Jacobs, B. (1986). Single unit activity of locus coeruleus neurons in the freely moving cat: I. During naturalistic behaviors and in response to simple and complex stimuli. *Brain research*, 371(2), 324-334.

Raux-Demay, M., Pierret, T., d'Yvoire, M., Bertagna, X. & Girard, F. (1990). Transient inhibition of RU 486 antiglucocorticoid action by dexamethasone. *The Journal of Clinical Endocrinology & Metabolism*, 70(1), 230-233.

Reimer, J., McGinley, M., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D. & Tolias, A. (2016). Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. *Nature Communications*, 7. doi: 10.1038/ncomms13289.

Reul, J. & Kloet, E. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*, 117(6), 2505-2511.

Robinson, T. & Berridge, K. (1993). The neural basis of drug craving: an incentive sensitization theory of addiction. *Brain Research Reviews*, 18, 247-291.

Robinson, T. & Berridge, K. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1507), 3137-3146.

Robinson, T., Yager, L., Cogan, E., & Saunders, B. (2014). On the motivational properties of reward cues: individual differences. *Neuropharmacology*, 76, 450-459.

Robert, P., Lanctôt, K., Agüera-Ortiz, L., Aalten, P., Bremond, F., Defrancesco, M., ... & Manera, V. (2018). Is it time to revise the diagnostic criteria for apathy in brain disorders? The 2018 international consensus group. *European Psychiatry*, 54, 71-76.

Robert, P., Onyike, C., Leentjens, A., Dujardin, K., Aalten, P., Starkstein, S., ... & Byrne, J. (2009). Proposed diagnostic criteria for apathy in Alzheimer's disease and other neuropsychiatric disorders. *European Psychiatry*, 24(2), 98-104.

Rochat, L., Van der Linden, M., Renaud, O., Epiney, J., Michel, P., Sztajzel, R., ... & Annoni, J. (2013). Poor reward sensitivity and apathy after stroke: implication of basal ganglia. *Neurology*, 81(19), 1674-1680.

Rohleder, N., Joksimovic, L., Wolf, J. & Kirschbaum, C. (2004). Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biological Psychiatry*, 55(7), 745-751.

Russell, G., Durant, C., Ataya, A., Papastathi, C., Bhake, R., Woltersdorf, W. & Lightman, S. (2014). Subcutaneous pulsatile glucocorticoid replacement therapy. *Clinical Endocrinology*, 81(2), 289-293.

Ryan, R. & Deci, E. (2000). Self-determination theory and the facilitation of intrinsic motivation, social development, and well-being. *American Psychologist*, 55(1), 68-78.

Ryan, P., Ma, S., Olucha-Bordonau, F. & Gundlach, A. (2011). Nucleus incertus—an emerging modulatory role in arousal, stress and memory. *Neuroscience & Biobehavioral Reviews*, 35(6), 1326-1341.

Sahraei, H., Ghazzaghi, H., Zarrindast, M., Ghoshooni, H., Sepehri, H. & Haeri-Rohan, A. (2004). The role of alpha-adrenoceptor mechanism (s) in morphine-induced conditioned place preference in female mice. *Pharmacology Biochemistry and Behavior*, 78(1), 135-141.

Sänger, J., Bechtold, L., Schoofs, D., Blaszkewicz, M. & Wascher, E. (2014). The influence of acute stress on attention mechanisms and its electrophysiological correlates. *Frontiers in Behavioral Neuroscience*, 8. doi: 10.3389/fnbeh.2014.00353.

Sales, A., Friston, K., Jones, M., Pickering, A. & Moran, R. (2019). Locus Coeruleus tracking of prediction errors optimises cognitive flexibility: An Active Inference model. *PLoS Computational Biology*, 15(1). doi: 10.1371/journal.pcbi.1006267.

Samuels, E. & Szabadi, E. (2008). Functional Neuroanatomy of the Noradrenergic Locus Coeruleus: Its Roles in the Regulation of Arousal and Autonomic Function Part II: Physiological and Pharmacological Manipulations and Pathological Alterations of Locus Coeruleus Activity in Humans. *Current Neuropharmacology*, 6(3), 254-285.

Sapolsky, R., Romero, L. & Munck, A. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21, 55-89.

Sara, S. & Bouret, S. (2012). Orienting and reorienting: the locus coeruleus mediates cognition through arousal. *Neuron*, 76(1), 130-141.

Sasaki, M., Shibata, E., Tohyama, K., Takahashi, J., Otsuka, K., Tsuchiya, K., ... & Sakai, A. (2006). Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport*, 17(11), 1215-1218.

Schwabe, L. (2017). Memory under stress: from single systems to network changes. European *Journal of Neuroscience*, 45(4), 478-489.

Schwabe, L., Höffken, O., Tegenthoff, M. & Wolf, O. (2011). Preventing the stress-induced shift from goal-directed to habit action with a β-adrenergic antagonist. *Journal of Neuroscience*, 31(47), 17317-17325.

Schwabe, L., Tegenthoff, M., Höffken, O. & Wolf, O. (2010). Concurrent glucocorticoid and noradrenergic activity shifts instrumental behavior from goal-directed to habitual control. *Journal of Neuroscience*, 30(24), 8190-8196.

Schwabe, L., Tegenthoff, M., Höffken, O., & Wolf, O. (2012). Simultaneous glucocorticoid and noradrenergic activity disrupts the neural basis of goal-directed action in the human brain. *Journal of Neuroscience*, 32(30), 10146-10155.

Schwabe, L. & Wolf, O. (2009). Stress prompts habit behavior in humans. *Journal of Neuroscience*, 29(22), 7191-7198.

Schwabe, L. & Wolf, O. (2011). Stress-induced modulation of instrumental behavior: From goal-directed to habitual control of action. *Behavioural Brain Research*, 219(2), 321-328.

Shaham, Y., Highfield, D., Delfs, J., Leung, S. & Stewart, J. (2000). Clonidine blocks stress-induced reinstatement of heroin seeking in rats: an effect independent of locus coeruleus noradrenergic neurons. *European Journal of Neuroscience*, 12(1), 292-302.

Seeley, W., Menon, V., Schatzberg, A., Keller, J., Glover, G., Kenna, H., ... & Greicius, M. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience*, 27(9), 2349-2356.

Shepard, J., Bossert, J., Liu, S. & Shaham, Y. (2004). The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biological Psychiatry*, 55(11), 1082-1089.

Skosnik, P., Chatterton Jr, R., Swisher, T. & Park, S. (2000). Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *International Journal of Psychophysiology*, 36(1), 59-68.

Smith, S. & Vale, W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, 8(4), 383-395.

Söndergaard, H., Kushnir, M., Aronsson, B., Sandstedt, P. & Bergquist, J. (2012). Patterns of endogenous steroids in apathetic refugee children are compatible with long-term stress. *BMC Research Notes*, 5. doi: 10.1186/1756-0500-5-186.

Speer, K., Semple, S., Naumovski, N., D'Cunha, N. & McKune, A. (2019). HPA axis function and diurnal cortisol in post-traumatic stress disorder: A systematic review. *Neurobiology of Stress*, 11. doi: 10.1016/j.ynstr.2019.100180.

Spironolactone. (n.d.). National Institute for Health and Care Excellence. https://bnf.nice.org.uk/drug/spironolactone.html#sideEffects

Strassman, H., Thaler, M., & Schein, E. (1956). A prisoner of war syndrome: apathy as a reaction to severe stress. *American Journal of Psychiatry*, 112(12), 998-1003.

Strauch, C., Koniakowsky, I. & Huckauf, A. (2020). Decision Making and Oddball Effects on Pupil Size: Evidence for a Sequential Process. *Journal of Cognition*, 3(1). doi: 10.5334/joc.96.

Stavreva, D., Wiench, M., John, S., Conway-Campbell, B., McKenna, M., Pooley, J., ... & Hager, G. (2009). Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nature Cell Biology*, 11(9), 1093-1102.

Stokes, P. & Sikes, C. (1991). Hypothalamic-pituitary-adrenal axis in psychiatric disorders. *Annual Review of Medicine*, 42(1), 519-531.

Sussman, T., Jin, J. & Mohanty, A. (2016). Top-down and bottom-up factors in threat-related perception and attention in anxiety. *Biological Psychology*, 121, 160-172.

Swallow, K., Braver, T., Snyder, A., Speer, N. & Zacks, J. (2003). Reliability of functional localization using fMRI. *Neuroimage*, 20(3), 1561-1577.

Swallow, K., Jiang, Y. & Riley, E. (2019). Target detection increases pupil diameter and enhances memory for background scenes during multi-tasking. *Scientific Reports*, 9(1). doi: 10.1038/s41598-019-41658-4.

Szabadi, E. (2013). Functional neuroanatomy of the central noradrenergic system. *Journal of Psychopharmacology*, 27(8), 659-693.

Thau, L., Gandhi, J. & Sharma, S. (Eds.). (2019). *Physiology, cortisol.* StasPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK538239/.

Theleritis, C., Siarkos, K. & Politis, A. (2019). Unmet needs in pharmacological treatment of apathy in Alzheimer's disease: A Systematic Review. *Frontiers in pharmacology*, 10. doi: doi: 10.3389/fphar.2019.01108.

The Pulses Study. (2014). Health Research Authority. https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/the-pulses-study/.

Tiemensma, J., Andela, C., Kaptein, A., Romijn, J., van der Mast, R., Biermasz, N. & Pereira, A. (2014). Psychological morbidity and impaired quality of life in patients with stable treatment for primary adrenal insufficiency: cross-sectional study and review of the literature. *European Journal of Endocrinology*, 171(2), 171-182.

Timmermans, S., Souffriau, J. & Libert, C. (2019). A general introduction to glucocorticoid biology. *Frontiers in Immunology*, 10. doi: 10.3389/fimmu.2019.01545.

Turker, H., Riley, E., Luh, W., Colcombe, S., & Swallow, K. (2019). Estimates of locus coeruleus function with functional magnetic resonance imaging are influenced by localization approaches and the use of multi-echo data. *BioRxiv*.3(1). doi: 10.5334/joc.96.

Uddin, L. (2015). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience*, 16(1), 55-61.

Ulrich-Lai, Y. & Herman, J. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, 10(6), 397-409.

Unnerstall, J., Kopajtic, T. & Kuhar, M. (1984). Distribution of α2 agonist binding sites in the rat and human central nervous system: analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Research Reviews*, 7(1), 69-101.

Valentino, R. (1988). CRH effects on central noradrenergic neurons: relationship to stress. *Mechanisms of Physical and Emotional Stress*, 47-64.

Valentino, R. & Foote, S. (1987). Corticotropin-releasing factor disrupts sensory responses of brain noradrenergic neurons. *Neuroendocrinology*, 45(1), 28-36.

Valentino, R. & Foote, S. (1988). Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. *Journal of Neuroscience*, 8(3), 1016-1025.

Valentino, R. & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology*, 583(2-3), 194-203.

van Hulst, B., De Zeeuw, P. & Durston, S. (2015). Distinct neuropsychological profiles within ADHD: a latent class analysis of cognitive control, reward sensitivity and timing. *Psychological Medicine*, 45(4), 735-745.

Vankov, A., Hervé-Minvielle, A. & Sara, S. (1995). Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat. *European Journal of Neuroscience*, 7(6), 1180-1187.

Van Lippevelde, W., Vervoort, L., Vangeel, J. & Goossens, L. (2020). Can Parenting Practices Moderate the Relationship between Reward Sensitivity and Adolescents' Consumption of Snacks and Sugar-Sweetened Beverages?. *Nutrients*, 12(1). doi: 10.3390/nu12010178.

van Marle, H., Hermans, E., Qin, S. & Fernández, G. (2009). From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. *Biological Psychiatry*, 66(7), 649-655.

van Marle, H., Hermans, E., Qin, S. & Fernández, G. (2010). Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. *Neuroimage*, 53(1), 348-354.

Varazzani, C., San-Galli, A., Gilardeau, S. & Bouret, S. (2015). Noradrenaline and dopamine neurons in the reward/effort trade-off: a direct electrophysiological comparison in behaving monkeys. *Journal of Neuroscience*, 35(20), 7866-7877.

Vazey, E., Moorman, D. & Aston-Jones, G. (2018). Phasic locus coeruleus activity regulates cortical encoding of salience information. *Proceedings of the National Academy of Sciences*, 115. doi: 10.1073/pnas.1803716115.

Ventura, R., Cabib, S., Alcaro, A., Orsini, C. & Puglisi-Allegra, S. (2003). Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *Journal of Neuroscience*, 23(5), 1879-1885.

Ventura, R., Latagliata, E., Morrone, C., La Mela, I. & Puglisi-Allegra, S. (2008). Prefrontal norepinephrine determines attribution of "high" motivational salience. *PLoS One*, 3(8). doi: 10.1371/journal.pone.0003044.

Walsh, A., Carmel, D. & Grimshaw, G. (2019). Reward elicits cognitive control over emotional distraction: Evidence from pupillometry. *Cognitive, Affective, & Behavioral Neuroscience*, 19(3), 537-554.

Wang, C., McInnis, H., Brien, D., Pari, G. & Munoz, D. (2016). Disruption of pupil size modulation correlates with voluntary motor preparation deficits in Parkinson's disease. *Neuropsychologia*, 80, 176-184.

Ward, D., Grizzle, W. & Gann, D. (1976). Inhibitory and facilitatory areas of the rostral pons mediating ACTH release in the cat. *Endocrinology*, 99(5), 1220-1228.

Watson, C. (2012). Motor nuclei of the cranial nerves. In C. Watson, G. Paxinos & L. Puelles (Eds.), *The Mouse Nervous System* (pp. 490-498). Academic Press.

Weinshenker, D. & Schroeder, J. (2007). There and back again: a tale of norepinephrine and drug addiction. *Neuropsychopharmacology*, 32(7), 1433-1451.

Weitzman, E., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. & Hellman, L. (1971). Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *The Journal of Clinical Endocrinology & Metabolism*, 33(1), 14-22.

Whitton, A., Treadway, M. & Pizzagalli, D. (2015). Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Current Opinion in Psychiatry*, 28(1), 7-12.

Wiegert, O., Pu, Z., Shor, S., Joëls, M. & Krugers, H. (2005). Glucocorticoid receptor activation selectively hampers N-methyl-D-aspartate receptor dependent hippocampal synaptic plasticity in vitro. *Neuroscience*, 135(2), 403-411.

Windle, R., Wood, S., Shanks, N., Lightman, S. & Ingram, C. (1998). Ultradian rhythm of basal corticosterone release in the female rat: dynamic interaction with the response to acute stress. *Endocrinology*, 139(2), 443-450.

Young, E., Abelson, J. & Lightman, S. (2004). Cortisol pulsatility and its role in stress regulation and health. *Frontiers in Neuroendocrinology*, 25(2), 69-76.

Young, E., Lopez, J., Murphy-Weinberg, V., Watson, S. & Akil, H. (1998). The role of mineralocorticoid receptors in hypothalamic-pituitary-adrenal axis regulation in humans. *The Journal of Clinical Endocrinology & Metabolism*, 83(9), 3339-3345.

Young, K., Preskorn, S., Victor, T., Misaki, M., Bodurka, J. & Drevets, W. (2016). The effect of mineralocorticoid and glucocorticoid receptor antagonism on autobiographical memory recall and amygdala response to implicit emotional stimuli. *International Journal of Neuropsychopharmacology*, 19(9). doi: 10.1093/ijnp/pyw036.

Yoshitomi, T., Ito, Y., & Inomata, H. (1985). Adrenergic excitatory and cholinergic inhibitory innervations in the human iris dilator. *Experimental Eye Research*, 40(3), 453-459.

Zerbi, V., Floriou-Servou, A., Markicevic, M., Vermeiren, Y., Sturman, O., Privitera, M., ... & Bohacek, J. (2019). Rapid reconfiguration of the functional connectome after chemogenetic locus coeruleus activation. *Neuron*, 103(4), 702-718.

Zhang, J., Berridge, K., Tindell, A., Smith, K. & Aldridge, J. (2009). A neural computational model of incentive salience. *PLoS Computational Biology*, 5(7). doi: 10.1371/journal.pcbi.1000437.