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Driving the Locus Coeruleus: Pupillometry in a Driving Simulation Paradigm

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A naturalistic and continuous driving simulation paradigm is presented as a novel method for investigating the function of the locus coeruleus norepinephrine (LC-NE) system and its modulation of cognition and behaviour. The LC is one of the earliest sites of Alzheimer's Disease (AD) related pathology, while the LC-NE system modulates multiple cognitive functions known to decline in ageing and AD. This paradigm provides a novel way of probing the LC-NE under conditions requiring attention, memory, and decision-making with the use of pupillometry and embedded psychological tasks. Pupil diameter results suggest an LC role in monitoring task demands and responding to errors, as predicted by the Adaptive Gain Theory (Aston-Jones & Cohen, 2005). The novel finding of a *gradual* increase in pupil diameter in response to the approach of a cognitively demanding situation was made possible by our continuous design. Significant pupil dilation responses to decision-making events support a role of the LC-NE, and the ability of this paradigm to examine its function.

Chapter 1: Introduction

Alzheimer's disease (AD) is a prevalent neurodegenerative disease, which affects 850K people in the UK alone (World Health Organisation, 2017) and costs the UK economy £26B annually (Prince et al., 2014). Much of this cost can be attributed to late-stage care of AD patients. Currently, AD diagnosis relies on traditional pencil and paper neuropsychological assessments of already advanced and irreversible symptoms (such as the Mini Mental State Exam [MMSE] (Folstein, Folstein, & McHugh, 1975). These tests lack both ecological validity and ability to distinguish between different types of dementia. The reliance on advanced symptoms for diagnosis results in less effective treatment. A new tool to enable the early diagnosis of AD, before symptoms arise, would lead to an increase in the effectiveness of current treatments, both pharmacological and cognitive, and potentially result in a significant delay in the onset of symptoms. With less money being spent on late-stage care of AD patients, the focus could move towards new treatment and prevention plans.

Mild cognitive impairment (MCI) describes a condition in which an individual's cognitive abilities are more impaired than expected for that age yet does not notably interfere with daily activities. Individuals with MCI are at high-risk of going on to develop dementia, with AD being the most common form. Diagnosis of these disorders relies on a combination of time-consuming and expensive tests, such as clinical interview, neuropsychological tests, blood tests, lumbar puncture, and MRI, CT or PET scans. Analysing the results from these test combinations, along with evaluating an individuals' medical records is not only time-consuming but also subjective. Moreover, these tests rely heavily on clinical infrastructure, limiting their availability and making them unsuitable for testing the large population at risk.

Researchers are now seeking non-invasive, fast, cheap and reliable methods for early diagnosis of MCI and AD. This would enable timely treatment, improved quality of life, and support research into new therapies—all of which are crucial for addressing the growing impact of AD on society.

EEG directly measures neural activity with high temporal resolution and has shown potential for detecting neural biomarkers related to MCI and AD (for example, Meghdadi et al, 2021).

ERP components such as the P3, which reflects cognitive processing and attention, are often delayed or reduced in amplitude in individuals with AD. This delay is indicative of slowed neural processing and impaired working memory (Polich & Corey-Bloom, 2005). Changes in theta power have been observed in individuals with MCI, serving as predictors of conversion to AD (Babiloni et al, 2010).

The locus coeruleus (LC), a small nucleus in the brainstem, is one of the earliest sites of AD-related pathology (Mravec, Lejavova, & Cubinkova, 2014). Hyperphosphorylated tau is often evident in the brainstem of individuals in their twenties (Braak, Thal, D, Ghebremedhin, & Del Tredici, 2011). Although there is debate around how, or if, this contributes to AD, a strong correlation has been found between neurofibrillary tangles in the neocortex and cognitive impairment before death (Nelson et al., 2012). The locus coeruleus norepinephrine (LC-NE) system modulates many cognitive functions and is key in determining cognitive abilities in late life. Localisation of the LC is challenging using traditional neuroimaging techniques due to its position in the brainstem and small size (~14mm x 2mm, Fernandes, Regala, Correia, & Gonçalves-Ferreira, 2012), however, several methods are able to provide an insight into LC-NE function. First, pupil diameter has been established as a proxy measure of LC-NE system function and has been shown to change with advancing age (Kasthurirangan & Glasser, 2006). Second, phasic activity of the LC and resulting NE release have been suggested to be key in producing the P3 event-related potential, an EEG component related to decision-making events in visual search, and its amplitude has been shown to decline with ageing (van Dinteren, Arns, Jongsma, & Kessels, 2014). Third, theta oscillations (4-8 Hz) have been suggested to promote attention, decision-making and learning, and high resting theta power is associated with increased cognitive function in older adults (Finnigan & Robertson, 2011). There is evidence to suggest that LC activation facilitates hippocampal theta oscillations, promoting the efficacy of memory formation (Asaka et al., 2005).

The previously mentioned methods provide an insight into LC-NE system function and present an opportunity for the development of an early biomarker for AD-related changes.

The upcoming sections will discuss the biology and physiology of the locus coeruleus and current theories surrounding the function of the LC-NE system. These theories will form the basis from which we identify informative and innovative ways to study LC-NE function in a quantitative, yet ecologically valid and naturalistic manner.

LC Biology and Physiology

The LC is a small brainstem nucleus containing the majority of the brain's noradrenergic neurons. The LC is a highly interconnected region despite its small size, and projects to many anatomically and functionally diverse regions (Schwarz & Luo, 2015) including the majority forebrain regions, many midbrain and brain stem nuclei, as well as the cerebellum and spinal cord (for a review, see Berridge & Waterhouse, 2003; Sara & Bouret, 2012; Gary Aston-Jones & Cohen, 2005). The release of norepinephrine (NE) in the mammalian brain contributes to a number of important aspects of cognition including the modulation of attention (McGaughy, Ross, & Eichenbaum, 2008), behavioural flexibility (Bouret & Sara, 2005; Gary Aston-Jones & Cohen, 2005), learning (Hansen & Manahan-Vaughan, 2015) and memory (Hagena, Hansen, & Manahan-Vaughan, 2016). Although previous research has established that the projections of the LC are broad, little information about the organisation of these projections has been provided. Previously, the LC has been suggested to operate homogeneously, with transmitter specific cells regulating brain function and behaviour through axonal projections and transmitter-mediated modulatory influences on diverse neural targets within the central nervous system (CNS).

A more recent challenge of this view is presented by (Chandler, Gao, & Waterhouse, 2014), who instead argue for a segregation of the LC-NE. Results suggest biochemical and electrophysiological differences of LC cells innervating discrete cortical regions, for example, more excitable LC cells projecting to the PFC than the motor cortex. This evidence challenges the previously held view of a homogenous modulator, and instead argues for a more specific organisation of LC efferent projections, and asynchronous release of NE. The degree of synchrony is likely to vary across behavioural and cognitive states. Recent studies have also found evidence for a target-specific, ensemble-based LC neuronal code (Uematsu et al., 2017; Wagatsuma et al., 2017), suggesting that the LC may be segregated into sub-sets of neurons acting as separate ensembles to modulate specific brain circuits. Totah, Logothetis, & Eschenko (2019) provide a scenario to explain this ensemble-based neuronal code, and why the activity of LC neurons is still largely synchronous. They propose that multiple LC ensembles may activate together to simultaneously modulate only a sub-set of neuronal circuits important for the behaviour (i.e., not one-to-one neuromodulation, and not fully global). This view is supported by evidence of LC neurons being activated strongly or weakly, and homogeneously as an ensemble or heterogeneously depending on the demands of a task (Uematsu et al., 2017). The longstanding views of the LC as an evolutionarily conserved structure involved only in functions critical for survival are changing towards an emerging perspective that the LC plays a critical role in multiple cognitive functions.

Usher et al (1999) propose a model of LC role in cognitive performance, revealing two modes of LC activity resulting from alterations in electronic coupling among LC neurons. Variations in gain of cortical units receiving LC inputs result in either facilitation of task performance—*phasic mode*, or produce more exploratory, less goal-directed responding—*tonic mode*. The two modes of LC activity are discussed below in relation to arousal, and further theories building on this model are presented.

LC and Arousal

The LC-NE brainstem neuromodulatory system is one of several such nuclei with diverse, ascending projections to the neocortex, critical in regulating cortical function. NE, a so-called ‘classical neurotransmitter’, does not produce direct excitatory or inhibitory effects on postsynaptic neurons, but instead modulates such effects produced by other neurotransmitters, for example glutamate and GABA.

Previous work has investigated the relationship between LC-NE function and arousal, and how this relates to the optimisation of reward-seeking behaviours (for example Bouret & Richmond, 2015). Task performance on a majority of tasks is best with an intermediate level of arousal and is worse with too little or too much, best described by the Yerkes-Dodson inverted U curve (Yerkes & Dodson, 1908). LC-NE activity and task performance can be described in the same way, consistent with the view that the LC-NE system mediates arousal. Previous theories of LC function have suggested that the system primarily affects sensory processing, largely due to the observation that arousing and highly salient stimuli elicit activation of LC neurons and concomitant NE release (for example, Aston-Jones & Bloom, 1981). More recently, recordings of monkey LC during decision-making tasks have suggested distinguishable modes of LC activity (for example, Rajkowski, Majczynski, Clayton, & Aston-Jones, 2004). Phasic activity has been associated with the outcome of task-related decision processes and with highly accurate behavioural responses. LC tonic mode leads to higher baseline activity and an absence of phasic bursts, and has been linked to more distractible behaviour.

LC Theory

Existing evidence suggests that the LC-NE system modulates neural gain throughout the brain (Aston-Jones & Cohen, 2005; Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010). Eldar, Cohen, & Niv (2013) present evidence for the theory that neural gain focuses attention and learning on dimensions to which an individual is predisposed to attend. Global increases in gain are proposed to increase the strength of strong representations while decreasing the strength of weaker representations. The findings suggest that although the individuals' predispositions influence learning, they are not necessarily binding. Rather, modulation of attention and learning occur via different modes of neural communication induced by global fluctuations in neural gain.

Aston-Jones & Cohen (2005) propose the Adaptive Gain Theory of LC-NE function. During performance of a task, phasic activity facilitates behavioural responses to the outcome of task-specific decision processes, while filtering responses to irrelevant events, therefore optimising performance. When utility subsides, tonic activity facilitates behaviours other than the task, exploring alternative sources of reward. These modes of activation are achieved via the widespread but temporally specific release of NE at cortical target sites, modulating the gain of processing in the circuits responsible for task performance. The phasic and tonic modes of LC activity adaptively adjust the gain of the appropriate cortical circuits, resulting in the facilitation or disengagement of task-specific processes. This process optimises the exploitation-exploration trade-off: pursuing optimal performance in the current task, while considering its utility against alternatives that may be more valuable.

A further theory of LC-NE system function proposes that, under arousal, glutamate amplifies noradrenergic effects (GANE model, Mather, Clewett, Sakaki, & Harley, 2016) to enhance high priority representations and suppress or out-compete those of lower priority. 'NE hotspots' are suggested to be created by increases in local NE release from the LC, resulting from high glutamate at the site of prioritised representations. Suppression of lower priority representations is suggested to occur through two mechanisms: amplification of lateral inhibition at the site of NE hotspots; and in the absence of hotspots, NE levels high enough only to activate low-threshold inhibitory adrenoreceptors. These mechanisms result in widespread suppression, with only a few hotspots of excitation enhancing prioritised information. The NE mechanisms proposed in this theory promote selective attention and memory under arousal.

Both cognitive and behavioural fluctuations have been found to correlate with LC-NE activity. From activation over timescales of *50ms to 100ms* with a sudden reorientation away from the current task and towards something novel (Bouret & Sara, 2004), to timescales of a few seconds when pupil diameter can be used as a measure of LC-NE activity (Unsworth & Robison, 2015), and to several minutes when pupil diameter and release of NE correlate with adjustment of behavioural strategy (Urai, Braun, & Donner, 2017). The LC-NE is evolutionarily conserved across vertebrates, indicating its role in functions critical for survival (Szabadi, 2013). However, higher-order cognitive functions also involve the LC-NE system. The connectivity of brainstem regions and relatively modern forebrain regions allow the integration of survival functions with cognition (Szabadi, 2013).

LC and Pupil Diameter

As well as in response to changes in luminance, pupil diameter changes in line with levels of NE, for example during wakefulness compared with sleep (Lowenstein & Loewenfeld, 1964), aerobic exercise (Ishigaki, Miyao, & Ishihara, 1991) and emotional arousal (Bradley, Miccoli, Escrig, & Lang, 2008), along with multiple cognitive tasks which are also associated with activation of the LC. Functional neuroimaging research has uncovered a strong correlation between pupil dilation and LC activity (Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014). However, it is not known whether the LC is causally responsible for pupil dilation, or the pathway of such a connection. It is possible that the correlation results from a common presynaptic input from the paragigantocellularis nucleus (PGi) to LC and Erdinger-Westphal (E-W) nucleus (Nieuwenhuis, De Geus, & Aston-Jones, 2011), an indirect pathway, and the relatively slow time scale of pupil dilation following electrical stimulation of the LC (~500ms) (Joshi, Li, Kalwani, & Gold, 2016) points towards this.

Changes in pupil diameter predict behavioural outcomes in many cognitive tasks. Pupil diameter alone was able to predict a decision, both the timing of the response and the chosen digit, in a covert decision-making task (Einhäuser, 2010). Changes in pupil diameter have also been found to track decision-making in mice in a Go/NoGo task (Lee & Margolis, 2016). Large amplitude dilations were observed prior to and during Hit and False Alarm responses, and small amplitude pupil dilations followed by constriction were observed for Correct Reject responses. The pupil responses became more pronounced and earlier with learning, with pupil diameter predicting behavioural choice with around 80% accuracy. *These results provide evidence of pupil diameter as a measure to track covert decision-making and are consistent with engagement of neuromodulatory systems such as the LC-NE system.*

Pupil diameter at encoding has been found to predict subsequent recognition memory strength (Kafkas & Montaldi, 2011). A smaller pupil response at encoding led to more successful long-term memory formation for the item. A greater number of fixations, and a shorter first fixation, led to more successful encoding. Apparently contradictory results were found by Papesh, Goldinger, & Hout (2012) in a recognition memory task. Enlarged pupils signalled greater effort during encoding of subsequently recognised items, with strong memories resulting in the highest peak pupil diameters. However, the procedure employed by Kafkas & Montaldi (2011) eliminated task-evoked pupillary responses (TEPRs) during encoding, due to the learning being incidental (the participants were not asked to actively encode the material), leaving only *tonic* changes that reflect overall arousal levels during encoding. TEPRs correspond to phasic, or event-related, activity in the LC, indicating high levels of attention and occur independently of tonic changes which follow emotional arousal, stress or changes in luminance (Karatekin, Couperus, & Marcus, 2004).

Pupillary changes can reflect cognition dependent on neuromodulatory systems, such as LC-NE, however, they also reflect changes in luminance. To control for this potentially confounding factor, some studies have utilised auditory stimuli, involving no changes in visual stimulation (for example, Knapen et al., 2016). However, the question should be asked as to whether the parameters are the same for visual and auditory stimuli. Klingner, Tversky, & Hanrahan (2011) compared the effects of both visual and aural stimuli on pupil dilations in a range of cognitive tasks and found similar patterns of dilations in terms of onset timing, duration and shape. Auditory stimuli led to significantly larger pupil dilations, and visual presentation increased accuracy, suggesting that cognitive load is higher for auditory than visual presentation.

Measuring changes in pupil diameter has provided further insight into the exploration-exploitation trade-off. Larger baseline pupil diameters have been found to precede exploratory, rather than exploitative, choices (Jepma & Nieuwenhuis, 2011). Individual differences in baseline pupil diameter were found to predict an individual's tendency to make exploratory choices, with a larger baseline correlating with more exploratory choice behaviour. During the transition between exploration and exploitation, pupil diameter was correlated with changes in utility. Further, increased baseline pupil diameter is associated with decreased task utility and task disengagement (exploration), and decreased baseline pupil diameter, along with increases in task-evoked dilations, are associated with task engagement (exploitation) (Gilzenrat et al., 2010). Consistent with this are findings from Murphy, Robertson, Balsters, & O'connell (2011), that poor performance elicited phasic pupil dilations and task reengagement. *Taken together, the results support the Adaptive Gain Theory (Gary Aston-Jones & Cohen, 2005b) and that pupil diameter may be used to index LC-NE activation state.*

Naturalistic Paradigms

Recent research into ageing and AD has seen a move from tightly constrained cognitive paradigms to a more naturalistic approach enabling generalisation to human behaviour in natural settings. Virtual reality (VR) environments offer increased ecological validity, and if rigorously designed and controlled, may provide more complete information on how perceptual and behavioural capacities accumulate, with multiple stimuli and responses competing for attention in more complex tasks.

The LC-NE system has previously been studied with the use of classical paradigms such as the 'Oddball' task, with the focus on eliciting an LC response to the novel stimuli. These tasks involve repetitive trials with simple stimuli, often circles. However, taking into consideration the more recent theories of LC-NE function (neural gain, adaptive gain theory, GANE theory), it may be argued that such an adaptive system would be better understood with a more ecologically valid and active paradigm.

Classical experimental designs could be considered reductionist, with little relevance outside the laboratory (For example Nastase, Goldstein & Hasson, 2020). The brain has evolved in a complex and multidimensional world. Is it possible to provide real insight into the function of the brain 'in the wild' when complex phenomena are broken down into easily manipulated components? On the other hand, classic laboratory research methods ensure control over stimuli and environment and enable teasing apart neural mechanisms responsible for the studied tasks. Furthermore, classic experimental designs have enabled repeated testing, leading to the development of highly detailed theories of brain functional organisation and cognition.

The Rivermead Behavioural Memory Test (RBMT) has been proposed as an ecological assessment of AD. This short memory test battery can provide data about an individual's memory function in daily life. The RBMT does not follow an established memory model and does not focus on the individual's ability to complete the task but instead analyses their performance and the impact on daily functioning through a series of real-world-like assessments of prospective memory, retrospective memory, orientation, visuospatial memory, and short- and/or long-term memory. Tasks include remembering to ask for an appointment when an alarm sounds after 20 minutes; remembering a story with 21 ideas to test immediate recall (just after hearing it) and delayed recall (20 minutes later); immediate and delayed recall of a message (remembering to pick up an envelope and place it in a specific place); recalling the first and second name of a person in a picture 20 minutes after it was shown.

Tests such as the RBMT make a move towards more ecologically valid methods for evaluating cognitive functions, while still maintaining a level of control. Studies have assessed its ability to distinguish between healthy participants and those with MCI and AD (Bolló-Gasol, 2014).

However, despite its ability to ecologically evaluate cognitive functioning using tasks similar to those in everyday life, there is still a reliance on observable symptoms for diagnosis using this test. Johansson & Wressle (2012) found that the RBMT can help to distinguish between MCI and mild dementia, however more sensitive screening is needed to detect early cognitive impairment.

Virtual reality (VR) assessments have recently been employed as a method of delivering traditional neuropsychological tests with increased ecological validity. Maronnat et al (2020) created an immersive VR environment including multiple tasks adapted from the Mini-Mental State Examination (MMSE), the Monreal Cognitive Assessment (MoCA), and the five words of Dubois (F5B) to assess the feasibility of employing such methods in a patient population.

To test a more real-world assessment of episodic memory, Plancher et al (2012) utilised a virtual environment to characterise episodic memory profiles in an ecological fashion. Healthy older adults, patients with amnestic mild cognitive impairment (aMCI) and patients with early to moderate AD were immersed in two virtual environments. First as the driver of a virtual car (active exploration) and second as the passenger of that car (passive exploration), participants were asked to encode all elements of the environment as well as the associated spatiotemporal contexts. AD patients performed worse than aMCI and healthy aged groups in line with progression of cognitive decline. Spatial allocentric memory assessments were able to distinguish between aMCI and healthy older groups. Active exploration in the task enhanced patients' episodic memory performance, including those with AD, highlighting the importance of further research into active encoding in episodic memory. Of particular note, patients' daily memory complaints were more highly correlated with performance on the virtual task than with performance on a classic memory test.

VR studies are more likely to create multi-sensorial and self-relevant situations than typical laboratory conditions, and are relevant in research, diagnosis and rehabilitation.

It is the aim of the current study to strike a balance between experimental control and ecological validity and generalisability by incorporating adaptations of 'classical' tasks into a more ecological and active paradigm. To assist in this aim, it was necessary to create a quantifiable, yet naturalistic and continuous paradigm capable of probing the function of the LC and its influence on cognitive functions such as decision-making and memory.

LC, Emotional Memory, and Taboo Words

Memory strength can be enhanced by emotionally arousing conditions, and this appears to involve the release of NE. Previous research has found that NE plays a crucial role in emotional arousal and emotional memory (for example, Cahill, Prins, Weber, & McGaugh, 1994). Taboo words have been found to reliably evoke emotional arousal, and to better capture the attention of participants (Chan, Madan, & Singhal, 2016).

The LC activates when reorienting behaviour is required in response to a disruptive stimulus (Aston-Jones et al, 1991), with phasic LC-NE activity interrupting function to direct attention to salient or threatening situations (Johnson, 2003). Chan & Singal (2013) found better memory recall for negative information and suggested that emotion-based distraction can modulate attention and decision-making abilities. Further to this work, Chan, Madan & Singal (2016) found that taboo words were better remembered than moderately arousing positive and negative words, and non-arousing neutral words when viewed in a simulated driving task. Taboo words were also associated with better lane control, suggesting that taboo-related arousal can selectively enhance attentional focus during complex tasks. The use of taboo words as salient, novel and attention-grabbing stimuli is something the current study will explore.

Memory is an essential part of performing complex tasks such as driving, however creating an ecologically valid method for testing this is not as simple as presenting isolated words. In real-world scenarios, the brain is constantly required to weight and re-weight all incoming dimensions (relevant and non-relevant) in the context of the task at hand. Using our driving simulation paradigm, we are able to naturalistically test different aspects of memory using embedded psychological tasks that are encountered whilst driving around the simulated track. In order to test recognition memory for taboo and neutral stimuli specifically, it was essential to conduct a separate online study that allowed the creation of a new set of stimuli where the effects we assessed were appropriate.

LC, Pupil Responses and Anxiety

Anxiety has recently been identified as a potential risk factor for AD (for example Becker et al, 2018; Li et al, 2021; Santabárbara et al, 2020; Gracia-Garcia et al, 2023) and is also associated with LC activation (for example Browning et al, 2015). The LC-NE system modulates arousal, alerting and orienting functions, as well as memory systems, and these can be amplified in response to threat or a stressor. In an aversive learning task which manipulated environmental volatility, Browning, Behrens, Jocham, O'Reilly, & Bishop (2015) found that high-trait anxious individuals showed a smaller effect of environmental volatility on post-outcome pupil dilation. This finding suggests a deficit in adjusting learning rate associated with the changing environment in participants with high anxiety. Processing of environmental changes and the use of guided learning is closely linked to the activity of the LC-NE, and this is supported here by the finding that pupil dilations tracked environmental volatility and were significantly correlated with adjusted learning rates.

Several other studies have also suggested that pupil dilation may track changes in environmental features (Preuschoff, 't Hart, & Einhäuser, 2011; Nassar et al., 2012), reflecting LC/NE activity and modulation of learning of environmental uncertainty.

The Current Study

The current study involves the use of a continuous, naturalistic driving simulation paradigm, allowing the LC to be challenged in an ecologically valid fashion and allowing for generalisations to human behaviour in natural settings. This paradigm probed the function of the LC-NE system under conditions requiring decision-making and memory with the use of embedded tasks within it. Eye movements and pupil diameter were recorded as participants attempted to complete the task with speed and accuracy.

Why a Driving Simulation Paradigm?

Driving is a highly demanding task and can be intense. It requires time-pressured decisions and places a high demand on working memory. A naturalistic and continuous task also provides good ecological validity and a highly flexible scenario—we are able to manipulate the task demands and include a number of psychological tests along the track. This paradigm includes the use of naturalistic adaptations of memory and decision-making tasks. The LC-NE system should dynamically modulate behaviour and decision-making in this task.

Hypotheses

The behavioural data collected here is expected to show a significant positive effect of previous driving and gaming experience on the outcome of the driving simulation (i.e. simulation score). High anxiety is expected to negatively impact a participants' driving simulation score. There is not expected to be a significant effect of age on simulation score due to the narrow age range in the current study, however, further research may look to include a group of older participants, and a significant difference in simulation score would be expected between the two age groups.

In relation to the embedded, naturalistic adaption of an n-back memory and decision-making task (described later as the *road-sign task*), it is expected that a correct decision will be accompanied by a higher confidence rating; a correct decision will result in a lower reaction time; more correct decisions will be made based on information remembered from '1-back' than from '2-back'; reaction times will be lower for decisions based on information '1-back' than '2-back'; confidence ratings will be higher for '1-back' decisions than '2-back' decisions; and that higher confidence in a decision will lead to a quicker reaction time.

In relation to the embedded recognition memory task (described later as the *pub-sign task*), it is expected that correctly recognised items will have been rated as more taboo (taboo ratings collected in a separate online study); and that incorrect recognition (i.e. incorrectly recognising a decoy item) will be more likely if the item has been previously rated as more taboo.

The data collected relating to pupil diameter is expected to show dilation when the participant makes a decision, and this effect will likely be modulated by the difficulty of the decision. This pupillary response should predict the participant's performance on the task. In relation to the embedded decision-making task, a different pupil response is expected between conditions, and this is expected to be modulated by a participants' confidence in that decision.

Chapter 2: Method

Method

Design

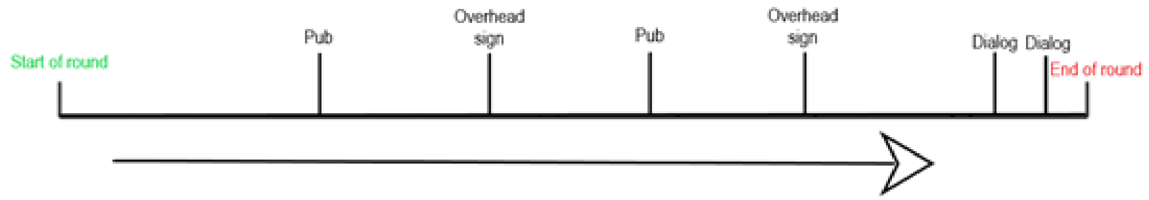
The main driving task consisted of a naturalistic highway driving simulation task, rendered using a 3D graphics library and presented on a 1900 x 1080 px computer screen. The display provided a view from the driver position in the driver vehicle, and included a rear-view mirror for observing approaching traffic, a speedometer and time bonus gauge to encourage risk-taking to gain points, and a points display (positive and negative) (Figure 1). The driver vehicle was controlled using a 10-button controller, with buttons controlling lane changes (left/right), speed (cruise control, with increments of 10km/h), and braking (continuous press) (Figure 2). RPM modulated engine noise enhanced realism.

Figure 1. A screenshot of the driving task, showing the placement of the rear-view mirror, time bonus gauge, speedometer, and point display.

Figure 2. The SNES™-style 10-button controller used to control the driver vehicle.

The driving task consisted of 16 rounds (laps around a driving track). The time taken to complete each round varied according to performance. A round was restarted following a collision event, and more than two collisions in a given round resulted in the simulation skipping ahead to the following round. A round containing no collision events took around two minutes to complete. Each round included: (1) Traffic events, requiring the participant to make lane change or brake decisions in order to avoid collisions, which were logged as button presses; (2) Two sets of overhead road-signs spaced out along the track. Each road-sign contained place names with corresponding directions (four place names per sign, two associated with a left turn and two with a right turn). This information was to be remembered by the participant and will be referred to as the *road-sign task*; (4) Two pop-up dialog boxes at the end of each round corresponding to the road-sign task (described in more detail below); (5) Two pub buildings displaying large name signs at the side of the track (described in more detail below—*pub-sign task*). See Figure 3 for illustration of round design.

Figure 3. The order and approximate position of the stimuli presented in each round of the driving simulation task. Traffic events requiring overtake or braking decisions occurred throughout the task and were logged by button presses.



Road-sign task. This task aimed to test memory and decision-making with the use of a naturalistic adaptation of an *n-back* style task. The overhead road-signs containing place names and corresponding directions (Figure 4) were placed at intervals around the track. The participant was required to remember information displayed on these signs, whilst navigating traffic and attempting to gain points. At the end of each round, a pop-up dialog box was displayed, asking “Which way to X”, where X was a place name randomly selected from the eight displayed in that round. The ‘n’ was varied from 1 to 2 to increase/decrease difficulty. The participant was required to press a button (left or right) as quickly as possible according to the remembered information. Following the decision, a second dialog box asked the participant to rate their confidence in the response from four options: very sure, fairly sure, not sure, complete guess (divided into *high confidence* [‘very-’ and ‘fairly-sure’] and *low confidence* [‘not-sure’ and ‘complete guess’] for the purposes of analysis).

Figure 4. An example of an overhead sign containing the place names and corresponding directions displayed in the driving simulation, as part of the *road-sign task* (**A**), and an example of a pub name sign (**B**).

Pub-sign task. Pubs displaying large name signs were placed at intervals around the track (two per round) (Figure 5). In each round, a name was randomly assigned to each pub from a list of 80 pub names (rated on their perceived salience in a separate online study). Participants were not explicitly asked to remember this information. Following completion of the driving task, participants were asked to complete a forced-choice recognition memory task implemented in PsychoPy 2020.1.0. In each trial, one of the previously displayed taboo/neutral names was presented alongside a new, unseen name. participants were required to indicate which names they recognised from the driving task. The pub names used in the driving task were selected by the researchers and subsequently rated for funniness, memorability, and taboo-ness by participants in a separate experiment using a Qualtrics questionnaire (below-Online word rating study).

Points were assigned to events occurring in the round and were displayed to the participant as they occurred. This aimed to provide reinforcement to encourage risk-taking while remaining vigilant. Points were positive [+] or negative [-], and were assigned for:
(1) Overtaking vehicles safely [+15 or +30]

(2) Minimising the time taken to complete a round [+100 for a fast lap, +50 for a medium lap, -50 for a slow lap, -100 for a very slow lap]

(3) Colliding with another vehicle [-200]

(4) Impeding traffic [-50]

(5) Driving too closely to other vehicles [-25]

(6) Overtaking in the wrong lane [-50].

Participants were instructed prior to the start of the task to drive as quickly as possible while avoiding other vehicles, and to remain in the right lane unless overtaking. They were informed of the points scheme and encouraged to maximise their final point count for the full 16 rounds.

Eye tracking and EEG were recorded simultaneously throughout the whole experiment. All events were logged by the simulation, and corresponding markers sent synchronously to the eye tracker (via a CAT cable) and EEG (via a Cedrus StimTracker) hardware such that the time series from these devices could be synchronised with the driving simulation log.

Participants

Participants (N=25, age 19-27, mean 21.08, SD 2.34, 17 female) were recruited from the University of Nottingham and surrounding areas using advertisement posters and email, as well as from the university's participant recruitment system for undergraduate students. Consent was given prior to taking part in the experiment. Ethical approval was gained from the University of Nottingham, School of Psychology Ethics Committee.

Apparatus

The study involved the use of a driving simulation task, a Java-based custom software built of JMonkeyEngine v.3.1 (jmonkeyengine.org) and OpenDS (opends.eu) libraries. XML-based simulation specification allowed customisability of the simulation environment, game parameters and traffic interactions. Monocular eye movements and pupil diameter were measured using an Eyelink ® 1000 eye tracker in remote mode with a sampling rate of 1000Hz, and without the use of a chin rest. BioSemi ActiveTwo electroencephalogram (EEG) with 64 electrodes recorded EEG data while participants were seated in a faraday cage. A SNES™-style USB controller was used to control the driver vehicle. Blender software (blender.org) was used to edit pub buildings (sourced from publicly available online sources such as https://3dwarehouse.sketchup.com) and road-signs within the simulation. PsychoPy 2020.1.0 software (psychopy.org) was used to create the pub-sign recognition memory task. All participants were required to complete an online questionnaire (Appendix 1) to indicate their past and current levels of experience in active video games, city driving and highway driving; from which a composite score was derived (scaled 0-8, 0= virtually no prior driving or gaming experience, 8= substantial driving and gaming experience). Participants were also required to complete an online version of the Zung Anxiety Scale (Zung, 1971) (Appendix 2) prior to the main task to assess anxiety.

Procedure

Prior to the start of the task, all participants were asked to complete an online questionnaire indicating their prior driving and gaming experience, as well as the Zung Anxiety Scale. Participants were fitted in an EEG cap before being informed about the operation of the driver vehicle and the objective of the task: to drive as fast as possible, while remaining vigilant and avoiding collisions or unsafe interactions with other vehicles, in order to gain points. They were seated in a faraday cage approximately 70cm from the computer monitor without the use of head fixation. Calibration and validation of the eye tracker was performed prior to the start of the task using a 9-point calibration grid. If validation was unsuccessful, a participant was excluded from the pupil analysis. Participants then completed the driving simulation task followed by the recognition memory task while simultaneous eye tracking and EEG were recorded.

EEG data was collected simultaneously to the eye tracking data in this study, however, for the purposes of this report, only behavioural and eye tracking data was analysed. Future analyses and investigations into the EEG data will be discussed at the end of this report.

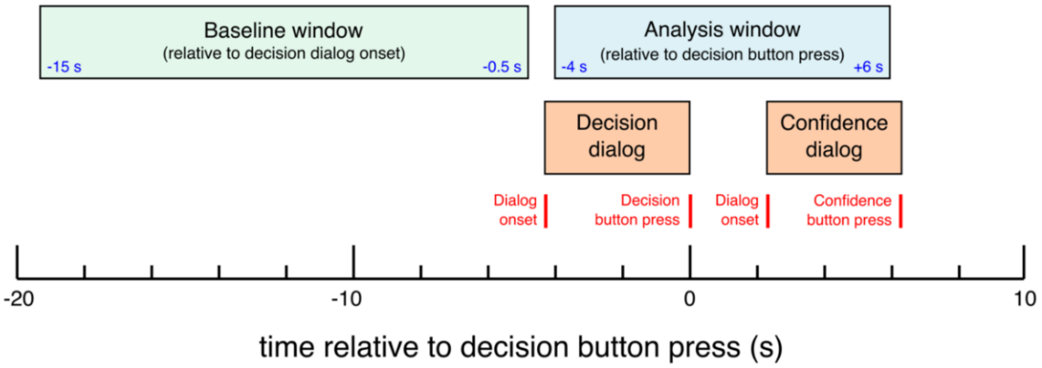
Data Analysis

Pre-processing- eye tracking

Pupil time series were plotted and visually inspected to assess potential signal drop-out, and data was removed from further analysis if this exceeded 20% during driving intervals. Pre-processing consisted of identification of saccade and fixation intervals, removal of eye blinks (blink periods filled by means of linear interpolation), calculation of pupil diameter z-scores within each participant, and synchronisation of the driving log and eye tracking time series. This was conducted with the use of custom MATLAB code, built on Fieldtrip version 20190419 (https://github.com/neurocoglab/opends-matlab).

Road-sign decision task— Pupil diameter was locked to the decision button press for each participant. A baseline window in this task was defined as the period from -15s to -5s relative to the dialogue box onset. Pupil diameter was extracted in a time window from -4s to 6s relative to the decision button press event, for each of the 16 rounds. The mean pupil diameter of the baseline window was subtracted from the windowed pupil diameter, producing a baseline-corrected pupil diameter for each round. See Figure 5 for the time window for analysis.

Figure 5. This figure displays the timeline around the decision button press for the *road-sign decision task*.



Analysis- eye tracking

Pupil diameter was compared across time for the following conditions: (1) decision vs. baseline; (2) correct vs. incorrect; (3) 1-back vs. 2-back; (4) confident vs. non-confident, by testing a null hypothesis of the form H_0: $PD_A = PD_B$, where A and B are the two conditions. A single *t*-test was performed per time point for the 10s analysis window. Cluster-based inference was used to correct for multiple comparisons and control family-wise error, and asked: what is the probability of finding a cluster of *t*-scores this large in our random sample, if there is actually no difference in the population?

Pupil diameter (decision vs. baseline)—Null hypothesis $\mathbf{H_o: PD_{decision} = PD_{baseline}}$ was tested to analyse the comparison of PD during the baseline window and PD during the decision-making window. This decision-making window was around road-sign decisions (road-sign decision task) at the end of each round. $PD_{decision}$ is estimated by averaging across all rounds for each participant, resulting in a grand mean and SD across participants. $PD_{baseline}$ was a 'null distribution' from the baseline window. 10000ms windows were made around 50 random points within the baseline window, and mean PD was computed across these random windows for each round, and the mean across rounds for each participant. Resulting distributions were compared with dependent *t*-tests.

Pupil diameter (correct vs. incorrect)—Null hypothesis $\mathbf{H_0: PD_{correct} = PD_{incorrect}}$ was tested to analyse the comparison of PD during the decision-making window when decisions were correct versus when they were incorrect. Trials were separated into "correct" and "incorrect" decisions, and PD was averaged over those trials. A participant was excluded from this analysis if they have fewer than two trials for each condition.

Pupil diameter (1-back vs. 2-back)—Null hypothesis $\mathbf{H_0: PD_{1\text{-}back} = PD_{2\text{-}back}}$ was tested to analyse the comparison of PD during the decision-making window when place names were encountered on the first overhead road sign (2-back) versus on the second sign (1-back). Trials were separated into "1-back" and "2-back", and PD was averaged over those trials. As before, a participant was excluded from this analysis if they have fewer than two trials for either condition.

Pupil diameter (confident vs. non-confident)—Null hypothesis $\mathbf{H_0: PD_{confident} = PD_{nonconfident}}$ was tested to analyse the comparison of during the decision-making window for confident ("very" or "fairly" sure) and non-confident ("not sure" and "complete guess") decisions. Trials were separated into "confident" and "non-confident", and PD was averaged over those trials. A participant was excluded from this analysis if they have fewer than two trials for either condition.

Box 1. Cluster-based inference.

Pupil diameter data collected in this study was analysed using cluster-based inference. The data here was collected at an extremely large number of time points. This large number of statistical comparisons (5000 in the current study) means that a method is needed to correct for multiple comparisons and to control family-wise error. A non-parametric framework (Maris, 2004; Maris & Oostenveld, 2007) is able to solve the multiple comparisons problem and control the family-wise error rate at the critical alpha-level (0.05). Pupil diameter time series are smooth over time, and therefore, adjacent t-tests are not independent from one another. Instead, we can cluster together adjacent time-samples that all exhibit a similar difference. The signal on the two types of trials is compared for every sample by means of a t-value, and all samples with a t-value larger than a predefined threshold are selected. The selected sample is clustered in connected sets on the basis of temporal adjacency, and the sum of t-values within a cluster is calculated. Each cluster is considered an individual comparison, rather than each single time-point, and so the number of comparisons is greatly reduced. This method tests the probability of finding a cluster of t-scores this large in a random sample, assuming the null hypothesis is true. If the p-value is below the critical alpha-level (p<.05), we are able to reject the null hypothesis for that cluster.

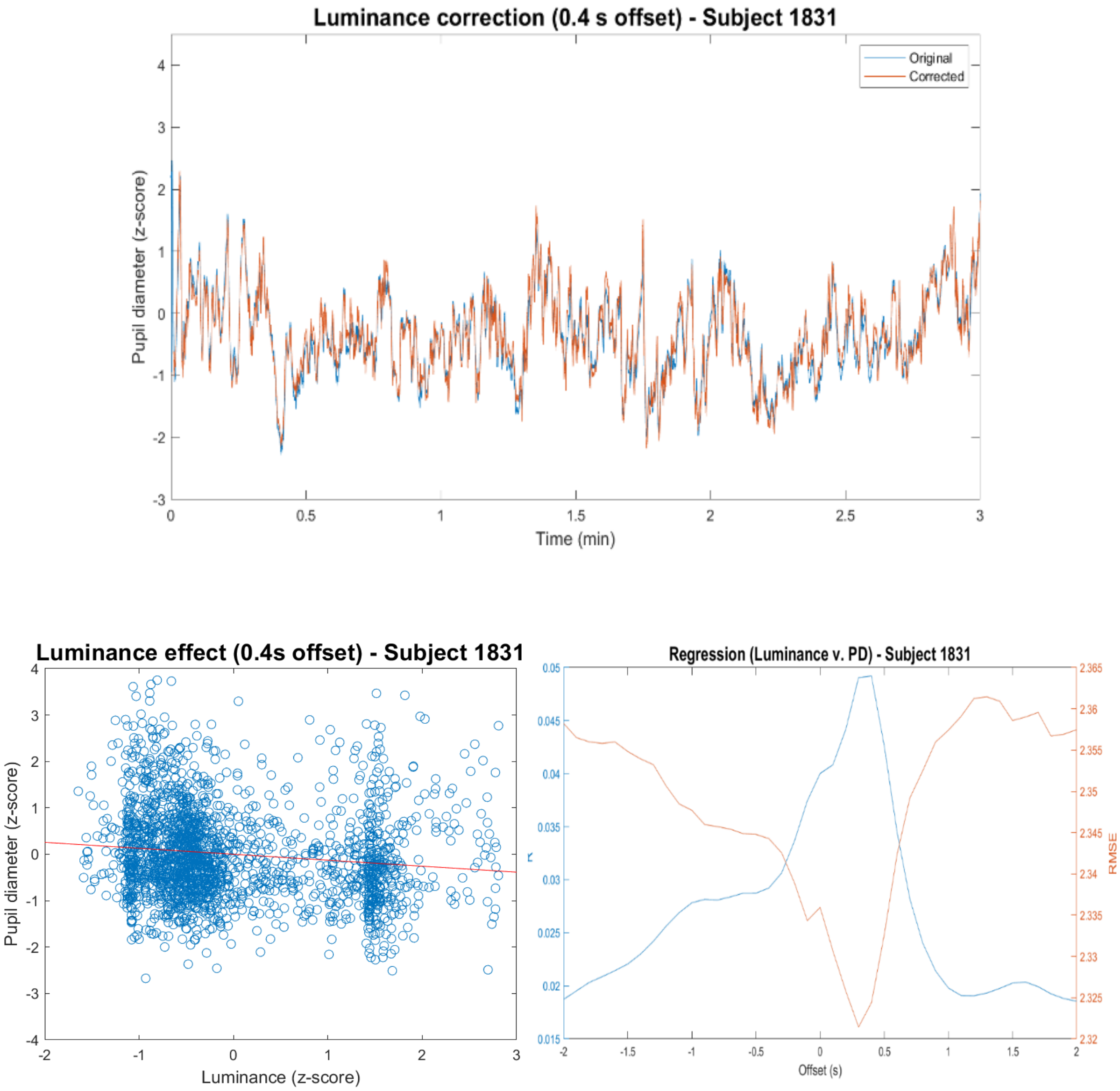
Luminance

Pupil diameter is also driven by luminance, and our stimuli are not equiluminant. It is possible to estimate luminance post hoc from the participants' gaze position and red-green-blue (RGB) values taken at that position, using a circular mask with diameter derived from a visual angle of 10 degrees, assuming an eye-screen distance of 70cm. The formula to estimate luminance (L) at time (t) is:

$$L(t) = 0.2126*R(t)^{2.2} + 0.7152*G(t)^{2.2} + 0.0722*B(t)^{2.2}$$

Where R, G and B are the average red, green and blue pixel values in the mask, respectively, for the screenshot acquired at time t. (International Telecommunication Union Recommendation BT.707, 2015).

Figure 6. Luminance correction in a single participant.



The pupil diameter data displayed in this report are limited by the fact that they are not yet corrected for luminance, however, the above figures show how luminance correction occurs in a single participant. The simulation event log was replayed and synchronised gaze position was used to estimate relative screen luminance, which was then regressed against pupil diameter.

Chapter 3: Online Word Rating Study

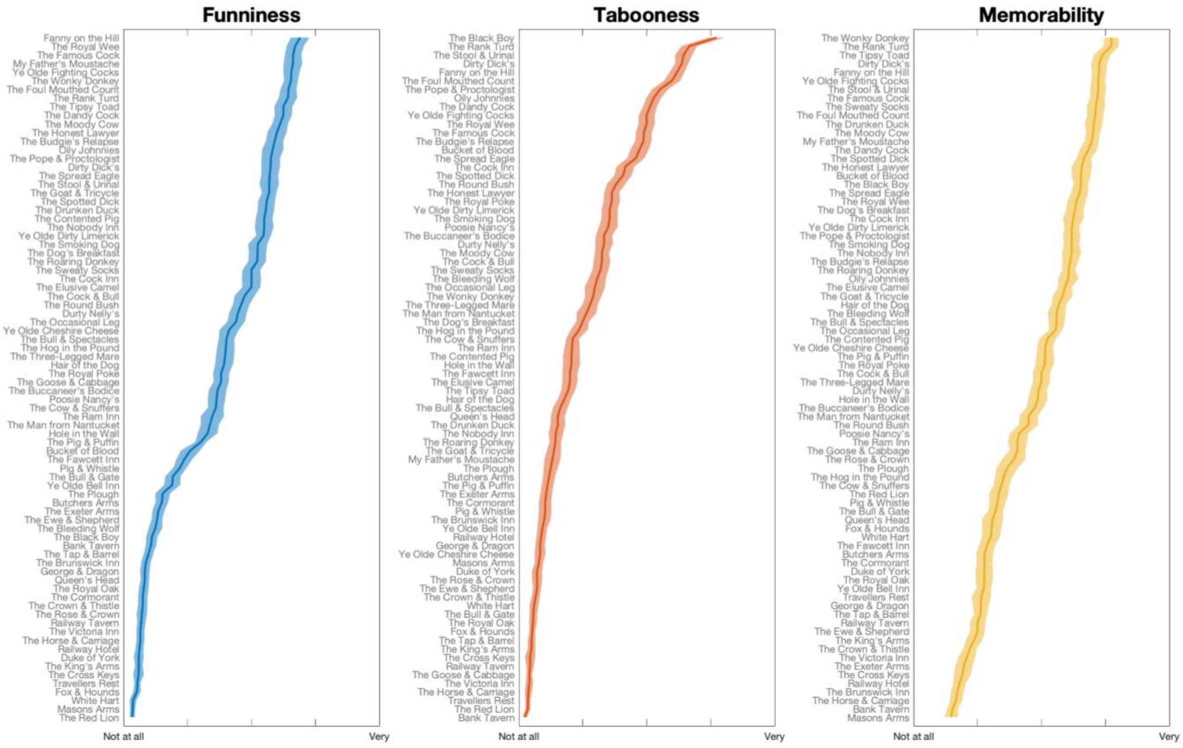
A separate online study was conducted in order to determine the perceived salience of a list of 80 randomly ordered pub sign names for the taboo word/recognition memory section of the task (described above as the *pub-sign task*; list of names in Appendix 3).

Participants were recruited through the Prolific system (https://www.prolific.co/) (n=89, 49 female, 39 male, 1 non-binary; mean age 27, range 18-60). Ethical approval was gained through the University of Nottingham, School of Psychology Ethics Committee. All participants gave informed consent prior to taking part.

Participants were directed to a Qualtrics questionnaire and asked to rank each name on a 5-point Likert scale (0= “Not at all X”, 4= “Very X”), with X being: Taboo-ness; Funniness; Memorability.

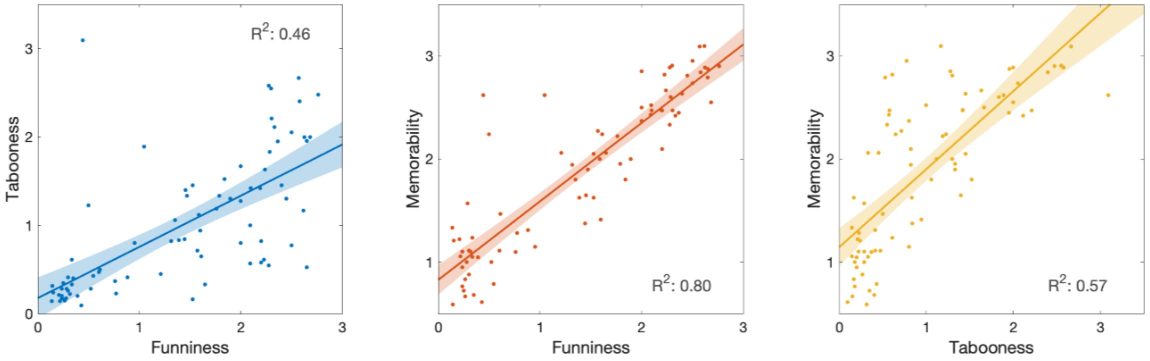
Results

Figure 7. Funniness, taboo-ness and memorability ratings.



Pub names were rated on their perceived ‘funniness’, ‘memorability’ and ‘taboo-ness’. Memorability and funniness ratings were significantly correlated (*r(78)=.834, p<.001*), as were memorability and taboo-ness ratings (*r(78)=.689, p<.001*), and taboo-ness and funniness ratings (*r(78)=.545, p<.001*).

Figure 8. Regression of pub name ratings.



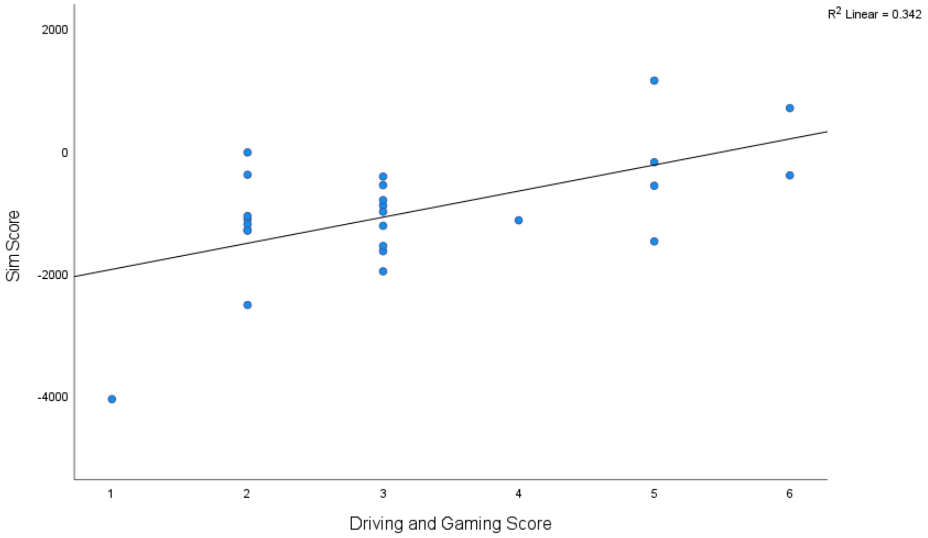
These ratings were averaged to create a ‘mean pub score’ which was used in the analysis of the pub-sign recognition task. The pub names, along with their scores, form the basis of a new set of stimuli suitable for use in a naturalistic paradigm aimed at probing the function of the LC and its influence on memory in an ecologically valid way.

Chapter 4: Behavioural Results

Behavioural Results

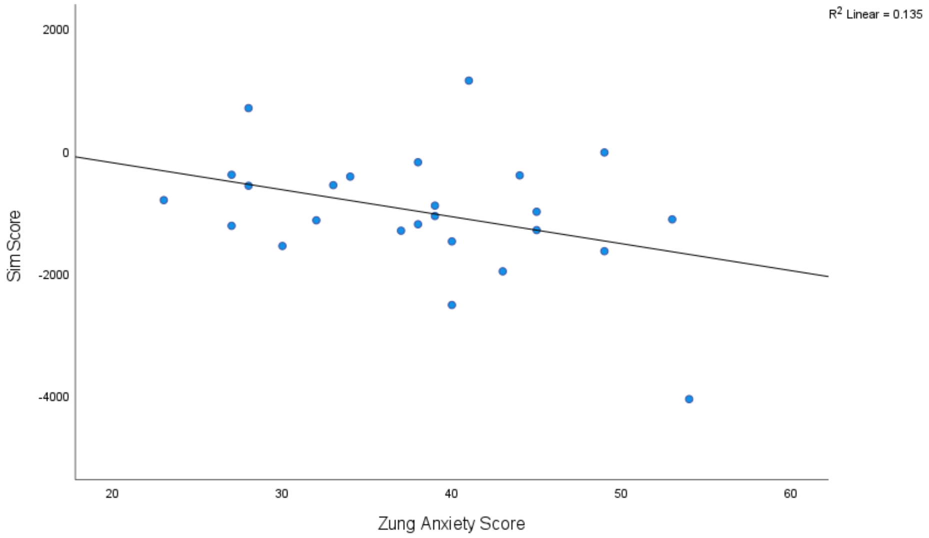
Results of a multiple linear regression indicated that there was a collective significant effect between driving and gaming experience, age, anxiety score, and total simulation score (*R^2=.392, F(3,21)=4.516, p=.014*). The individual predictors indicated that driving and gaming experience (*β=.512, t=2.859, p=.009*, Figure 6) was a significant predictor of total score, and anxiety score (*β=-.231, t=-1.270, p=.218*, Figure 7) and age (*β=-.104, t=-.596, p=.557*, Figure 8) were not.

Figure 9. Composite driving and gaming experience score plotted against total simulation score for all participants.



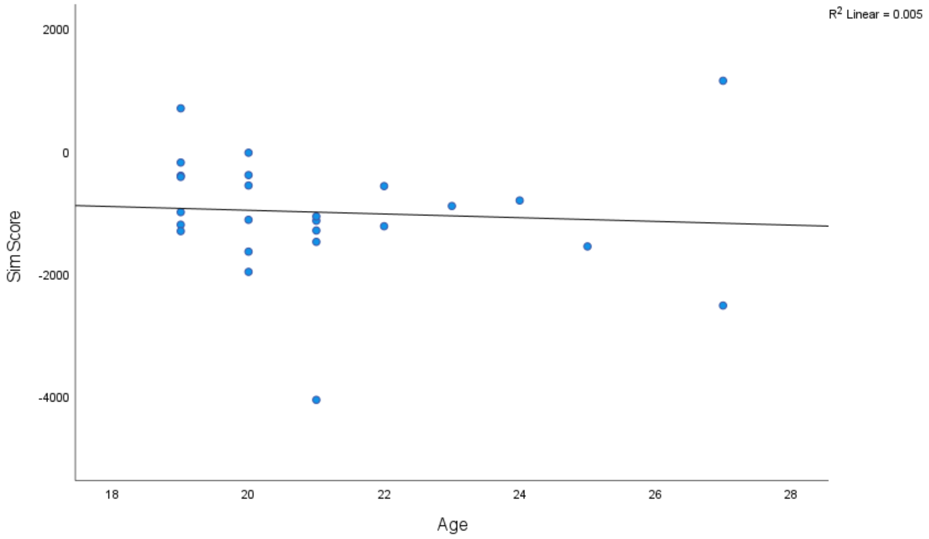
A significant positive correlation is observed. As expected, previous driving and gaming experience strongly predicted total simulation score, providing external validity for the driving simulation, and allowing prior experience to be controlled for in further analyses.

Figure 10. Zung Anxiety Scale score plotted against total driving simulation score for all participants.



Anxiety score was not a significant predictor of simulation score, although a downward trend is observed in the data.

Figure 11. Age plotted against the total driving simulation score for all participants.



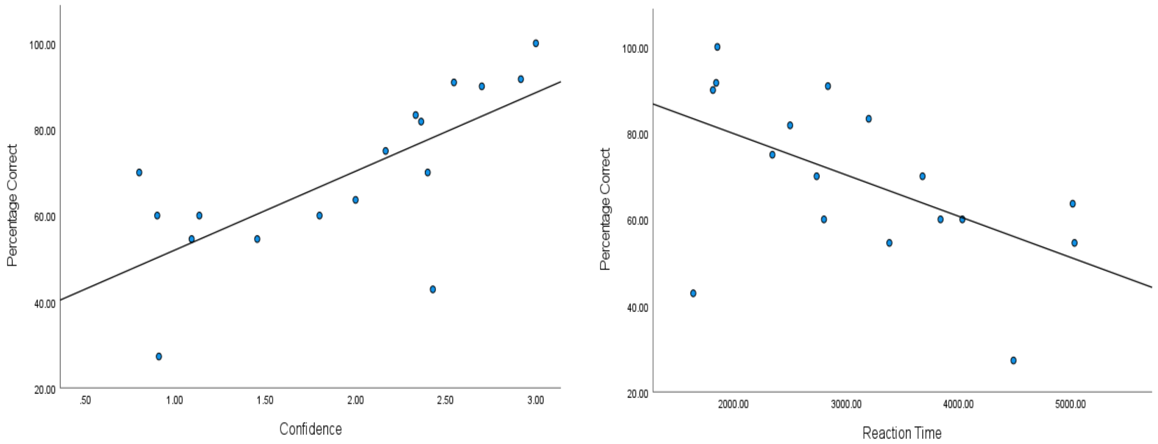
Age was not found to be a significant predictor of simulation score. In this phase of the study, only younger (18-30 years) participants were tested, and so this result was expected.

Road-Sign Decision Task Results

The road-sign decision task required a participant to remember information presented on overhead road signs while driving around the track. At the end of a round, they were asked to make a navigation decision based on the remembered information from either 1- or 2-signs back and rate their confidence in that decision.

Results of paired t-tests revealed that there was a significant difference in confidence ratings relating to correct and incorrect decisions (t(16)=3.946, p<.001), in reaction time for correct and incorrect decisions (t(16)=-1.815, p<.05), and in reaction times for confident and non-confident decisions (t(6)=-2.736, p<.05).

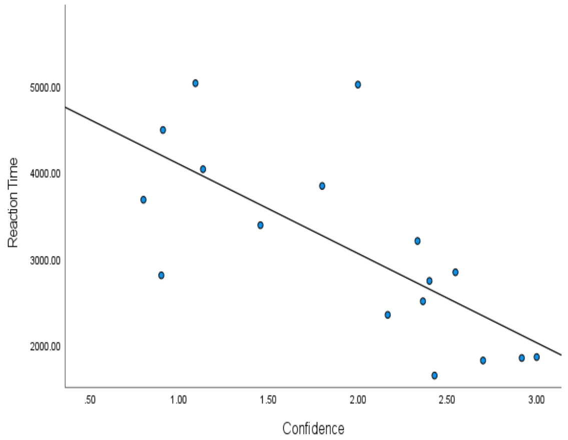
Figure 12. Scatterplots displaying the percentage of correct decisions and confidence ratings (**A**), and the percentage of correct decisions and reaction times (**B**).



A. **B.**

Correct decisions were accompanied by higher confidence ratings (r^2=.470, F(1,15)=15.188, p<.001) and quicker reaction times (r^2=.253, F(1,15)=6.409, p<.05).

Figure 13. Scatterplot displaying confidence ratings and reaction time.



Confident decisions were accompanied by quicker reaction times (r^2=.463, F(1,15)=14.786, p<.05).

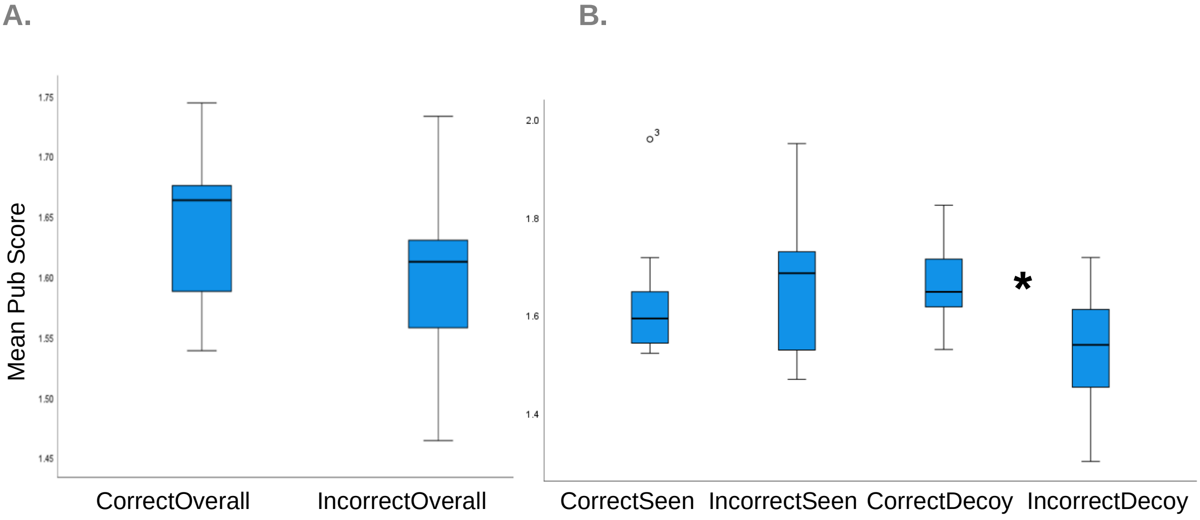
No significant differences were found in paired t-tests between reaction times for 1-back and 2-back decisions (t(16)=.180, p=.430), confidence in 1-back and 2-back decisions (t(16)=.804, p=.216), or the percentage of correct answers for 1-back and 2-back decisions (t(16)=1.373, p=.094).

Pub-Sign Task Results

In the pub-sign task, participants viewed pub buildings with large name signs as they navigated around the driving track. They were not explicitly asked to remember the information but were later asked to complete a forced-choice recognition task including the pub names that were presented in the driving simulation, alongside new unseen names. The separate online study enabled each pub name to be assigned with a ‘mean pub score’ relating to its perceived funniness, memorability and taboo-ness, and this score is utilised in these analyses.

Results of paired t-tests (Figure 14) revealed no significant difference in ‘mean pub score’ for correctly recognised and incorrectly recognised names that were displayed in the simulation (t(12)=-.595, p=.281), or in ‘mean pub score’ for correctly and incorrectly recognised items overall (i.e. both seen and decoy stimuli) (t(12)=1.254, p=.117). A significant difference was observed in the ‘mean pub score’ between correctly and incorrectly recognised decoy stimuli, however (t(12)=2.985, p<.05).

Figure 14. Boxplot displaying the ‘mean pub score’ for correctly and incorrectly recognised pub names (**A**) and separated into seen versus decoy stimuli (**B**).



* = significant difference

Discussion: Behavioural Results

As expected, driving and gaming experience strongly predicted a participant’s score on the driving simulation task. Participants were instructed to drive as fast as possible, while avoiding collisions and unsafe interactions with other vehicles in order to gain points. Completing the driving task with a high score was stressed as the participants’ main goal. This required well-timed lane changes and braking, being aware of the surrounding vehicles and making use of the rear-view mirror, all of which are likely to be second nature to more experienced drivers, enabling them to gain high scores. The driving simulation task was displayed on a normal computer screen, and the driver vehicle was controlled with the use of a SNES™-style 10-button controller. Although the function of each button was fully explained to each participant prior to the start of the task, participants with previous gaming experience were likely more familiar and comfortable with the use of such a controller and therefore able to obtain higher scores on the task. One possible limitation regarding the use of the gaming controller is a possible impact on reaction time in less experienced participants. As the current study recruited participants aged 18-30 years, this is not expected to be a significant issue here, however, further research using this paradigm will aim to recruit older participants (age 55-70 years) and participants with mild cognitive impairment or early-stage Alzheimer’s Disease. The ease of use of the gaming controller for participants unfamiliar with such a device should be considered. Overall, the finding that previous driving and gaming experience predicted a participant’s score on the driving simulation task provides external validity for the paradigm.

In this study, the Zung Anxiety Scale measured a participant’s trait anxiety prior to completing the driving task. High trait anxiety has been suggested to impede an individual’s ability to adjust their updating of outcome expectancies between stable and volatile environments (Browning, Behrens, Jocham, O’Reilly, & Bishop, 2015). Decision-making is guided by our expectations of the outcome, an individual with high trait anxiety may focus disproportionately on potential negative outcomes and have difficulty estimating the likelihood of outcomes when uncertainty is a complicating factor. On the driving task, high anxiety may be expected to result in poor decisions, and therefore poor performance, particularly when there is uncertainty around a decision. In the current dataset (Figure7), anxiety ratings appear to be skewed toward the higher end of the scale (a score of 36 and over suggests the need for further medical assessment of GAD; Zung, 1971). Although statistically non-significant, a downward trend can be seen in the data, and it is possible that a relationship between anxiety and performance on the task may become more clear with the collection of further data and a possible inclusion of more participants from outside the university setting.

Age was not found to be a significant predictor of simulation score (Figure 8). In this phase of the study, only younger (18-30 years) participants were tested, and so this result was somewhat expected. It is possible that these results may change with the collection of more data (possibility of increased driving experience of a 30-year-old compared to an 18-year-old, for example), however, it is expected that the narrow age range of this group will result in a small effect of age on simulation score. Future work may look to include a group of older (55-70 years) participants, and a significant difference between simulation scores for younger and older groups would be expected.

Road-sign decision task

The road-sign decision task involved participants remembering information presented on overhead road signs presented throughout the driving simulation (two signs per round, four possible destinations) and making a navigation decision at the end of each round based on the remembered information. Following the navigation decision, participants were also asked how confident they were in that decision. A significant difference was found in confidence between correct decisions and incorrect decisions, and in reaction time for correct versus incorrect decisions and high versus low confidence decisions. These findings provide support for the task as a successful naturalistic and continuous test of memory and decision-making in the driving simulation. The finding of no significant differences in correct versus incorrect decisions, confidence, or reaction time in relation to the 1-back/2-back aspect of the task may suggest a need to update or rethink this. It may be that there is not a significant difference in cognitive load between 1-back and 2-back information, or that participants were able to make use of strategies for remembering information not taken into account by the design. The serial position effect should also be considered. The current study included only younger (18-30 years) participants, and it is possible that primacy and recency effects worked equally among this sample. Further research using this paradigm will look to recruit older (55-70 years) and cognitively impaired (MCI and early-stage AD) participants and, particularly in the latter group, there is expected to be significant effects of 1-back versus 2-back information on decision-making in this task.

Pub-sign recognition task

In this task a new set of stimuli, which were rated on their perceived ‘funniness’, ‘taboo-ness’ and ‘memorability’ in a separate study, were presented. Previous research has found that taboo items are more effective in capturing a participants’ attention and evoking emotional arousal than positive arousing, negative arousing, and neutral words (Chan, Madan, & Singhal, 2016). Pupil responses are modulated by the amount of attention paid to the stimulus (Johnson, 1993), and the ‘shock value’ of a taboo stimulus is likely to capture that attention.

Performance on the forced-choice recognition task was analysed to establish whether there was a difference in recognition between items rated as more taboo versus neutral items. No significant difference in ‘mean pub score’ for correctly versus incorrectly recognised items suggests that taboo stimuli were not successful in capturing a participant’s attention in the driving simulation. A potential limitation of this task is that participants were not explicitly asked to remember the name signs on the pubs they saw when driving around the track. This decision was made so as to better establish the attention-capturing capability of taboo over neutral stimuli, and to add another layer of ecological validity wherein the LC-NE system was challenged to adaptively respond to multiple inputs. Considering the Adaptive Gain Theory (Aston-Jones & Cohen, 2005), and the fact that participants were not asked to remember this information, it is possible that the name signs encountered in the simulation were filtered out as irrelevant events, unimportant in pursuing optimal performance in the driving task (i.e. exploitation of driving task rather than exploration of alternative sources of reward). In future iterations of this paradigm, it should be considered whether informing participants of the existence of the pub stimuli, but not the salient/neutral nature of the names, would produce differing results. A significant difference in ‘mean pub score’ for correctly versus incorrectly recognised decoy items may suggest that although the taboo stimuli were not able to draw a participants’ attention away from the driving task, they were successful in doing so in the recognition memory section of the task. Further analysis of the pub-sign recognition task could utilise the pupillometry data collected. Assessing pupil diameter at the point of fixation on the stimulus could provide further information on the role of LC-NE in this task. It may be hypothesised that a pupil dilation upon fixation of the stimulus would predict correct recognition later.

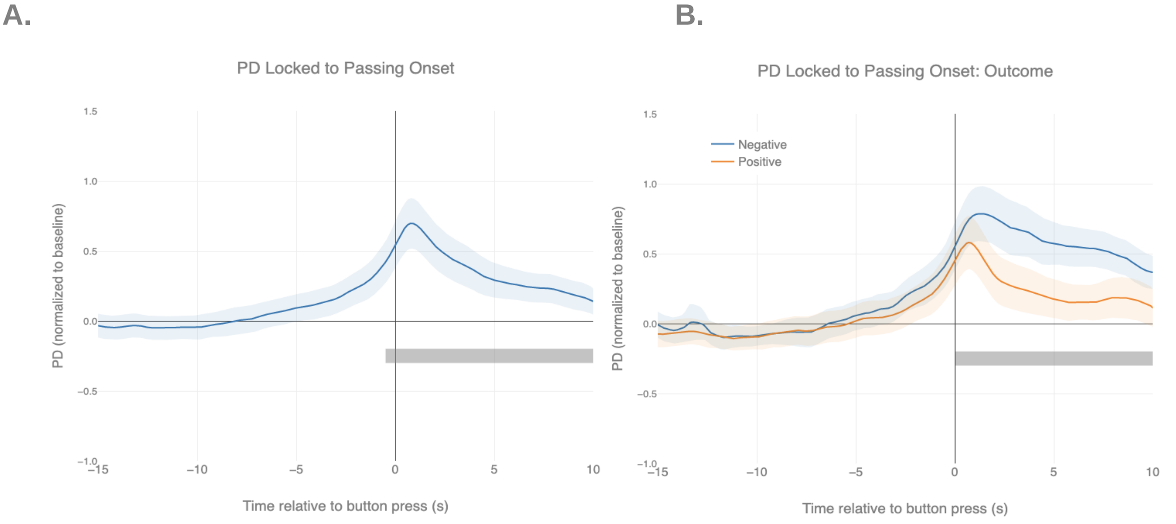
With the use of this embedded recognition task, we aimed to adapt and update more ‘classical’ methods of studying the LC and its effects on memory processes, presenting salient and neutral stimuli in a continuous and naturalistic paradigm.

Chapter 5: Eye Tracking Results

Pupil Diameter

Pupil diameter was analysed in a window around traffic decisions. Traffic decisions included the decision to change lanes (left button press to overtake, *passing onset*, Figure 9; right button press to return to correct lane following overtake, *passing offset*, Figure 10), and the road-sign decision at the end of each round, in which the participant selects a direction (left or right) according to information remembered from overhead road signs in the round (*road-sign decision*, Figure 11). Pupil diameter was also compared between correct and incorrect (Figure 12), 1-back and 2-back (Figure 13), and confident and non-confident (Figure 14) decisions for the road-sign decision task. Fieldtrip (version 20190419, http://www.fieldtriptoolbox.org/) was used to perform a dependent *t*-test at every time point, and cluster-based inference (as described above) was applied to determine significant clusters (p<.05) (Maris & Oostenveld, 2007).

Figure 15. Pupil diameter locked to passing onset (left button-press to initiate overtake) showing a comparison with baseline (**A**) and between positive and negative outcome (**B**), averaged across trials.

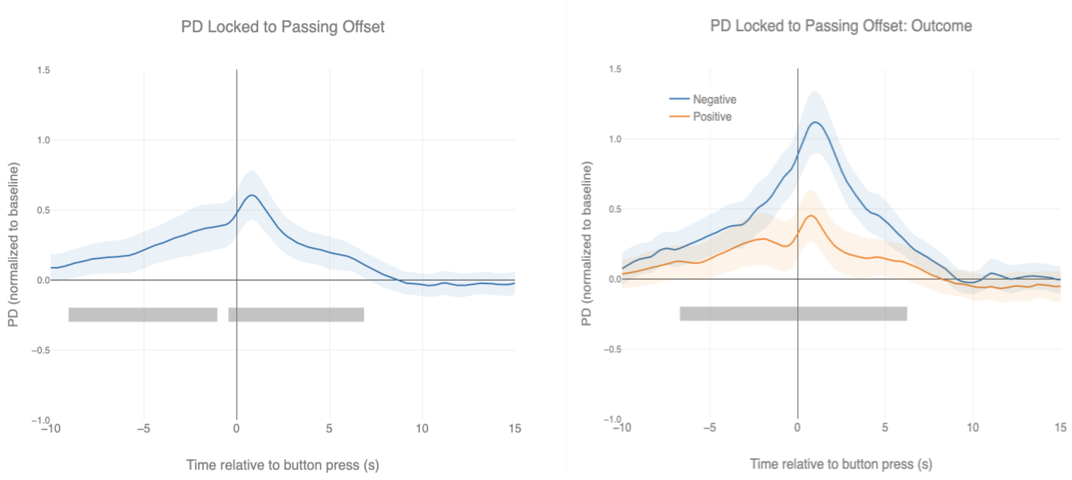


The blue and orange lines represent the mean pupil diameter time series, the shaded area around this line represents the confidence interval (95%). The grey box at the bottom of the figure shows intervals in which the mean pupil diameter is significantly different (A) from the baseline, and (B) between conditions using cluster-based inference (p<.05).

A significant increase in pupil diameter in the seconds prior to the lane change decision, peaking at approximately 1s after the decision button press is seen. In Figure 15A, pupil diameter is significantly larger than baseline between -1s (relative to button press) and the end of the analysis window for this event (+10s). The left button-press to initiate an overtake manoeuvre resulted in a significant pupil response, possibly reflecting the decision-making process. Figure 15B shows a significant difference in pupil diameter between positive and negative outcomes of the overtake manoeuvre. A negative outcome (i.e. a manoeuvre that lost a participant points in the task) resulted in a larger peak pupil diameter and a significantly slower return to baseline than for positive outcomes. The two conditions are significantly different from the time of the decision button press (0s) until the end of the analysis window (+10s).

Figure 16. Pupil diameter locked to passing offset (right button-press to end overtake manoeuvre) showing a comparison with baseline (**A**) and between positive and negative outcomes (**B**), averaged across trials.

A. **B.**

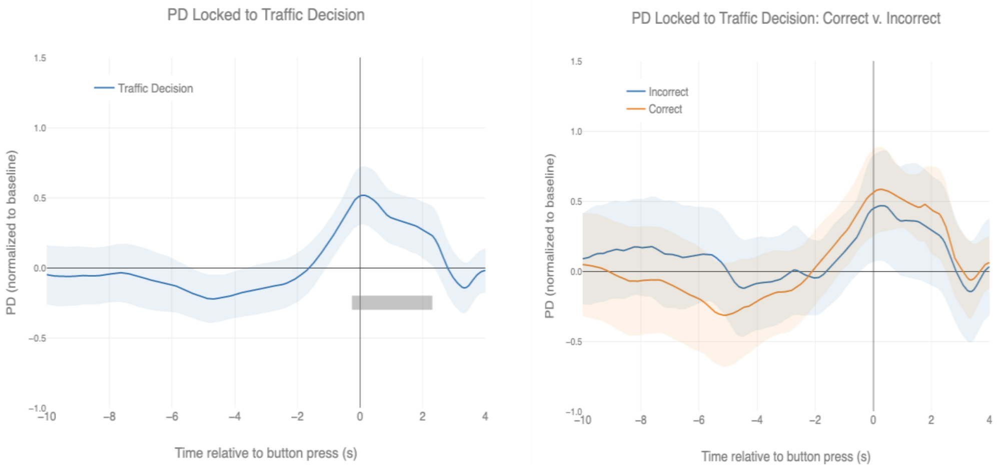


The blue and orange lines represent the mean pupil diameter time series, the shaded area around this line represents the confidence interval (95%). The grey box at the bottom of the figure shows intervals in which the mean pupil diameter is significantly different (A) from the baseline, and (B) between positive and negative outcomes, using cluster-based inference (p<.05).

A significant increase in pupil diameter, again with a peak approximately 1s after the lane change button press is seen. Pupil diameter is significantly larger than baseline from -9s until 6.6s (relative to button press), indicating that the decision to change lane elicited a significant pupil response (Figure 16A). Figure 16B shows a significant difference in pupil diameter between positive and negative outcomes of the overtake manoeuvre between -7s and 6.4s relative to the button press. Negative outcomes lead to significantly larger peak pupil diameters than positive outcomes.

Figure 17. Pupil diameter locked to the road-sign decision (decision button-press) showing a comparison with baseline (**A**) and between correct and incorrect decisions (**B**), averaged across trials.

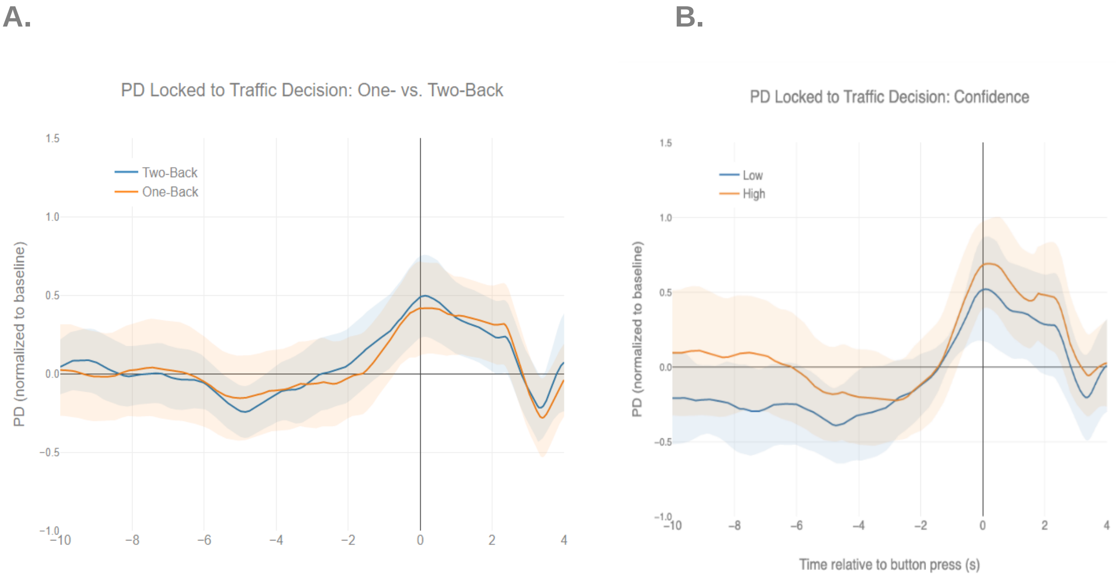
A. **B.**



The blue and orange lines represent the mean pupil diameter time series, the shaded area around the lines represent the confidence interval (95%), and the grey box at the bottom of the figure shows intervals in which the mean pupil diameter is significantly different (A) from the baseline, and (B) between correct and incorrect decisions, using cluster-based inference (p<.05).

Pupil diameter increases significantly in the seconds before the decision is made and peaks around the decision button press, before decreasing more slowly in the seconds following the decision. Pupil diameter is significantly larger than baseline between -0.5s and 2.4s (relative to button press). The road sign decision elicited a significant pupil response (Figure 17A), although the shape of this response is different from that of the overtake decisions. Figure 17B shows a very similar pupil response for correct and incorrect decisions with no intervals in which the two conditions are significantly different from one another. A significant increase in pupil diameter, with a peak shortly following the decision button press, is seen for both correct and incorrect decisions.

Figure 18. Pupil diameter locked to the road-sign decision, showing comparisons between 1-back and 2-back decisions (**A**) and high and low confidence decisions (**B**), averaged across trials.

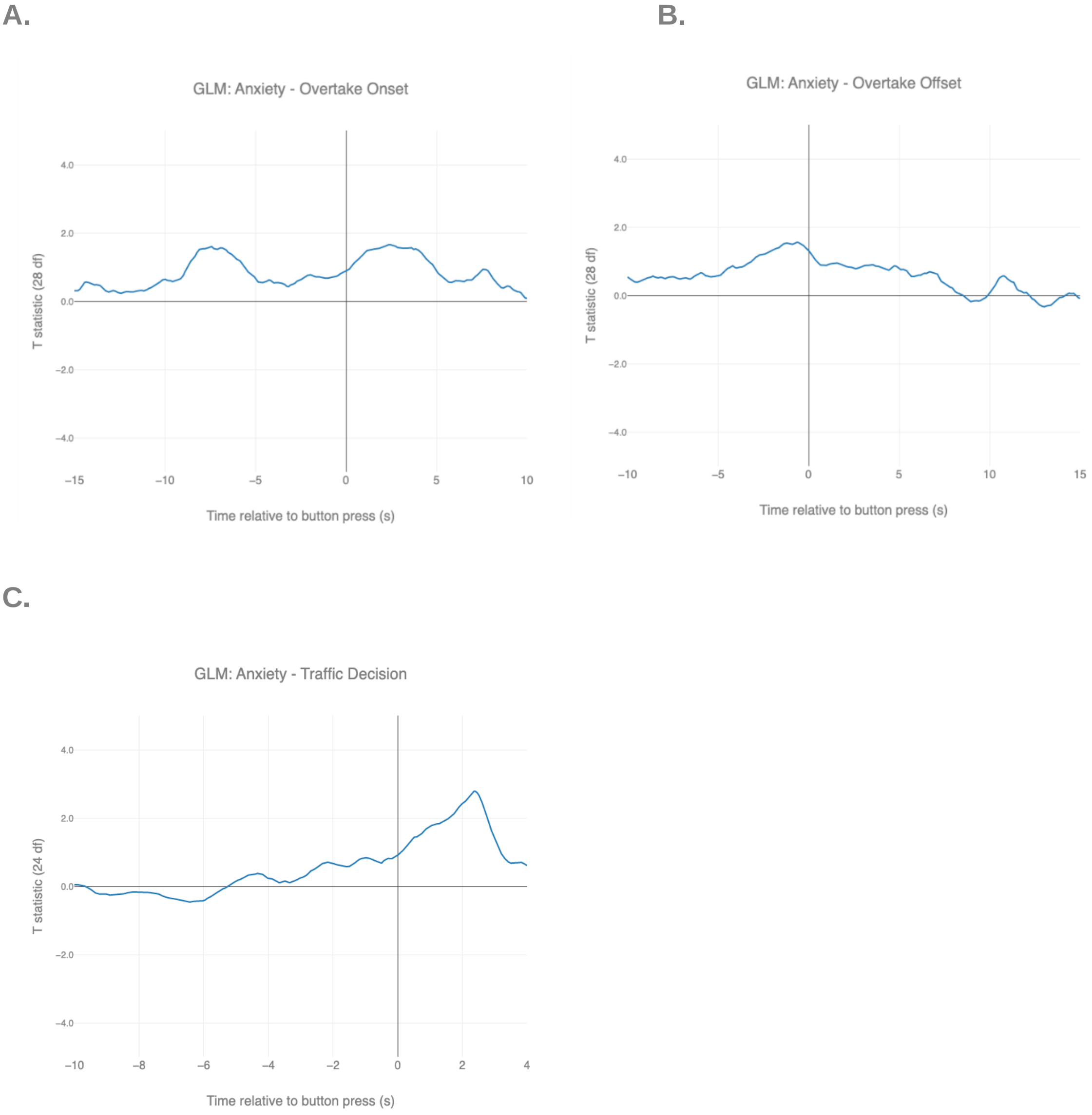


The blue and orange lines represent the mean pupil diameter time series, the shaded area around the lines represent the confidence interval (95%). No grey shaded box at the bottom of the figure indicates that there are no intervals in which the mean pupil diameter was significantly different between the conditions, using cluster-based inference (p<.05).

A similar pupil response between 1-back and 2-back decisions, and high and low confidence, with no intervals in which the two conditions are significantly different from one another is observed. Although both figures show no significant difference in pupil response between conditions, there are intervals for all conditions in which the pupil diameter is significantly different from baseline.

A generalised linear model (GLM) was used to assess pupil diameter in relation to anxiety score on overtake manoeuvres (passing onset/passing offset) and the navigation decision event.

Figure 19. GLM of anxiety for overtake onset (**A**), overtake offset (**B**), and navigation decision (**C**).

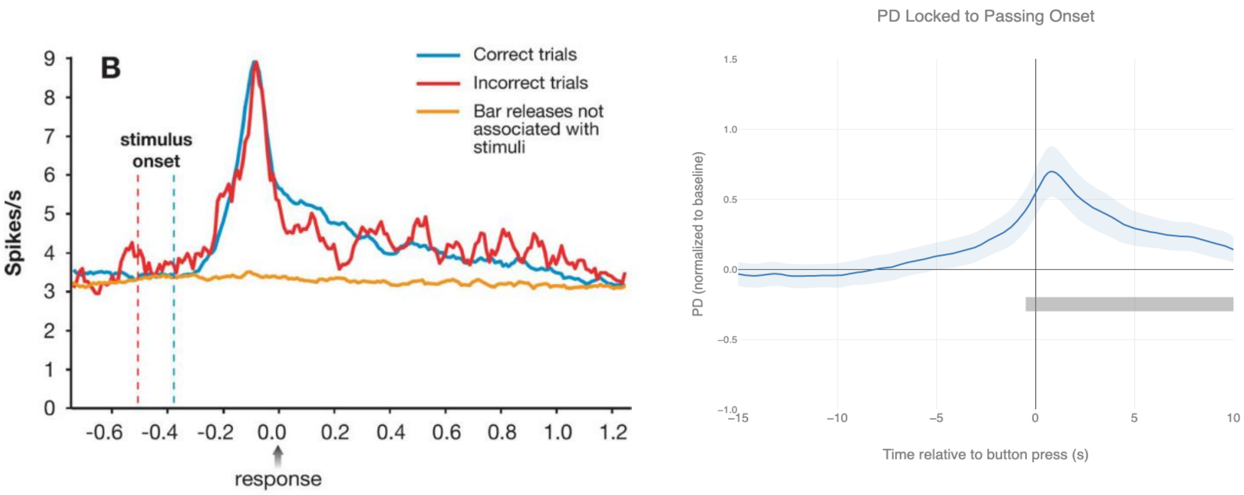


These figures show the t-statistic for a relationship between anxiety (as assessed by the Zung Anxiety Scale) and pupil diameter at every time point surrounding decision-making events. At no time point is this relationship significant, both with and without cluster correction.

Discussion: Pupil Diameter Results

Overtake manoeuvres in the driving task (passing onset; passing offset) elicited a significant pupil dilation, with peak pupil diameter shortly following the lane change button press. Previous research has shown that pupil diameter changes in line with activity of the LC and levels of NE in the brain (for example, Lowenstein & Loewenfeld, 1964; Ishigaki, Miyao, & Ishihara, 1991; Bradley, Miccoli, Escrig, & Lang, 2008; Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014), and it is likely that the observed pupil dilation is reflecting the decision-making process to change lane in order to avoid a collision. The figures above show a *gradual* increase in pupil diameter as a cognitively demanding situation approaches, with a peak shortly after that demand has passed, and then a return to baseline. This novel finding is made possible by the continuous nature of the driving simulation. Previous studies based on trial-based designs have assumed a phasic LC response to a stimulus (for example Aston-Jones & Bloom,1981; Murphy et al, 2011) and given less consideration to the longer-term time-course of neural activity and its correlation with behaviour. Given the adaptive nature of the LC and its influence on learning and memory, studying LC activity over longer timescales in a continuous task is likely to capture that information typically written off as unexplained variance or “noise”, creating a more informative, broadband picture of neural activity and its relation to behaviour in a naturalistic setting. Huk et al (2018) provide a convincing argument in favour of quantitative neuroscience experiments utilising a naturalistic and continuous approach, to enhance our understanding without losing quantitative rigor.

Figure 20. LC activity in macaques, from Aston-Jones & Cohen (2005) (**A**). Pupil response to overtake manoeuvre in our driving paradigm (**B**).


A.

B.

The above figures show LC activity during a 2-alternative forced-choice flanker task in macaques (Aston-Jones & Cohen, 2005). A similar pupil response can be seen in our data relating to overtaking manoeuvres in the driving simulation paradigm, although we show a more gradual increase in pupil diameter prior to the stimulus onset due to the more continuous nature of the task, and as participants are anticipating an event. This is consistent with Adaptive Gain Theory as it demonstrates a selective increase in phasic LC firing around moments of high long-term utility (i.e., safely overtaking a vehicle), which returns to baseline immediately after the decision has been made.

The pupil dilation response was significantly different depending on the outcome of overtake the decision. This suggests the LC is monitoring ongoing task demands *and* responding to errors. Adaptive Gain Theory proposes two modes of LC-NE function according to levels of task utility. With high utility phasic activation facilitates exploitation of the task, and with low utility tonic mode favours exploration of other sources of reward (Aston-Jones & Cohen, 2005). Further to this, it has also been suggested that LC plays a role in error monitoring, adaptively adjusting control state when errors are made (for example Gilzenrat et al, 2010). Gilzenrat et al (2010) present pupillometry results consistent with the influence of task utility on control state, as predicted by the Adaptive Gain Theory. In conditions when the amount of both conflict and reward were low, and when both conflict and reward were high, smaller baseline pupil diameters and larger dilations suggested that LC phasic mode promoted task engagement in conditions in which the additional effort put into the task was rewarded. In conditions of protracted conflict and low reward, larger baseline pupil diameters and smaller dilations suggested that LC tonic mode promoted disengagement from the task, hence an adaptive reduction in the recruitment of control in conditions in which effort was not rewarded.

In relation to the road-sign decision task, we found significant pupil dilations around the decision events, although these did not appear to be modulated by either correctness or confidence. During traffic manoeuvres, a difference in pupil diameter was found between positive and negative outcomes, but not between correct and incorrect decisions on this task. It is possible that, when completing driving manoeuvres, it was immediately obvious to participants that they had made an incorrect decision as this resulted in a collision (i.e. an obvious negative outcome). In the road-sign task, a participant does not know whether they have made a correct choice as there is no feedback on this, they were only asked to rate their confidence in the decision. This may relate to the Gilzenrat et al (2010) paper discussed above, where feedback was immediate and pupil changes in subsequent trials depended on this feedback. Points are earned for successful driving manoeuvres and deducted for unsafe vehicle interactions, and therefore it may be this main task that participants were exploiting. A decision was required at the end of each of the 16 rounds of the driving simulation, however participants did not receive any feedback as to whether their ongoing effort was paying off. This may have resulted in disengagement from the road-sign decision task, favouring exploitation of the driving aspect in which they earned points. In future experiments utilising this paradigm, we suggest the addition of earning points for a correct decision and deducting points for an incorrect decision to promote engagement with this task. The different temporal patterns between the decision to overtake a vehicle and the navigation decision at the end of each round should also be considered. In an overtake manoeuvre, a participant is anticipating a gradually approaching cognitive demand (i.e., an approaching vehicle), and here we see a significant difference in pupil dilation response between outcomes. The navigation decision is more comparable to a trial-based design, whereby a participant is required to quickly respond to a stimulus (i.e., a pop-up dialog box), and no difference in pupil dilation response was seen here between conditions. A future modification of this task could look to increase the naturalistic and continuous nature further. Instead of a pop-up dialog box requiring a response, the participant could encounter an overhead sign reading ‘Go to X’ (with X referring to a previously seen destination), followed by a fork in the road. This would require the participant to make an actual, naturalistic navigation decision, as well as providing the anticipation of an upcoming cognitively demanding situation.

We did not observe a significant difference in pupil response for confident versus non-confident decisions. Here, the pupil data is averaged across trials and the means are compared. It is possible that there may be an effect of confidence, however, this analysis has not fully teased it apart. Due to the design of the study, data from only 17 participants was analysed here, and it is possible that an effect may become more apparent as additional data is collected. It may also be that alternate analyses would better investigate this possible effect. As this research has not been conducted before, and expectations are not based on similar research, multiple tests may need to be investigated in order to establish the most appropriate test for the current data. Rather than comparing means averaged across trials, future analyses may instead look at the *dilation slope*, the steepness of the increase in pupil dilation (as in Naber & Marburg, 2017, for example). This method allows for the use of more powerful, parametric tests to compare the pupil dilation slope for each trial. Non-confident decisions may be marked by a shallower slope, with a slower rise signalling more uncertainty in the decision.

Although the decision-making event elicited significant pupil dilations, we found a similar pupil response for both 1- and 2-back decisions. This increase from baseline may signal the decision-making process or retrieval from memory but does not appear to differ between conditions. Further analysis of the behavioural data may indicate an effect of confidence or accuracy here. It is also possible that there is not a strong difference in cognitive load between the 1-back and 2-back decisions, as discussed in the discussion of behavioural results above, and indicated by pupillometry results here.

Further investigation of the road-sign decision task data could look to identify the point at which the original stimulus was perceived to establish whether pupil dilation at encoding predicts subsequent memory for that item. Previous research has found that pupil diameter at encoding can predict recognition memory strength (Papesh, Goldinger, & Hout, 2012). Enlarged pupils signalled greater effort during encoding of subsequently recognised items, with strong memories resulting in the highest peak pupil diameters. We would expect to find that a strong pupillary response at the moment a navigation cue is perceived would predict whether a correct choice is made later in the task.

No effect of anxiety was found on pupil diameter in the time points surrounding decision-making events. Browning, Behrens, Jocham, O'Reilly, & Bishop (2015) found evidence that high trait anxiety impeded adjusting of learning rates in uncertain environments, with pupil diameter found to track this. Taking this into account, we expected to see an effect of anxiety here. However, the conditions of the driving simulation paradigm were not as punitive as electrical shocks, only consisting of earning and losing points. Perhaps this did not create such environmental volatility as displayed in the Browning et al (2015) paper. It must also be mentioned that no participants in this study were assessed as having clinically relevant anxiety, as tested by the Zung Anxiety Scale, although the distribution of anxiety scores was toward the higher end of the scale. It could be that an effect of anxiety may be seen in participants with a diagnosed anxiety disorder, however, it was not the aim of this study to investigate this but mainly to control for potential effects of anxiety on outcomes, as a link between anxiety and LC-NE function has been reported.

Chapter 6: General Discussion and Conclusions

The current study aimed to assess the ability of the driving simulation paradigm in probing the function of the LC-NE system under conditions requiring memory and decision-making. The simultaneous recording of monocular eye movement and pupil diameter data aimed to provide further insight into the functioning of the LC-NE system throughout.

The main findings can be summarised as follows. Significant pupil dilation responses to decision-making events support a role of the LC-NE, and the ability of this paradigm to examine its function. The novel finding that as a participant approached a cognitively demanding situation, the pupil gradually dilated, peaked after the demand had passed, and then returned to baseline was made possible by our continuous design. Evidence of LC role in monitoring task demands *and* responding to errors is provided by significantly different pupil dilation responses to positive and negative outcomes during traffic manoeuvres, with results in line with the Adaptive Gain Theory (Aston-Jones & Cohen, 2005) of LC-NE function.

The road-sign decision-making task and the pub-sign recognition memory task were tested as new ways of investigating the relationship between pupil dilation, LC-NE function, and memory encoding and retrieval. Our finding of a significant pupil dilatory response around decision events supports the hypothesis that phasic LC activity accompanies memory retrieval. No significant difference in pupil dilatory response between conditions (correct/incorrect, confidence, 1-/2-back decisions) suggests that information about these properties are not conveyed here. This lack of effect may be due to a lack of reward. Reward, or lack thereof, appeared to modulate the LC response in the Gilzenrat et al (2010) paper. Without feedback to reassure participants' that their ongoing effort is paying off, we see no effect. With regard to the driving aspect of the paradigm, during overtake events, there *is* intrinsic feedback—both in the perceived danger of close proximity to other vehicles and in the score penalty displayed on the screen—and it is during these events that we see significant pupil dilatory responses. This suggests a future modification of the embedded tasks should include the addition of points and penalties for performance.

The driving simulation paradigm is proposed as a new approach to combine quantitative methods with a naturalistic and continuous approach. Nastase et al (2020) argue that classical experimental designs are reductionist, with little relevance outside of the laboratory. This paper advocates for observational research methods in order to gain insight into how the brain functions in the real world. However, with little to no experimental control, it is not possible to test hypotheses using these methods. The argument between ecological generalisability and experimental control should be seen as a spectrum and it is our opinion that a balance between both is optimal. Matusz et al (2019) acknowledge that simple paradigms have been an important building block in improving our knowledge of the brain, however, ever more complex research questions would be more effectively pursued with new methods capable of capturing a broader picture of cognition. Traditional methods include short trials aimed at investigating quick phenomena (i.e. reactions rather than deliberations). As brain activity is ongoing, each trial cannot be completely independent, even considering inter-trial intervals, and this is evidenced by trial-to-trial correlations and trial-to-trial serial dependence (Huk, Bonnen & He, 2018). Naturalistic paradigms not only improve ecological validity, but also allow slow processes to be characterised rather than discarded as noise. Adaptive Gain Theory (Aston-Jones & Cohen, 2005) states that the LC-NE system contributes to the optimisation of behavioural performance by monitoring task-related utility and adapting the mode of activation to either facilitate task-performance (phasic mode, exploitation) or disengage from the task and search for alternate sources of reward (tonic mode, exploration). Considering the LC-NE is a modulatory system, it is likely that it functions across longer timescales. Adaptations to ongoing environmental stimuli will therefore be more effectively captured in a continuous and naturalistic scenario.

One limitation to our approach is the relatively small number of events compared to trial-based designs. This is especially true for the navigation decision events (16), and is inherent in the design and not easily increased. It is possible that the continuous and naturalistic design of this paradigm may help to reduce the number of trials required to attain a comparable level of statistical power as trial-based designs, however it does prove difficult to interpret a negative finding, as we may just lack the power to find an effect. The luminance confound is another limitation to the results presented here. A strategy is in place to control for this, however, due to technical difficulties this has not yet been implemented. Based on initial observations using the proposed correction, luminance is not expected to substantially change the results.

This is a first step into developing this paradigm and although there are limitations and negative findings, these all inform future iterations of this task. Overall, the driving simulation paradigm was successful in probing the function of the LC-NE system during completion of a complex task requiring attention, memory, and decision-making.

Chapter 7: Future Research

This chapter covers further analyses of data collected in the current study, as well as future directions for research using the continuous, naturalistic driving simulation task.

LC, Ageing and AD

The LC-NE system is thought to be key in determining cognitive abilities in late life. Both the structural integrity of the LC (Takahashi et al., 2015) and levels of NE throughout the brain (Kaddurah-Daouk et al., 2011) have been associated with cognitive function, ability and age-related decline. The LC is particularly vulnerable to toxins and infections due to its position on the lateral edge of the fourth ventricle, high exposure to blood flow, and long, unmyelinated axons. LC degeneration is highly correlated with cognitive decline, amyloid-beta and neurofibrillary tangles (Nelson et al., 2012). Hyperphosphorylated tau in the LC is among the first detectable signs of AD neuropathology (Chalermpalanupap, Weinshenker, & Rorabaugh, 2017) and research has supported the idea that the LC is central to both the development and the expression of AD (Mravec et al., 2014). AD-related pathology often appears first in the LC, with tau pathology evident in some people by their mid-twenties (Braak et al., 2011). Although AD-related pathology is evident in most people at a relatively early age, the process remains unclear. If tau pathology contributes to AD, it progresses extremely slowly before crossing the threshold to clinically recognisable AD. Much of the previous research into the relationship between the LC and AD has focused on pathological changes, however there is also evidence of significant degeneration of the LC, along with other brainstem nuclei, in AD (Zarow, Lyness, Mortimer, & Chui, 2003). Density of monoaminergic neurons in the LC post-mortem have been found to have a significant relationship with cognitive decline in the years preceding death, unlike neurons in the dorsal raphe nucleus, substantia nigra and ventral tegmental area (Wilson et al., 2013). These findings strongly suggest a role for the LC in cognitive function in late life.

NE released from the LC has been shown to protect the brain from factors that may accelerate AD, such as inflammation, excitotoxicity and aggregated β-amyloid (Mravec et al., 2014; Feinstein et al., 2002; Heneka et al., 2010). NE supplied to the hippocampus was found to reverse age-related deficits in long-term potentiation (LTP) and memory in aged rats (Mei et al., 2015), and increased NE levels in older mice improved spatial memory performance (Kalinin et al., 2012; Kummer et al., 2014). However, evidence suggests that AD not only involves spreading neuropathology, but simultaneous reduction in the ability of the brain to combat threats. AD patients have lower NE levels than do age-matched controls in both brain tissue and ventricular fluid (Adolfsson, Gottfries, Roos, & Winblad, 1979; Kaddurah-Daouk et al., 2011). NE stimulates microglial cells involved in clearing β-amyloid (Heneka et al., 2010) and inhibits amyloid-induced oxidative stress, mitochondrial depolarisation and caspase activation (Counts & Mufson, 2010). AD is able to remove the brain's defence mechanism against β-amyloid, potentially contributing further to AD pathogenesis.

The numerous neurobiological and neurochemical changes that occur with advancing age can also help explain cognitive changes in ageing. Maintaining the integrity of the LC in the ageing brain may also help to maintain cognition. NE is not only important in protecting the brain against age-related threats, but also in modulating cognitive processes such as working memory and inhibiting irrelevant information, as well as facilitating attention and behavioural flexibility. Impairments in the LC-NE system are therefore likely to disrupt these processes.

Decision-making and working memory are two functions known to be impaired in advanced ageing and AD. A comparison of young and middle-aged participants has found underlying age-related differences in working memory (Wild-Wall, Falkenstein, & Gajewski, 2011). Young participants showed increased neuronal activity prior to a successfully detected target under high working memory load, whereas middle-aged participants displayed the same neuronal pattern regardless of whether the subsequently presented target will be detected or missed. Consistent, but more pronounced deficits in working memory have been observed in AD patients (Baddeley, Bressi, Della Sala, Logie, & Spinnler, 1991). Tasks involving decision-making have found that older, cognitively normal, adults show impaired performance compared to younger adults. In an Iowa Gambling Task, older participants failed to shift their selections toward advantageous outcomes (Denburg et al., 2007). A similar task revealed that participants with early AD shifted more frequently than cognitively normal older participants between safe and risky outcomes, indicating random decision-making and a lack of overall strategy. These measures have been successful in detecting differences between healthy ageing and early AD.

Average pupil diameter decreases linearly with age (Winn, Whitaker, Elliott, & Phillips, 1994; Pfeifer et al., 1983) and evidence suggests this decrease is not due to impaired biomechanics of the eye. An α-noradrenergic α1-agonist phenylephrine, administered as eye drops, was able to dilate the pupils of younger and older adults to approximately the same diameter (Korczyn, Laor, & Nemet, 1976). It has been suggested that pupillary responses may also be used as a biomarker for early risk for AD. Pupil diameter has been linked to the activity of the LC, and LC pathology is evident in the earliest stages of AD. Pupil dilation during performance of cognitive tasks has been shown to reflect cognitive effort, however task performance declines with AD. Compensatory effort may be increased in early stages and reduced as the disease progresses, and it is likely that this is reflected by pupil diameter. Differences in pupil diameter have been found on a digit-span task between cognitively normal participants, single- and multi-domain MCI participants (Granholm et al., 2017). Pupil responses were able to differentiate cognitively normal participants and those with MCI, with reduced phasic dilation responses to increased cognitive load in participant with MCI. Further evidence suggests that early damage to LC results in persistent high tonic LC activity at rest and a disruption of task-related phasic activity during task performance, and that this is reflected in pupillary responses (Elman et al., 2017). These results provide strong evidence that pupillary responses can reflect early dysfunction of the LC and may be utilised as a biomarker for early AD risk.

Relevance of hippocampus and entorhinal cortex in ageing and AD

The hippocampus plays a pivotal role in memory, learning and spatial navigation (Bird & Burgess, 2008; Miller et al, 2014). This region undergoes significant structural changes with ageing and is one of the earliest sites affected in AD. Loss of hippocampal volume has been extensively documented in AD, with studies such as Josephs et al (2017) providing evidence of this relationship. The accumulation of amyloid-beta and tau proteins in the hippocampus, as discussed by Chu (2012), contributes to its structural and functional deterioration. Atrophy in the medial temporal lobe, including the hippocampal regions, serves as a structural marker of AD, observable through magnetic resonance imaging (MRI). Furthermore, a correlation between age-related decline in hippocampal-dependent learning and memory and reduced hippocampal volume has been observed in female rats (Driscoll et al, 2006). This decline is accompanied by diminished synaptic plasticity and neurogenesis, which are critical for maintaining cognitive function in ageing. Hippocampal theta oscillations, which are associated with memory processing and spatial navigation, are also disrupted in these conditions.

The entorhinal cortex (EC) is another critical region implicated in AD. as noted by Knierim (2015), the EC is often the first site where amyloid plaques and neurofibrillary tangles appear in AD patients. During the early stages of the disease, tau protein accumulates in the EC before spreading to the hippocampus (Asai et al, 2020). Functionally, the EC serves as the primary interface between the hippocampus and the neocortex, receiving input from the neocortex and transmitting processed information to the hippocampus (Muñoz & Insausti, 2005). Located in the medial temporal lobe, the EC is a key component of the memory system and also plays a role in navigation and time perception. It contains specialised neurons called grid cells, which fire at regular intervals to help animals understand their position in space. AD-related damage to the EC disrupts the function of grid cells, impairing spatial navigation and memory.

Prokopiou et al (2023) have reported that lower novelty-related LC activity is associated with greater tau deposition in medial and lateral temporal lobe regions, and with steeper memory decline in older individuals. Findings suggest an LC-medial temporal lobe pathway that is particularly vulnerable to the progression of tau, and associated with downstream cognitive decline, particularly in individuals with elevated amyloid-beta deposition.

LC, P3 ERP and Theta Oscillations

The P3 event-related potential (ERP) is a positive, large-amplitude potential with a peak latency of around 300 to 400 ms following stimulus presentation and is thought to be elicited by the decision-making process (Nieuwenhuis, Aston-Jones, & Cohen, 2005). Frequently used to study the P3 is the *oddball task*, in which a participant is required to respond to (or passively attend to in the case of many animal studies) infrequent target stimuli, presented in a sequence of more frequent non-target stimuli. An inverse relationship, best described by the classic Yerkes-Dodson inverted-U curve (Yerkes & Dodson, 1908), has been identified between P3 amplitude and the probability of the rare event in the task sequence (Murphy et al., 2011). P3 amplitude is also sensitive to local probabilities- a larger P3 is elicited to a target stimulus that is preceded by non-target stimuli than when preceded by other target stimuli (Duncan-Johnson & Donchin, 1982); and emotionally valent stimuli, both positive and negative, elicit a larger P3 response than emotionally neutral stimuli (Keil A. et al., 2002). These effects are modulated by the amount of attention paid to the stimulus (Johnson, 1993). A reliable P3 is only elicited by stimuli that is attended to. These antecedent conditions are similar to those of the LC phasic response, and due to this, the LC-NE system has been linked to the P3 ERP. According to the LC-P3 hypothesis (Nieuwenhuis et al., 2005) the P3 is generated via phasic activity of the LC and the resulting release of NE. Task-relevant stimuli evoke robust P3 components. Stimuli that elicit a large P3 response are more likely to be detected and appropriately responded to compared to stimuli that do not elicit a P3, suggesting a relationship between the P3 component and task performance consistent with the phasic LC response (Hillyard, Squires, Bauer, & Lindsay, 1971; Parasuraman & Beatty, 1980).

The P3 can be divided into sub-sections known as the P3a and P3b. The P3a is elicited by unexpected or deviant stimuli (such as target stimuli in the *oddball* task) and is thought to reflect frontal lobe activity related to the hippocampus (Knight, 1996); and the P3b is elicited in response to task-relevant information. The P3a has a central maximum and the P3b has a parietal maximum, with different peak latencies and distinct topographic amplitude distributions. The difference in latency between the P3a and P3b may be explained by the anatomy of NE afferent projections, which innervate the frontal cortex before continuing to more posterior areas, unlike the majority of cortical afferents (Nieuwenhuis et al., 2005). P3 amplitude and latency change across the lifespan. From childhood to adolescence, P3 amplitude increases and P3 latency decreases, likely reflecting brain maturation. A plateau is reached during adulthood, before degenerative effects begin-- a decrease in amplitude, possibly reflecting decreasing neural power or cognitive resources, and an increase in latency, possibly indexing a slowing of neural speed or efficiency (van Dinteren et al., 2014).

P3 amplitude has been found to decline with ageing (Wild-Wall, Falkenstein, & Gajewski, 2011; van Dinteren, Arns, Jongsma, & Kessels, 2014), and to decline even further in AD (Polich & Corey-Bloom, 2005). Smaller P3 amplitudes are associated with decreased task performance and may reflect age-related cognitive decline. It may be possible, therefore, to distinguish early AD from healthy ageing by analysing the P3 ERP. Evidence has been found to suggest that P3a amplitude and P3b latency are related to differences in cortical thickness and cognitive function in an elderly group of participants (Fjell, Walhovd, Fischl, & Reinvang, 2007). AD patients have been found to demonstrate lower peak amplitude, less amplitude difference between target and non-target stimuli, and longer latencies than age-matched controls in an auditory discrimination task (Polich, Ladish, & Bloom, 1990). This is mostly consistent with more recent research comparing visual and auditory P3 amplitude and latency in participants with mild AD, moderate AD and age-matched controls, and correlating these measures with the results of neuropsychological tests (Mini-Mental State Exam [MMSE], Global Deterioration Score [GDS] and Alzheimer's Disease Assessment Scale [ADAS-cog]). Significantly longer latency and lower mean amplitude of auditory P3 was observed in the AD patients compared with controls, and MMSE score was positively correlated with auditory P3 latency and visual P3 amplitude (Pokryszko-Dragan, Słotwiński, & Podemski, 2003). However, P3 alone was not able to distinguish between mild AD and controls or mild AD and moderate AD. It is possible, therefore, that additional, simultaneous, measures should be employed if the aim is to detect early AD-related changes.

Theta rhythm, an oscillation of ~4-8 Hz, is associated with several neural functions and behaviours including the modulation of plasticity processes in the hippocampus, memory consolidation and arousal states such as exploratory behaviour (Walling, Brown, Milway, Earle, & Harley, 2011; McNaughton & Gray, 2000; Ma et al., 2008). Walling et al (2011) present evidence linking hippocampal theta oscillations to the release of NE from the LC in rats. Relative power of mid-theta frequencies (7.5-8.5 Hz) in rats expressing NE-LTP was selectively increased by glutamate activation of the LC, potentially contributing to plasticity processes and the efficacy of memory formation in awake, behaving animals. This is consistent with findings from Brown et al (2005), that activation of the LC by glutamate results in an increase of the 4-8 Hz theta rhythm and the promotion of long-term synaptic plasticity. The results here, from awake and behaving, as well as anaesthetised rats, are consistent with the adaptive gain theory (Aston-Jones & Cohen, 2005) of LC-NE function—that the LC phasic mode promotes the optimisation of the current behaviour, and the LC tonic mode facilitates task disengagement and promotes the inclusion of new features.

Consistently lower power in frontal theta frequencies have been found in older adults compared to younger adults in an n-back task (Gajewski & Falkenstein, 2014), a potential indication of decreasing ability to recruit frontal regions with increasing task demand. This finding may also relate to age-related deficits in memory functions, for example in encoding and working memory (Missonnier et al., 2011; Sederberg, Kahana, Howard, Donner, & Madsen, 2003; Jensen & Tesche, 2002). The reduction in frontal theta in older adults in this study was also accompanied by a reduction of the P3a, suggesting the involvement of attentional processes in the decline of performance. This combination of ERP and spectral analysis may also prove useful in the search for methods able to distinguish between cognitively normal ageing and early AD-related changes.

Manipulations of theta activity have been suggested as a means to counteract age-related impairments. Older rabbits given eye-blink conditioning trials learnt as fast as young controls when trials were given during episodes of hippocampal theta (Asaka et al., 2005a). Theta-triggered training was found to benefit both age groups, but the enhancement persisted in older animals. The results suggest the age-related deficits in learning and memory can be overcome by giving trials when the animal is exhibiting hippocampal theta activity. This finding may have implications into cognitive interventions for mild cognitive impairment (MCI) and AD patients, and potentially into the prevention of AD symptom onset.

Analysis of EEG data

EEG data was collected simultaneously with eye tracking data in this experiment. The next steps will involve the analysis of this data, with particular focus on the P3 event-related potential (ERP) and theta oscillations. The P3 event-related potential (ERP) is a positive, large-amplitude potential with a peak latency of around 300 to 400 ms following stimulus presentation and is thought to be elicited by the decision-making process (Nieuwenhuis, Aston-Jones, & Cohen, 2005). A reliable P3 is only elicited by stimuli that is attended to. These antecedent conditions are similar to those of the LC phasic response, and due to this, the LC-NE system has been linked to the P3 ERP. According to the LC-P3 hypothesis (Nieuwenhuis et al., 2005) the P3 is generated via phasic activity of the LC and the resulting release of NE. Task-relevant stimuli evoke robust P3 components. Stimuli that elicit a large P3 response are more likely to be detected and appropriately responded to compared to stimuli that do not elicit a P3, suggesting a relationship between the P3 component and task performance consistent with the phasic LC response (Hillyard, Squires, Bauer, & Lindsay, 1971; Parasuraman & Beatty, 1980).

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Pre-processing of EEG data collected in the current experiment will be performed with the use of custom Matlab code and Fieldtrip, and will consist of applying highpass (1Hz) and lowpass (60Hz) filters to the raw EEG data, and removing ocular artefacts based on the eye tracking time series. Independent component analysis (ICA) will be used to remove major artefacts using Fieldtrip. For event-related analysis, a bandpass filter between 0.1Hz and 40 Hz will be applied, and z-scores calculated as with the eye tracking data. For time-frequency analysis, a bandpass filter between 0.5Hz and 60Hz will be applied, and time-frequency decomposition will be performed for frequencies ranging from 2Hz to 40Hz, at 0.5Hz intervals. Pre-processing for Hilbert transform analysis will involve the application of separate bandpass filters: 1-4Hz (delta), 4-8Hz (theta), 8-15Hz (alpha), 15-32Hz (beta), 32-60Hz (gamma).

Epoch-based analysis—*Baseline epochs* will be defined as periods in which no other traffic is in sight of the driver. *Overtake epochs* will be defined as intervals beginning with a button press to initiate a lane change to the right and ending with a button press to initiate a lane change to the left. *Pre-overtake epochs* will be defined as the 10 seconds of the baseline epoch immediately prior to the lane change button press. *Post-overtake epochs* will be defined as the

20 seconds immediately following the lane change button press. *N-back epochs* will be defined as the 10 seconds prior to, and 3 seconds after, fixation on the n-back navigation sign. *Recognition epochs* will be defined as the 10 seconds prior to, and 3 seconds after, fixation on the recognition memory signs. *Decision epochs* will be defined as the 10 seconds prior to, and 3 seconds after, the decision button press.

Z-scored pupil diameters and Hilbert envelopes for each frequency band will be analysed in these epochs to answer the following questions: (1) Is there an age difference in spectral power and/or performance (positive or negative outcome) in overtake epochs? (2) Is there an age difference in spectral power and/or performance in n-back epochs? (3) Is there an age difference in spectral power and/or performance in recognition epochs? (4) Does spectral power in overtake epochs predict performance (positive or negative outcome on overtake)? (5) Does spectral power in n-back epoch predict performance (correct or incorrect decision at the end of the round)? (6) Does spectral power in the recognition epoch predict performance (positive or negative outcome on recognition memory test)?

Event-based analysis—Left and right lane change button presses, the moment of overtake, fixation on road signs (n-back and recognition memory signs), and the decision button press at the end of each round will be analysed to answer the following questions: (1) Is there an age difference in P3 and/or performance at the lane change button press? (2) Is there an age difference in P3 and/or performance on fixation of the n-back signs? (3) Is there an age difference in P3 and/or performance at the decision button press? (4) Is there an age difference in P3 and/or performance on fixation of the recognition memory signs? (5) Does P3 on fixation of the n-back sign predict performance on the n-back decision? (6) Does P3 on fixation of the recognition memory signs predict performance on the recognition memory test?

T-tests will be performed within each participant for time-frequency coordinates in the baseline z-scored power spectra to test the same contrasts.

EEG data is expected to show increased theta power in decision making epochs where pupil dilation occurs. Decreased theta power is expected when a poor decision is made, and an increase in low-theta poor decisions in older participants is likely. A P300 response to difficult decision-making epochs in which the pupil dilates should be seen. A decreased P300 response is expected when there is no pupil dilation, and when a poor decision is made. Both the theta and the P300 should increase at the moment a navigation cue is perceived and predict whether a correct choice is likely to be made later in the task.

We have presented a naturalistic and continuous driving simulation paradigm as a novel method for investigating the function of the locus coeruleus norepinephrine (LC-NE) system and its modulation of cognition and behaviour. The LC is one of the earliest sites of Alzheimer's Disease (AD) related pathology, while the LC-NE system modulates multiple cognitive functions known to decline in ageing and AD. This paradigm provides a novel way of probing the LC-NE under conditions requiring attention, memory, and decision-making with the use of embedded psychological tasks, and the scope for simultaneous recording of pupillometry and EEG measures. This is considered a first step in developing this paradigm as an early biomarker for age-related cognitive decline.

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Appendices

Appendix 1: Driving and Gaming Questionnaire

What is your age?

What is your gender?

Have you ever driven (a car or motorcycle)?

- yes/no

How many years of driving experience do you have (i.e., years in which you've driven at least once)?

- Less than 5 years
- 5-10 years
- More than 10 years

On average, over the past five years, how frequently have you driven in the city?

- 1 day or less per week
- 1-3 days per week
- 4-5 days per week
- 6-7 days per week

On average, over the past five years, how frequently have you driven on a highway (i.e., speed limit at least 100 kph, distance at least 30 km)?

- 1 day or less per week
- 1-3 days per week
- 4-5 days per week
- 6-7 days per week

How frequently do you ride a bicycle or scooter?

- 1 day or less per week
- 1-3 days per week
- 4-5 days per week
- 6-7 days per week

What best describes your previous experience (more than five years ago) with action video games (first-person shooter, racing, fighting, etc.)?

- I have virtually never played them
- I used to play them occasionally (less than 3 times per week)

- I used to play them a lot (more than 3 times per week)

What best describes your current experience (within five years) of action video games?

- I virtually never play them
- I play them occasionally (less than 3 times per week)
- I play them a lot (more than 3 times per week)

Appendix 2: Zung Anxiety Scale

	A Little Of The Time	Some Of The Time	Good Part Of The Time	Most Of The Time
1. I feel more nervous and anxious than usual.				
2. I feel afraid for no reason at all.				
3. I get upset easily or feel panicky.				
4. I feel like I’m falling apart and going to pieces.				
5. I feel that everything is all right and nothing bad will happen.				
6. My arms and legs shake and tremble.				
7. I am bothered by headaches neck and back pain.				
8. I feel weak and get tired easily.				
9. I feel calm and can sit still easily.				
10. I can feel my heart beating fast.				
11. I am bothered by dizzy spells.				
12. I have fainting spells or feel like it.				
13. I can breathe in and out easily.				
14. I get numbness and tingling in my fingers and toes.				
15. I am bothered by stomach aches or indigestion.				
16. I have to empty my bladder often.				

17. My hands are usually dry and warm.				
18. My face gets hot and blushes.				
19. I fall asleep easily and get a good night’s rest.				
20. I have nightmares.				

https://psychology-tools.com/test/zung-anxiety-scale

Scoring-
http://www.mentalhealthprofessionalsinc.com/Forms/Zung_Self-Rating_Anxiety_Scale.pdf

Appendix 3: List of Pub Names

The Dandy Cock

The Brunswick Inn

The Occasional Leg

The Nobody Inn

The Three-Legged Mare

The Horse & Carriage

Poosie Nancy’s

The Budgie's Relapse

The Bull & Spectacles

My Father’s Moustache

The Victoria Inn

The Goose & Cabbage

The Crown & Thistle

The Spread Eagle

The Red Lion

The Black Boy

Bucket of Blood

Hole in the Wall

Ye Olde Bell Inn

The Goat & Tricycle

The Pig & Puffin

The Cock & Bull

Durty Nelly's

Hair of the Dog

Dirty Dick's

The Wonky Donkey

The Elusive Camel

The King’s Arms

The Exeter Arms

The Hog in the Pound

Pig & Whistle
The Roaring Donkey
Bank Tavern
The Tap & Barrel
The Ram Inn
The Cow & Snuffers
The Stool & Urinal
The Bleeding Wolf
The Royal Wee
The Ewe & Shepherd
The Cormorant
The Contented Pig
The Smoking Dog
The Drunken Duck
The Tipsy Toad
The Cock Inn
The Famous Cock
Ye Olde Cheshire Cheese
The Bull & Gate
Fanny on the Hill
Ye Olde Dirty Limerick
Oily Johnnies
The Moody Cow
The Foul Mouthed Count
The Rank Turd
The Round Bush
The Royal Poke
The Fawcett Inn
The Spotted Dick
The Buccaneer's Bodice
Ye Olde Fighting Cocks
The Honest Lawyer

The Royal Oak

The Plough

The Rose & Crown

Railway Tavern

Queen’s Head

White Hart

Fox & Hounds

The Cross Keys

George & Dragon

Masons Arms

Duke of York

Travellers Rest

Butchers Arms

Railway Hotel

The Pope & Proctologist

The Dog’s Breakfast

The Man from Nantucket

The Sweaty Socks