Does nicotine improve attention? A pupillometry study

Effects of a single administration of low-dose nicotine on cognitive performance of non-nicotine users

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Abstract

We investigated the effects of low-dose nicotine on pupil size, eye blink rates (EBR), and visual attention amongst non-nicotine users while performing a multiple-object-tracking (MOT) task. Participants were tested with a double-blind, 2 (Drug: placebo vs. 2 mg nicotine) x 3 (Load: two vs. three vs. four) design. The drug manipulation was administered with chewing gums and divided into two sessions that were counterbalanced across participants with at least 72 hours in between the sessions. In the MOT task, participants were asked to track either two, three, or four circles out of 12 circles in motion. At the end of each trial, participants indicated whether a highlighted circle was one of the targets or not. Pupils and eye movement were recorded binocularly during the MOT task using the SMI RED500 eye tracker at a 60-Hz sample rate. Results revealed that pupils were relatively smaller in the nicotine than in the placebo condition. Pupil dilation increased proportionally to the target load. In contrast, EBR declined as the target load was higher. Response accuracy was higher in the small target load than in the medium and high target loads. Effects of nicotine and target load on response latency were not observed. We concluded that a small dose of nicotine was sufficient to constrict pupil size through its interaction with acetylcholine in the parasympathetic nervous system. However, a small dose of nicotine might not have a direct effect on dopaminergic system to stimulate eye blinks, enhance attention, and speed behavioural response.

Foreword

The year-long process of conducting the experiment and writing this thesis taught me a great deal of invaluable things. To mention a few, I learnt to manage time more efficiently and communicate more articulately in academic and non-academic contexts. The thesis experience allowed me to learn novel research and analysis techniques and refine my understanding of human cognition. This paper was written not merely for fulfilling the requirement to be an official Master of Philosophy in Cognitive Neuroscience. It was also written for purposes of pursuing intellectual accomplishment and creativity that was based on scientific method and critical thinking.

The amount of lessons one can learn by doing research and writing a scientific paper is immense. I have learnt that conducting research requires not only intelligence, but also agility, hard work, commitment, and passion. I trust that all the hard work I put in this study will be worthwhile. In the spirit of open and reproducible science, I hope that the knowledge derived from this study can be shared to a wider society and there are other studies attempting to replicate the current study.

I would like to thank significant people who helped me enormously during my studies and master's project. First, my knowledgeable and humble supervisor Professor Bruno Laeng. Thank you for your patience, constructive suggestions, humour, and prompt responses. You were also so generous with supervisory time! My working experience with you has built me as an independent and dedicated individual. Second, my internship supervisor Sebastiaan Mathôt. Thank you for introducing me to a lot of cutting-edge techniques, guiding me through the maze patiently, and believing in me. I really hope we can collaborate again in the future. Third, my whole family who lives far away but stays close in my heart. Thank you for being available all the time for me and always reminding me to stay modest and be grateful. Fourth, Eldar Lorvik and family. Your incessant support, positive energy, attention, and genuine care helped make the thesis writing really fun. Fifth, the Indonesian Endowment Fund for Education (LPDP) who provided financial support throughout my studies and living in Norway. It would not have been possible for me to come and study in Norway without the scholarship from LPDP. Lastly, all the people behind open-source software and open-access journals. Your hard work and dedication in supporting open science and producing amazing things for public have inspired many people to do the same. And of course, your tools and papers have indirectly helped me finish my thesis!

Contents

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Nicotine is a pharmacological stimulant that is widely available and has been well-studied. Albeit it is addictive and toxic (Benowitz, 2009; Herman, DeVito, Jensen, & Sofuoglu, 2014), several advantages of nicotine on cognitive processing have been observed (Rezvani & Levin, 2001), which could contribute to its widespread use. For instance, a facilitatory effect of nicotine on sustained attention has been found amongst healthy and non-smoking adults (Levin et al., 1998). This finding has also been replicated in different samples, such as smokers and non-smokers with attention-deficit hyperactivity disorder (ADHD; Levin et al., 1996a), Alzheimer's disease (White & Levin, 1999) and schizophrenia (Levin, Wilson, Rose, & McEvoy, 1996b).

Nevertheless, some other studies have failed to reproduce the facilitatory effect of nicotine on cognitive processing (e.g., Evans, Jentink, Sutton, Rensburg, & Drobes, 2014). Evans and colleagues argued that nicotine was only effective for long-term smokers or nicotine users. In chronic smokers, performance declined when they were deprived of nicotine and increased when the crave was satiated. Hence, Evans and colleagues concluded that boosts in performance were more likely due to the removal of withdrawal effects. By inspecting event-related potentials (ERPs) in the brain, they found that the effect of nicotine on non-smokers did not mirror its effect amongst smokers.

We surmise that nicotine may facilitate performance and improve attention, although it may not be manifested within all populations and under differing conditions. We believe that using current physiological approaches for the study of attention, such as pupillometry, would aid the understanding and development of nicotine involvement in cognitive processing. To date, there are not many studies investigating the effect of nicotine on cognitive processing *and* with the pupillometry method. Pupillometry is a very promising, non-invasive tool that can provide continuous measure of cognitive processing through pupillary changes to internal states (Laeng, Sirois, & Gredebäck, 2012). Thus, it will be useful to use pupillometry to elucidate basic properties of cognitive processing in the context of nicotine use. We briefly review the relevant research for our study by beginning with extensive elucidation of pupillometry, nicotine, and cognitive processing. Thereafter, we present the formulation of general research questions and the specific hypotheses of our study.

Pupillometry

Pupillometry refers to the study of pupillary fluctuations in size (Laeng et al., 2012). These fluctuations occur primarily to adjust retinal illuminance (pupillary light response or PLR) and can be indicative of normal or abnormal retinal and cortical functions (Beatty, 1982; Beatty & Lucero-Wagoner, 2000). However, other non-luminance-related factors can account for pupillary changes, including task-related activity (task-evoked pupillary responses or TEPRs) and the use of pharmacological substances. Comprehension of the physiological mechanism behind pupillary responses is important to understand the interaction between pupillary responses and pharmacologically active substances. It is also crucial to understand how pupil fluctuations can serve as a "unique psychophysiological index of dynamic brain activity in human cognition" (Beatty & Lucero-Wagoner, 2000, p. 142).

The physiological mechanism. The study of pupillary fluctuations began from clinical neurology as changes in pupil size could indicate lesions in peripheral or central nervous system, or the use of pharmacologically active substances (Beatty & Lucero-Wagoner, 2000). Anatomically, pupillary constriction and dilation are controlled by the balanced activity (e.g., excitation vs. inhibition) of two neural pathways in the autonomic nervous system (Beatty & Lucero-Wagoner, 2000; Mathôt, 2018; McDougal & Gamlin, 2008; Wang & Munoz, 2015). Pupillary constriction is controlled through the parasympathetic nervous system, which is involved in the control of biological processing during ordinary situations. The parasympathetic nervous system innervates the iris sphincter muscle and contraction of the iris sphincter causes pupillary constriction (i.e., myosis). The fibres of the iris sphincter muscle are arranged concentrically (Figure 1, left panel). This innervation originates from neurones in the Edinger-Westphal nucleus that travel to the ciliary ganglion located within the orbit of the eye before finally reaching the iris. The ciliary ganglion is a part of the oculomotor (III) nerve in which nicotinic cholinergic synapses are formed (Beatty & Lucero-Wagoner, 2000; McDougal & Gamlin, 2008). Then in the iris, the short ciliary fibres release acetylcholine that contracts the sphincter muscle. Thus, compounds that are acetylcholine agonists, such as pilocarpine, carbachol, bethanechol, and metoclopramide, will produce myosis (Beatty & Lucero-Wagoner, 2000; McDougal & Gamlin, 2008). The acetylcholine agonists, as well as noradrenergic antagonists, contract the iris sphincter muscle by binding acetylcholine to the m3 muscarinic receptor—the muscarinic receptor subtype that is expressed 60 - 75% out of the total muscarinic receptors (Gil, Krauss, Bogardus, & WoldeMussie, 1997; McDougal & Gamlin, 2008). This binding initiates a series of chemical events that will yield sphincter muscle contraction. Pilocarpine, for example, produces

myosis through depolarisation of the effector cells located in the Edinger-Westphal nucleus (Beatty & Lucero-Wagoner, 2000). Another example is carbachol, an agonist that releases acetylcholine spontaneously at preganglionic cholinergic nerve endings within the ciliary ganglion (Beatty & Lucero-Wagoner, 2000; McDougal & Gamlin, 2008). Myosis or pupillary constriction can also be caused by inducing noradrenergic antagonists to inhibit activities in the sympathetic nervous system (explained below).

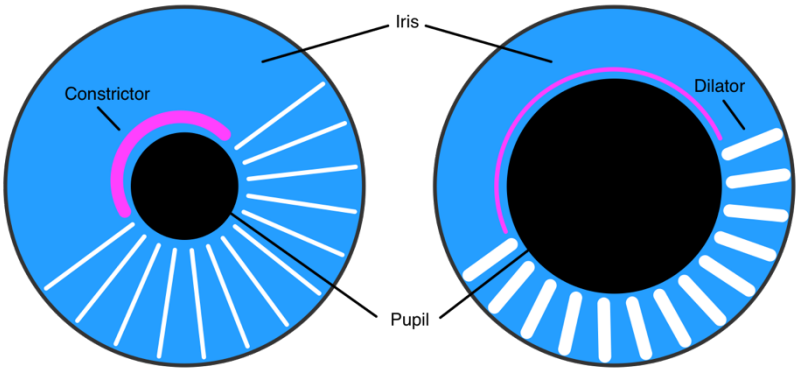

Figure 1. An illustration of the eye showing the pupil (black), iris (blue), sphincter muscle (the constrictor; in magenta), and dilator muscle (the dilator; in white). Left side: constricted pupil, right side: dilated pupil. This illustration is adapted from Bron et al. (1997) and Mathôt (2018).

Pupillary dilation is controlled through the sympathetic nervous system, which is characterised by the 'fight or flight' function. The sympathetic nervous system innervates the iris dilator muscle, of which fibres are composed in radial orientation (Figure 1, right panel). The activation of the iris dilator causes pupils to dilate (i.e., mydriasis). The dilation pathway starts from neurones in the ciliospinal centre, then the neurones travel to the superior cervical ganglion. From the superior cervical ganglion, adrenergic innervation goes through the long ciliary nerves (also called the ophthalmic nerves) to the iris and releases norepinephrine that contracts the dilator muscle. Compounds that are noradrenergic agonists, as well as acetylcholine antagonists, will produce mydriasis. The noradrenergic agonists produce dilation by binding norepinephrine to the alpha 1a adrenergic receptor (Beatty & Lucero-Wagoner, 2000; McDougal & Gamlin, 2008). The binding will then generate a series of signals from the long ciliary nerves to the dilator muscle. For example, ephedrine, a noradrenergic agonist, causes mydriasis by releasing norepinephrine spontaneously from the postganglionic sympathetic nerve endings and directly stimulating the alpha-adrenergic receptor (Beatty & Lucero-Wagoner, 2000; McDougal & Gamlin, 2009). Other noradrenergic agonists, such as phenylephrine and hydroxyamphetamine, also work in a similar procedure causing mydriasis (McDougal & Gamlin, 2008). Mydriasis is also caused

by inducing acetylcholine antagonists to inhibit activities in the parasympathetic nervous system. Unfortunately, the physiological dilation pathway is understood less well than the constriction pathway (Barbur, 2004). This is probably because the dilation pathway involves several neural areas, such as locus-coeruleus norepinephrine (LC-NE) system and hypothalamus (Mathôt, 2018), and superior colliculus (van der Wel & van Steenbergen, 2018), with which many cognitive domains are related.

Task-evoked pupillary responses (TEPRs). Cognitive pupillometry studies have demonstrated that small-scale fluctuations in pupil size may reflect variations in central cognitive processing. These variations include, but are not limited to, differences in visual attention and perception, memory capacity, and inhibition of cognitive control (see van der Wel & van Steenbergen, 2018, for a review of TEPR studies). Task-evoked pupillary responses are also referred to as "reporter variables" (Beatty & Lucero-Wagoner, 2000, p. 147). It is argued that TEPRs, just like the reporter genes in molecular biology, are important in reporting measured cognitive processing or events in the central nervous system albeit cognitive processing or cortical events itself may not directly cause pupillary constriction or dilation. One concern in measuring TEPRs precisely is to differentiate TEPRs from pupil light response (PLR; e.g., Laeng & Sulutvedt, 2013). It is crucial that pupillary fluctuations that occur during the task interval are not caused by differing luminosity levels of the stimuli. Thus, the luminosity level of the stimuli within and between tasks must be equated. Another concern is to eliminate oculomotor factors that can corrupt the data. To eliminate oculomotor confounds, an explicit eye-movement control can be implemented to reduce gaze shifting before task onset. For example, we can utilise a fixation point that requires participants to fixate their gaze within, for example, a two-degree window (Woodman, 2010).

Studies on TEPRs within psychology have a root on Hess and Polt (1960) who reported changes in pupil size during the presentation of emotional images. However, studies of TEPRs started blooming after a seminal study by Kahneman and Beatty (1966) on pupillary responses and the load of information. In their study, participants were asked to do mental calculations with a varying degree of difficulties. It was demonstrated that pupils were more dilated under the more difficult than the easier condition. In TEPR studies, there can be other confounding factors or non-cognitive artefacts, such as emotional reactions. Emotional arousal is expressed in the autonomic nervous system (Beatty, 1982; Bradley, Miccoli, Escrig, & Lang, 2008) and this can affect pupillary fluctuations. However, emotional arousal generally lasts longer compared to the brief *phasic* pupil responses, or responses during the interval of interest evoked by cognitive processing (Lang, Rice, &

Sternbach, 1972). Hence, factors such as emotional arousal and individual differences are more likely to influence the *tonic* or baseline pupil diameter (Beatty, 1982). For this reason, it is important to correct average phasic pupil diameter relative to average tonic pupil diameter.

More studies have since then utilised pupillary responses as an index of cognitive processing. For instance, Geng, Blumenfeld, Tyson, and Minzenberg (2015) used pupillary fluctuations as a metric of ongoing uncertainty during visual search for a target. Participants were asked to select a target presented alone or amongst distractors with various salience levels, compared to the target, over four blocks (0, 30, 70, and 100%). Rather than measuring differences in pupil diameter based on the accuracy of target selection per se, pupil diameter was assessed to observe uncertainty in target selection. They found that changes in pupil size were reliably related to uncertainty during attentional selection. Their findings demonstrated that pupillary responses reflected fluctuations in individual internal attentiveness. Pupils were more dilated when there was an increase in uncertainty, which reflected an increase in mental effort and cognitive control. When uncertainty was low, pupil size was smaller, reflecting decreased cognitive control.

The relation between increased cognitive control and pupil dilation is also shown by studies using the inhibition paradigm. Laeng, Ørbo, Holmlund, and Miozzo (2011) investigated the effect of Stroop task on pupillary responses. Stroop task is a colour-naming task with congruent and incongruent conditions, in which the colour of the letters corresponds to the word or not, respectively. Participants in Laeng and colleagues' study performed on the congruent, incongruent, and non-colour words (as a control and baseline pupil measurement). They found that, relative to non-colour words, pupil size increased significantly in the incongruent condition. More interestingly, pupil size in the congruent condition was smaller than pupil size in the non-colour condition. The Stroop effect on pupillary responses also predicted the Stroop effect on response time. Laeng and colleagues' finding was supported by van Steenbergen and Band (2013) who used a Simon task—a cognitive-conflict-related paradigm that shows stimuli ipsilaterally or contra-laterally to the required response. Van Steenbergen and Band found that the Simon effect modulated pupil dilation and the dilation mirrored the conflict-adaptation pattern observed in response latency. D'Ascenzo and colleagues (2016) also lent support to van Steenbergen and Band's study. D'Ascenzo and colleagues measured pupil dilation before and after practice with spatially incompatible displays. It was revealed that the Simon effect modulated pupil dilation not only before but also after practice. This finding provided converging evidence that pupil

dilation reflected response-conflict monitoring and cognitive acquisition of novel stimulus-response associations.

Crucial pupillometric evidence for attention and high-level cognitive processing can also be derived from two studies. First, Smallwood and colleagues (2011) investigated pupillary dilations and cortical activity during online and offline processing. Online processing is the deliberate information processing as stimuli are encoded. This is usually easily disrupted by concurrent perceptual information. In contrast, offline processing is spontaneous, imaginative in nature, and characterised as mind-wandering, which is not easily disrupted by external events. The minimisation of disruption in offline processing is supported by the ability of the mind to reversibly "decouple" attention from sensory input (see Frith & Frith, 2006, and Raichle, 2010, for reviews on cerebral intrinsic activity and mentalising). Smallwood and colleagues tested the decoupling hypothesis against task-based activation through the pupil diameter dynamics. Online processing was induced by a working memory task wherein participants had to retain the last presented stimulus. To make participants engage in offline processing, participants had to report only the current stimulus in a choice reaction-time task with intermittent probes. Their findings showed that in the online mode, pupil dilations were evoked by task-relevant stimuli only. In the offline mode, however, there was spontaneous activity of pupil diameter that was decoupled from task events (i.e., during periods of offline processing). They also found a stepwise relation between response time and pupillary dilation in both processing types, suggesting that online and offline processing represented distinct cognitive mechanisms.

Second, Kang, Huffer, and Wheatley (2014) replicated Smallwood and colleagues' (2011) paradigm by controlling for the luminance level in the stimuli. In the absence of luminance changes, they were able to rule out low-level changes influencing pupillary dilation. Kang and colleagues' findings demonstrated that pupillary dilations during online processing differed from dilations during offline processing. They also observed that the fluctuations of pupillary responses followed the fast moment-by-moment fluctuations between online and offline processing. It suggested that pupillary responses provided a high resolution of temporal measure of attention. Taken together, all of the TEPR studies described here present strong evidence that pupillary responses are a reliable and valid index of cognitive processing.

Nicotine and Acetylcholine

Nicotine ($C_{10}H_{14}N_2$) is the chemical extract from the dried leaves of *Nicotiana tabacum* and *Nicotiana rustica* that can be absorbed through the respiratory tract, alimentary canal, and intact skin before entering the bloodstream instantly (Luttrell & Vogel, 2014). It is widely available in tobacco products, liquid form (present in electronic cigarettes), chewing gums, transdermal patches, and *snus* (a small moist smokeless tobacco pouch placed under the upper lip, popular in Scandinavian countries; Lund & Lund, 2014). Nicotine is the prototypic nicotinic acetylcholine receptor (nAChR) agonist (Kumari et al., 2003; Luttrell & Vogel, 2014; Rezvani & Levin, 2001). It mimics and binds to a subset of acetylcholinergic receptors. Nicotine reception into the body system varies from one person to another due to the variation in genetics, such as the diverse expression of the *CHRNA4* gene (Espeseth, Sneve, Rootwelt, & Laeng, 2010). The *CHRNA4* gene codes for the α4 subunit in the nicotinic α4β2 receptor, which is the most common nAChR (Espeseth et al., 2010) and richly expressed in fronto-parietal areas and thalamus (Gotti, Zoli, & Clementi, 2006). The *CHRNA4* gene is known to interact with medium to high load processing in visual search and multiple-object-tracking tasks (Espeseth et al., 2010). This demonstrates that nicotinic and cholinergic neurotransmission can be involved in cognitive performance.

Acetylcholine is a chief neurotransmitter that is found throughout the nervous system, such as in ganglia, neuromuscular connections, central nervous system, and autonomic nervous system (Whitehouse, 2014). Acetylcholine pervasiveness implies that it plays an important role in the regulation of peripheral organs, basic physiological activities, and neuroprotection. In the autonomic nervous system, acetylcholine helps control the involuntary body functions as well as interacts with psychopharmacological substances. For example, when nicotine—an acetylcholine agonist—interacts with acetylcholine receptors, a signal is sent through the parasympathetic pathway to constrict pupils. Conversely, when acetylcholine receptors bind with an acetylcholine antagonist (e.g., atropine), a signal is sent through the sympathetic pathway to dilate pupils. In the central nervous system, the work of acetylcholine and its interaction with nicotine is a bit more complex because it involves excitation of acetylcholine and inhibition of muscarinic receptors, or vice versa, at the same time (Kimura, 2000). In general, acetylcholine in the central nervous system contributes to brain plasticity (Perry, Walker, Grace, & Perry, 1999; Sarter & Bruno, 1999), arousal and alertness (Perry et al., 1999), and sustaining attention as well as learning and memory (Hasselmo & Giocomo, 2006). Damage to the cholinergic system is associated with learning

and memory deficits in Alzheimer's disease, and treatment usually involves nicotine to stimulate acetylcholine release (e.g., White & Levin, 1999).

The relationship between acetylcholine and attention can be seen on the augmented release of acetylcholine in the prefrontal cortex during performance under a challenging and detrimental sustained-attention task (Kozak, Bruno, & Sarter, 2006). It is argued that augmented acetylcholine release is more related to attentional effort than to task performance. However, task performance has a strong dependency on attentional capacity and effort (Espeseth et al., 2010), such that higher capacity is related with lower attentional effort and vice versa. Thus, the release of acetylcholine should also be associated with task performance because attentional effort increases when capacity—and consequently, performance—is being challenged (Sarter, Gehring, & Kozak, 2006). To enhance sustained attention and the performance, nicotine has also been used to excite acetylcholine release (e.g., Levin et al., 1998).

Nicotine and Pupillary Responses

Up until now, there have been only a few studies investigating the effect of nicotine on pupillary responses. A classic study was conducted by Lie and Domino (1999) to test the effects of cigarette smoking on the human pupils. They compared the pupil size of smokers and non-smokers who had been abstained from caffeine products at least eight hours prior to the experiment. Both participant groups were in the placebo (i.e., sham cigarette) and tobacco (i.e., containing nicotine) conditions. Pupil diameter was recorded under a mesopic, or medium lighting, situation. Their results revealed that, first, there was no baseline differences between smokers and non-smokers prior to sham- or tobacco-smoking. Second, after sham- and tobacco-smoking, there was pupillary constrictions both amongst non-smokers and smokers. This constriction, though, was more notable in the tobacco-smoking condition. This finding was supported by Erdem and colleagues (2015) who assessed the acute effects of cigarette smoking on pupil size under mesopic and photopic (i.e., well-lit) situations. These effects were tested on smokers who smoked ten or more cigarettes a day for at least five years. Prior to the experiment, all smokers were abstained from smoking and caffeine products for the minimum of 12 hours. Experimental smoking was administered through a cigarette containing 1 mg nicotine. Mesopic and photopic pupil size was recorded before and after experimental smoking. Their results showed that pupils were constricted remarkably even amongst smokers under both the mesopic and photopic situations post-smoking. It

suggested that iris sphincter muscles were innervated by the binding of cholinergic receptors with nicotine, the only active pharmacological agent in tobacco.

Nevertheless, the effects of nicotine through smoking on pupillary constriction are still equivocal. Sobaci and colleagues (2013) evaluated the effect of chronic smoking on pupillary responses amongst smokers and non-smokers. Smokers were abstained from smoking and caffeine prior to the experiment for the minimum of 12 hours. Participants' pupil size was measured under the scotopic (i.e., low lighting) and photopic situations. They found that there was no difference in the pupil size of smokers and non-smokers under the scotopic situation. Under the *photopic* situation, the pupil size of smokers was *larger* than the pupil size of non-smokers. However, Sobaci and colleagues' study was unclear in several ways. They did not explain the dose of nicotine and how it was induced to the participants. It was unclear if baseline pupil size was measured and if the measurement of pupil size was collected before and after nicotine administration. Consequently, they were unable to compare pupillary constrictions pre- and post-nicotine administration. They only compared the pupil size of smokers and non-smokers in scotopic and photopic situations, which could be attributed by and large to pupil light response.

Findings of Bardak and colleagues (2017) who evaluated, amongst others, pupil size after cigarette smoking were in line to that of Sobaci and colleagues (2013). Participants in Bardak and colleagues' experiment were all chronic smokers and abstained from smoking for at least 12 hours. Following the abstinence, participants were induced with 1 mg nicotine through tobacco smoking. Participants' ocular and pupillary data were collected before and after smoking under the same room illumination. They found that there were no differences in pupil size between pre- and post-smoking. However, this study could be improved by including non-smokers. Also, it was a little unclear whether or not participants were abstained from caffeine and other stimulants that compensated the nicotine abstinence, and were kept away from smoking-related visual cues (see Chae et al., 2008, for autonomic responses to smoking-related visual cues). Yet taken together, more studies evaluating the relationship between nicotine and pupillary responses are needed to provide a clear, well-established answer.

Nicotine and Cognitive Processing

Nicotine in alternative forms to smoking have been commonly used in smoking cessation treatment programme in spite of the equivocal findings on their effectiveness (e.g., Cepeda-Benito, 1993; Kim & Baum, 2015; Yudkin et al., 2004). It is also common in

cognition and neuroscience studies investigating the effects of nicotine on cognitive performance and central nervous system (see Keith, Kurti, Davis, Zvorsky, & Higgins, 2017 for a review). Some other studies have investigated the role of genetics and its interaction with nicotine on visual attention, cognitive control, motoric skill, and spatial processing both in humans and mice (e.g., Espeseth et al., 2010; Ortega, Tracy, Gould, & Parikh, 2013; Romano, De Angelis, Ulbrich, De Jaco, Fuso, & Laviola, 2014). For the relevance of this thesis, however, literature reviews will be focussed on nicotine and cognitive processing in humans.

One of the cognitive tests that assesses attention is the Conners' Continuous Performance Test (CPT; Conners, 1994, 1995; cited Egeland & Kovalik-Gran, 2010). The Conners' CPT, amongst others, measures sustained attention. Sustained attention is retaining a consistent focus while attending to continuous stimuli (Egeland & Kovalik-Gran, 2010). In CPT, response accuracy is defined by correct commissions (i.e., responding to targets) and correct omissions (i.e., ignoring non-targets). From this, we can calculate the weighted formula of correct and incorrect responses to get a composite measure of attentiveness, which is useful in indexing individual's perceptual sensitivity. We can also calculate response time variability by recording response latency during the inter-stimulus intervals since stimulus onset. Levin and colleagues (1998) used computerised CPT and interval-timing task (assessing the accuracy and precision in estimating time periods) to find the baseline effects of nicotine on attention in non-smoking adults without pre-existing attentional deficits. Nicotine was administered through a nicotine transdermal patch (7 mg) and a placebo patch. They found that attention improved when participants were induced with nicotine. The improvement was marked by increased response accuracy and reduced response time variability. Furthermore, the composite attentiveness was also higher in the nicotine-induced participants.

Improved attention in nicotine-induced participants was also found in studies with smokers and non-smokers who were clinically diagnosed with attention-deficit/hyperactivity disorder (ADHD). Levin and colleagues (1996a) used computerised CPT, the Stroop task, and an interval-timing task to measure participants' attention. Smokers were induced with 21 mg/day nicotine following nicotine deprivation, while the non-smokers were induced with 7 mg/day nicotine, both through a transdermal patch. In another session, they were treated with a placebo patch. Levin and colleagues found that nicotine significantly reduced response time and response time variability in CPT and interval-timing task amongst smokers. Adding to this finding, Potter and Newhouse (2004) found that nicotine treatment significantly improved

cognitive inhibition during the Stroop task in non-smokers with ADHD. Taken together, these two studies demonstrated that nicotine could improve the symptoms of ADHD.

Nicotine treatment have also been administered to patients with Alzheimer's disease. Alzheimer's disease is marked by debilitating loss of cognitive function and has typical onset after age 60 (Levin, McClernon, & Rezvani, 2006). Thus, it is important to find whether nicotine can help improve cognitive functioning in patients suffering from Alzheimer's disease. White and Levin (1999) treated adults ranging from 62 to 87 years old, who were all non-smokers and had Alzheimer's disease, with placebo and nicotine transdermal patches (5 and 10 mg/day). Cognitive function of the participants was assessed by, amongst others, the Conners' CPT. White and Levin reported that, compared to the placebo condition, participants who were induced with nicotine had a notable decline in errors and response time variability. Additionally, the composite attentiveness in nicotine condition was evidently higher than in placebo condition. This facilitatory effect of nicotine on patients with Alzheimer's disease supported an earlier pilot study showing a decline in intrusion errors and improvement in recall consistency during the recall and category retrieval tests (Newhouse et al., 1988).

Interestingly, the effects of nicotine have also been investigated in patients with schizophrenia. A study was conducted with participants who were all smokers, had been clinically diagnosed with schizophrenia, and consumed the antipsychotic drug haloperidol (Levin et al, 1996b). Participants were smokers such that the effect of nicotine abstinence could be observed. Patients with schizophrenia were chosen because schizophrenia was associated with impaired attention and memory. Haloperidol was administered to assess the impact of an antipsychotic drug on cognitive function in schizophrenics and its interaction with nicotine. Nicotine was administered in four levels (0, 7, 14, and 21 mg/day) to each participant using a nicotine transdermal patch. Levin and colleagues found that moderate and high doses of haloperidol lengthened response times during spatial memory test and CPT. However, nicotine not only improved performance when administered alone, but also attenuated the adverse side effects of haloperidol. This finding was supported by a recent study investigating cognitive effects of very low nicotine content (VLNC; < 0.05 mg) in smokers with and without schizophrenia (AhnAllen, Bidwell, & Tidey, 2015). AhnAllen and colleagues reported that performance on motor speed, visual working memory, and sustained attention tasks was slower and poorer in the VLNC condition for both groups. Nonetheless, performance improved after participants were induced with 42 mg of nicotine through a transdermal patch.

More recent studies have tested the cognitive effect of nicotine using components of event-related potential (ERP). Evans and colleagues (2013) observed two specific P300/P3 components that were related to attention-orientation of target and task-irrelevant stimuli: the P3b and P3a, respectively. The P3b and P3a components had been found to be negatively associated with a wide range of maladaptive traits and behaviours, such as substance abuse (Iacono et al., 1999) and disinhibitory disorder (Iacono et al., 2002). Therefore, the P3b and P3a components were hypothesised to index cortical activities during a series of oddball tasks and nicotine abstinence. Participants were all smokers and deprived of nicotine overnight. Performance was tested and ERP waveforms were recorded under the nicotine (4 x 0.60 mg) and placebo (< 0.05 mg nicotine yield) conditions. Results of this study revealed that there was a significant reduction on the P3b amplitude in the nicotine-deprivation condition. Additionally, the P3a amplitude declined when smokers with lower cognitive control trait were deprived of nicotine. Together, the results indicated that nicotine deprivation could have a detrimental effect on neural cognitive indices. Unfortunately, it was not clear whether or not the nicotine treatment restored the adverse effect of nicotine deprivation.

A functional magnetic resonance imaging (fMRI) study by Kumari and colleagues (2003) sought to determine the neural correlates of nicotinic effects on attention and working memory function. Participants were all non-smokers with no pre-existing attentional deficits and were administered nicotine (1 mg) and placebo (saline) through subcutaneous injection. Attention and working memory function were assessed using the "*n*-back" task—a working memory task requiring participants to memorise the stimulus from *n* trials ago. It was found that response accuracy improved when participants were induced with nicotine. More importantly, improvement in behavioural performance was congruent with increases in blood-oxygen-level dependent (BOLD) response in the frontal region, anterior cingulate, parietal region, and superior colliculus. Activations in these regions suggested arousal by both nicotine and utilisation of cognitive strategies during the task. This result demonstrated that nicotine had promotive effects on attention and activated its neural correlates.

Thus far, it has been shown that nicotine enhances cognitive functions in normal and healthy people as well as people with clinical disorders. However, a number of studies have shown the opposite. For example, Evans and colleagues (2014) did not replicate their previous findings (Evans et al., 2013). Evans and colleagues (2014) measured P3b and P3a amplitudes to index neural correlates of attention in healthy non-nicotine users without pre-existing attentional deficits. Participants were tested under the nicotine (7 mg) and placebo (0 mg) conditions administered through a transdermal patch. The results of behavioural

measures revealed that response accuracy and latency during the oddball tasks in the nicotine condition did not significantly differ from the placebo condition. On top of that, nicotine did not enhance the P3b and P3a amplitudes. From this finding, they concluded that there was no facilitatory effects of nicotine on cognitive processing in healthy non-nicotine users. The improvement in task performance amongst nicotine users might be caused by the removal of irritable effects from nicotine withdrawal instead of from the nicotine itself.

The findings of Evans and colleagues (2014) were supported by Ernst and colleagues (2001). Ernst and colleagues investigated the nicotine effects on performance during a 2-back task and recorded cortical activations using positron emission tomography (PET). Participants were ex-smokers and smokers who were abstained from nicotine overnight. Nicotine was administered through nicotine chewing gum (4 mg) and placebo chewing gum (taste-matched, 0 mg nicotine). Their findings revealed that there were no differences in task performance amongst ex-smokers between the placebo and nicotine conditions. Task performance improved only amongst abstinent smokers after nicotine administration. Results of the PET scan showed that, in the placebo condition, cortical activation during performance amongst smokers was as strong as the ex-smokers' albeit they were different with respect to hemispheric lateralisation. In the nicotine condition, activation was diminished in smokers, while there was an enhanced activation in ex-smokers. These findings indicated that cognitive strategies might have a stronger influence than nicotine (as shown in differing hemispheric lateralisation) on task performance. Moreover, task performance of smokers might be influenced more strongly by withdrawal and tolerance effects to nicotine (as shown in different cortical activation pre- and post-nicotine administration).

The Present Study

Our primary question in the present study was whether nicotine could improve performance in a divided attention task, namely in the Multiple-Object-Tracking (MOT) paradigm (Pylyshyn & Storm, 1988). We also tested the effect of nicotine on pupillary responses during MOT, since this could provide information about the physiological correlates between nicotine and cognitive processing. Performance itself was measured by response accuracy and latency. Nicotine gums were chosen because it was widely available, but the general effect of nicotine in TEPRs was not well-established. We used only healthy non-nicotine users to eliminate withdrawal and tolerance effects. We also employed a placebo condition to control for drug expectancy effects.

Although not explicitly stated before, we also measured eye blink rates (EBR) for two main reasons. First, EBR were shown to correlate with the processing of cognitive load whereby reduced EBR predicted higher cognitive processing (Siegle, Ichikawa, & Steinhauer, 2008). Second, nicotine was known to mediate dopamine release (Herman et al,, 2014; Imperato, Mulas, & DiChiara, 1986) and EBR could index activity in the dopaminergic system, whereby higher EBRs indicated higher dopamine function (Jongkees & Colzato, 2016). Therefore, it seemed relevant to include EBR measurement.

In the present study, we formulated four hypotheses:

1. We hypothesised that pupil dilation would be larger in the higher task load. However, given the innervation of the iris sphincter muscle by the nicotine-acetylcholine interaction, we hypothesised that nicotine would constrict pupil size. Specifically, we predicted that pupils would be more dilated in the high-load condition than the medium load, which in turn would be more dilated than the low-load condition. In relation to the nicotine treatment, we predicted that pupil dilation would be smaller in the nicotine condition than in the placebo condition.
2. If higher cognitive load reduced EBR, then we hypothesised that EBR would be smaller in the higher task load. We also hypothesised that EBR could index the activation of dopaminergic system by nicotine. Specifically, we expected that EBR would be higher in the low-load condition than in the medium load, which in turn would be higher than the high-load condition. Additionally, we expected that EBR would be relatively higher in the nicotine condition than in the placebo condition.
3. We hypothesised that performance would be better in the lower task load than in the higher task load. Furthermore, we hypothesised that nicotine would enhance task performance. Specifically, we predicted that response accuracy would be higher in the low-load condition than in the medium- and high-load conditions. However, we predicted that response accuracy would be higher in the nicotine condition than in the placebo condition.
4. Given that a lower task load was relatively easier than a higher task load, we hypothesised that response would be faster in the lower load than in the higher load. This response, we hypothesised, would be speeded when people were induced with nicotine. Specifically, we expected a shorter response latency in the low-load condition than in the medium- and high-load conditions. Furthermore, we expected to find a shorter response latency in the nicotine condition compared to the placebo condition.

Method

Ethics Statement

Approval of the current study was obtained from the Department of Psychology's Research Ethics Committee before participant recruitment (Ref. number: 1730636; Appendix 1). Participants read the experiment information (Appendix 2) and signed an informed consent (Appendix 3) at the beginning of the experiment. Participants were treated according to the Declaration of Helsinki. Each participant was debriefed at the end of the final session.

Participants

Thirty-one participants volunteered in the current study. Data from one participant was excluded from the analysis due to a technical failure in one session. The complete data analyses included 30 university students in Oslo, Norway, who were recruited through social media (M_{age} = 24.57 years, SD = 3.33, age range = 19-34 years, n_{female} = 20). Participants had visual acuity of normal or corrected-to-normal (by contact lenses, not their prescription spectacles, to optimise the quality of eye recording). Each participant was compensated with NOK 300 in a form of universal gift cards for completing both sessions.

Prior to data collection, eligibility criteria of participation had been set due to the nicotine treatment. To eliminate the nicotine effect from previous consumptions, participants ought not to be users of any types of nicotine products, such as cigarettes, e-cigarettes, *vape* (an inhale-exhale vapour device), and *snus*. To avoid negative side effects, participants should not be pregnant or have self-reported clinical problems with heart, allergies, blood pressure, ulcers, thyroid, dentures, and anxiety during data collection (American Society of Health-System Pharmacists, 2013). To avoid interference in the eye-tracking recording, participants were required not to apply eye makeup (e.g., eyeliner, mascara, eyeshadow).

Design

The current study had a 2 (Drug: 0 mg nicotine or placebo vs. 2 mg nicotine) x 3 (Load: two vs. three vs. four) within-participants, double-blind design. Two bottles covered in dark tapes and labelled A and B were prepared for the allocation of placebo and nicotine gums. A third party allocated each type of the gums in either one of the bottles. The experimenter and participants were blind to the type of the gum administered in every session. The experimenter remained "blind" to the Drug manipulation until data collection was completed, and all statistical analyses were done with the blind labels. The order of Drug manipulation was fully counterbalanced, such that a number of participants took a gum from

bottle A and another from bottle B in their first session. In the second session, participants took the gum from another bottle that was not assigned to them in the first session. All participants did the same visual attention task with three Load levels in both placebo and nicotine conditions. The number of presented stimuli was kept constant in all trials.

The MOT task consisted of four blocks with 18 trials in each block. Out of the 18 trials, there were six two-, six three-, and six four-target trials of which the presentation order was partially counterbalanced. The block always begun with the presentation of targets in three gradual trials (i.e., two-target trial followed by three-, then four-target trial). The order of the trials in the rest of the block was fully randomised. The entire order of trials in the first block was then repeated in the second, third, and fourth blocks. This design made 24 trials for each Load level and 72 trials in total. On all trials, targets always appeared with distractors in a group of 12 disks that were uniform in size and isoluminant. Stimuli were made isoluminant by adjusting the RGB pixel values of the animations. There were no practice trials preceding the task.

Materials and Apparatus

Drug manipulation. Drug was manipulated by administering nicotine (2 mg) and placebo (0 mg nicotine) chewing gums. The choice of the dose was dictated by ethical and experimental considerations. Participants were healthy non-nicotine users, so higher doses might cause adverse side effects such as nausea, dizziness, and sore throat. As comparison, 2 mg of nicotine is higher than the permitted maximum content of nicotine per cigarette in Norway (i.e., 1 mg; The Ministry of Health and Care Services, 2003). The experimental consideration concerned the paradoxical nature of the nicotine psychoactive effect (i.e., the inverted U-shaped dose-effect curve; Levin et al., 1998). At low-to-medium doses, nicotine can cause improvement in cognitive processing performance. However, at higher doses, nicotine will cause impairment and ineffectiveness.

Chewing gums were chosen as the medium of Drug manipulation because it was available in lower doses. The doses in most of nicotine transdermal patches started from 5 mg (e.g, Nicotrol®) and 7 mg (e.g., Nicoderm®, Nicotinell®). Therefore, Extra® peppermint and Nicorette® ice mint chewing gums were used as the placebo and nicotine gums, respectively. The colour of both types of the gum was identical off-white. These two types were selected because of their similarity in flavours and aroma, although the shapes were slightly different. The placebo gum was rectangular, while the nicotine gum was square. It

was assumed that participants would remember the flavour and the aroma more than the shape of the gums after a few days in between the sessions.

Multiple-object tracking task. The current study implemented the attentional dynamic tracking paradigm. The task consisted of video animations created by Thomas Hagen at the University of Oslo (personal communication, September 18, 2017). A similar task is described in Espeseth and colleagues (2010) and Alnæs and colleagues (2014; 2015). Stimuli were 12 circular disks (20 mm) positioned onto a grey background (RGB: 128, 128, 128). The radius of the disks was 29 pixels with a minimum distance of 2 pixels between the disks. The size of the display area was 800 x 800 pixels stretched to fill the vertical size of the screen. Of the 12 disks, either two, three, or four were made to be the targets in their corresponding trials. At the beginning, all of the disks were presented in orange (RGB: 255, 140, 0), then the distractors were superimposed with a green colour (RGB: 0, 255, 0) while the targets remained orange. After 3000 ms, the targets flickered in the same green as the distractors and everything returned to orange as previously shown. In this uniform colour, each of the disks began moving in a random, independent, and non-overlapping fashion for 7000 ms. The motion was presented at a rate of 30 frames per second at a speed of 8.5 pixels per frame. Participants were allowed to move their eyes following the moving disks during the tracking. When the disks had stopped moving, one of the disks was circled in red with 50% probability that it would be one of the targets (Figure 2). The participants' task was to decide as quickly and accurately as possible if this red-circled disk was one of the targets assigned in the beginning. Participants had to press the corresponding button on the keyboard for a "yes" or a "no" answer. This task was self-paced, during which participants could start the next trial by pressing the space bar at will. Accuracy and response latency were recorded for each given response by the eye-tracking software described in the following sub-section.

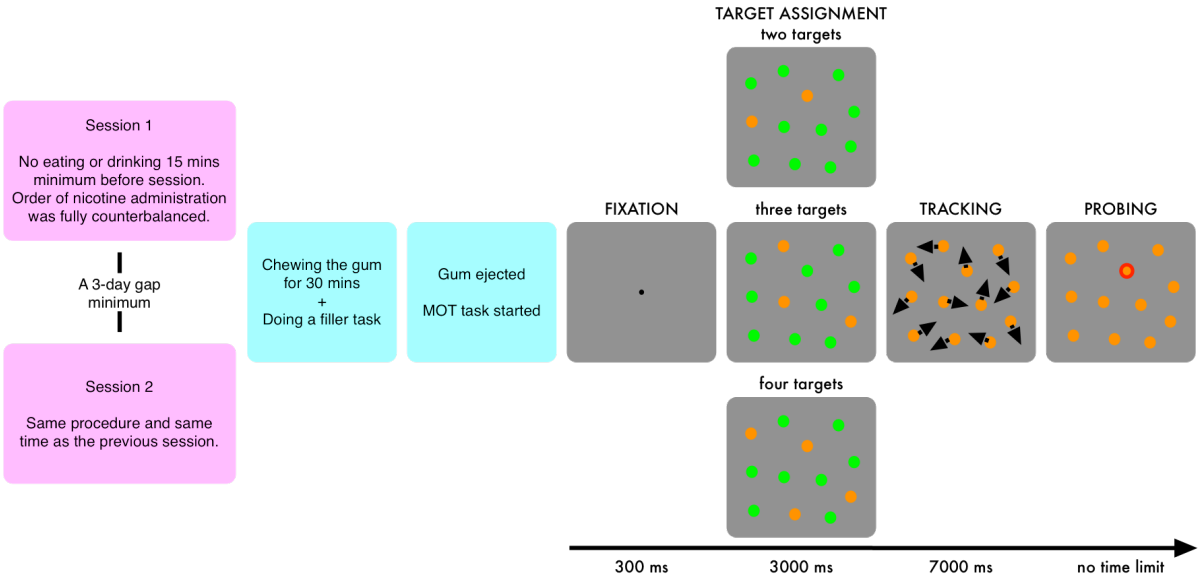

Figure 2. The experiment procedure (in magenta and cyan boxes) and the trial sequence of the MOT task (on a grey background).

Apparatus. The task was controlled by Experiment Centre® 3.2.17 software developed by SensoMotoric Instrument (SMI®, Berlin, Germany). A flat Dell P2213 VGA LCD monitor (47 x 29.40 cm) was used to present the stimuli. The display resolution was 1680 x 1050 pixels. Participants' head placement rendered a 60-cm distance between the screen and the cornea. Eye events and pupil diameter were recorded by the SMI RED500® remote eye-tracking device running on the iView X® 2.8 software at a sample rate of 60 Hz. The testing room was illuminated in constant and standard indoor lighting level throughout the experiment in both sessions for all participants. This illumination allowed the pupils to maintain an average size of about 4.64 – 4.76 mm at rest. The infrared-light-sensitive recording allowed binocular tracking in this illuminated room.

Manipulation check. A set of questionnaires was handed out to the participants at the end of the first and second sessions (Appendix 4). Data from the questionnaires were collected as a measure of participants' subjective assessments of the following aspects: (1) mood states; (2) concentration level; (3) estimate of the chewing gum identity (with vs. without active ingredient); (4) confidence level in estimating the gum identity; (5) rate of the similarity between the two types of the gum. Responses were given in a form of written verbal descriptions and Likert scales ranging from 1 to 10. For the statistical analyses, responses were categorised into several groups (see Results, pp. 21-22)

Procedure

Data collection from each participant was divided into two sessions and only one gum of either type was taken in each session (Figure 2). On average, there were eight days in between the sessions of the experiment (M_{day} = 8.57 days, *SD* = 10.24, range = 3-42 days). According to previous studies, there must be at least three days in between the sessions, such that the nicotine would have been fully eliminated from the previous session (Benowitz, 2009; Levin et al., 1996a; Levin et al., 2006). Participants were also required to refrain from eating or drinking, including water, at least 15 minutes before the start of each session. Oral consumption of food or drinks could change the neutral pH level in the mouth and, consequently, it could inhibit the absorption of nicotine through the lining of mouth (American Society of Health-System Pharmacists, 2013).

Upon arrival, each participant was ushered into the experiment room. There was only one participant at a time. In every session, each participant was instructed to take a gum from bottle A or B according to the participant's group assignment and chew the gum three or four times to break the gum sufficiently (Nicorette, 2016). Immediately after, participants had to place the gum in between the cheek and mouth gum or at the palate for approximately one minute. This process was to be repeated for 30 minutes so as to let the nicotine be completely absorbed into the bloodstream. The experimenter placed a digital timer in front of the participants, thus the participants could keep track of the time.

During the chewing process, participants did a filler perceptual task with face stimuli on the computer for about ten minutes. After completing the filler task, participants viewed some printed furniture catalogues or read their own book until the chewing period elapsed. Subsequently, participants ejected the gum into a plastic cup provided by the experimenter and were seated in front of the computer monitor. Participants used a head rest to remain stable posture during the eye recording. Prior to performing on the MOT task, participants' eye movements were calibrated and validated. The accepted angle of deviation was around 0.50° for both *x* and *y* axes. After the calibration and validation procedure, participants read the MOT task instruction (Appendix 5). The task begun after they pressed the space bar on the keyboard. At the end of the experiment, participants filled in self-reports. The whole procedure was held constant across sessions.

Data Processing and Analyses

Behavioural and eye data recorded by the eye tracker were released in the IDF format. This was converted into a TXT format to be readable to processing software. Measured

dependent variables were pupil diameter (mm), eye blink rates, response accuracy (%), and response latency (ms). Data processing was executed in Jupyter Notebook—an application that allows users to code, perform the computation, and produce results ("What is the Jupyter Notebook?", 2018). Data was then saved in a three-dimensional matrix. Custom-made coding in Python 3 language was used throughout the data processing. All statistical analyses were executed in JASP (JASP, 2018). The complete modules and codes to run the processing and other materials are made available on the Open Science Framework (see Appendix 6 for the link).

Questionnaires. Self-reports from the questionnaires were categorised into response groups and analysed with non-parametric tests. In one question, participants answered whether the gum taken in the second session was "with active ingredient" or "without active ingredient". These choices of answer were recoded as 1 and 0, respectively. The answers were then scored according to the assigned condition to check how many participants identified the chewing gum correctly. There were no reversed scales for questions posed with Likert-scale choices. Thus, responses were categorised accordingly.

Pupillometric and eye blink data. Binocular pupillometric data resulted in 600 samples of pupil size per participant per trial (i.e., 60 samples/second in a 10-s epoch). Pupil diameter from both left and right eyes were averaged into a single measure. Baseline epochs, for both placebo and nicotine conditions, were 700-ms long located between 1300 and 2000 ms (Figure 3). The trial epoch of interest was 8000-ms long starting at 2000 ms and lasted until 10,000 ms. Baseline and trial pupil sizes were then averaged separately for each task load level in the placebo and nicotine conditions. Baseline correction was carried out using the subtractive method, meaning that the average baseline pupil size was subtracted from the average trial pupil size. The eye-blink events were counted per trial (10,000-ms epoch) then averaged for each task load in the placebo and nicotine conditions. There was no baseline correction for eye blink rates.

Response accuracy. The average accuracy was calculated separately for placebo and nicotine conditions, and also for different target loads. Each trial was either a target (i.e., the probe highlighted a target) or a non-target trial (i.e., the probe highlighted a distractor). Participants had to press the "M" key for a "yes" response if the highlighted disk was one of the targets. If not, they had to press the "B" key for a "no" response. A response was scored 1 if the corresponding keypress was correct and 0 if it was not (i.e., if a "yes" response was made for a distractor or a "no" for a target). Responses from one participant had to be recoded due to keypress mistakes in one session as reported by the participant. First, the

participant pressed the "N" key instead of "M". Second, the "M" key was swapped for the "B" key. Thus, before the analysis, the "N" key was first recoded as "M". Then, the original responses in "M" were recoded to "B", and "B" to "M". Despite this mistake, data from this participant was retained because after correction, responses were reportedly consistent with mean accuracy of 86.11%.

Response latency. Participants' response latency for each trial was obtained through the SMI BeGaze software and recorded since the onset of the probe until a keypress indicating a yes-or-no response. Response latency was then averaged by participant and condition.

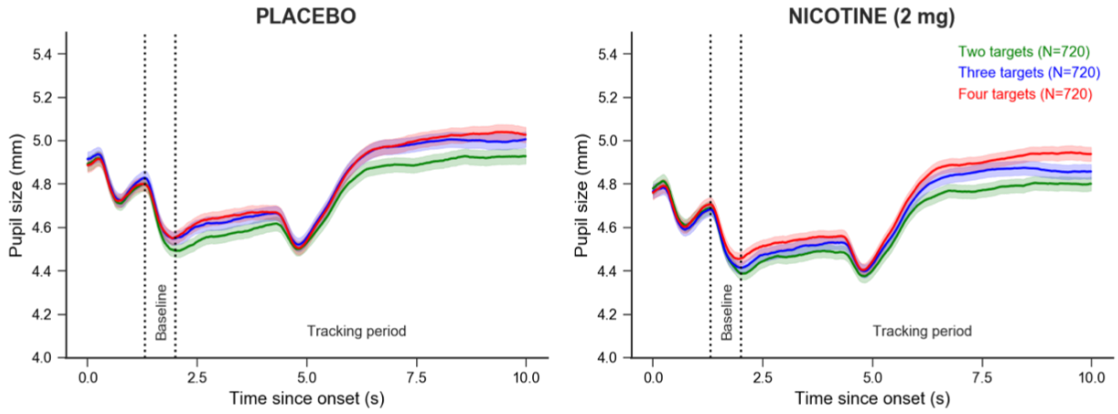

Figure 3. The graphs depict pupil trace since stimulus onset (0 ms). The dotted vertical lines separate absolutes of baseline and tracking pupil size (in mm) in placebo and nicotine conditions, across the two-, three-, and four-target loads. The 'valley' at around 5s corresponds to the start of the motion. Shaded area represents confidence interval of 95%.

Results

Participants' Subjective Assessment

The first aspect of the questionnaires (see Method, p. 18) was about participants' subjective assessment of mood states. Responses were categorised into three groups: positive (e.g., happy, interested, and enthusiastic), neutral (e.g., calm, relaxed, and "nothing special"), and negative (e.g., tired, anxious, and disinterested). The second aspect was participants' concentration level in placebo and nicotine conditions (range = 1 (*very distracted*) – 10 (*very focussed*)). Concentration levels were categorised into three groups: low (1-3), medium (4-7), and high (8-10). A contingency table chi-squared test was conducted to analyse whether or not there was a relationship between participants' mood states and concentration level. In the placebo condition, there was no relationship found between participants' mood state and concentration level, χ^2 (2) = 0.65, p = .724. Likewise, such relationship was not found in the

nicotine condition, χ^2 (2) = 0.43, p = .805. Additionally, a nonparametric paired-samples Wilcoxon signed-rank *t*-test was run to compare the concentration levels between the placebo and nicotine condition, regardless of participants' mood states. It was shown that there was no difference in the subjective concentration levels reported by participants in the placebo (M = 2.57, SD = 0.50) and the nicotine conditions (M = 2.73, SD = 0.45), W = 40, p = .208.

The third aspect concerned participants' discernment of the chewing gum identity taken in the second session. Participants were asked to estimate whether the gum contained an active ingredient or not. These estimates were scored (1 for correct estimate; 0 for incorrect estimate) and analysed using a binomial test. Result showed that the proportion of participants who discerned the gum identity (83.33%) was significantly higher than the proportion of participants who did not (16.67%), $p < .001$ (Table 1). Each participant's discernment of drug treatment was included in an additional analysis. Results of this analysis can be found in the last subsection.

The fourth aspect enquired participants' confidence level in estimating the identity of the chewing gum (range = 1 (*very doubtful*) – 10 (*very confident*)). Confidence levels were categorised into three groups: low (1-3), moderate (4-7), and high (8-10). The last aspect in the questionnaire concerned participants' rate of the similarity between the two types of the gum (range = 1 (*very dissimilar*) – 10 (*very similar*)). Again, responses were categorised into three groups: dissimilar (1-3), unsure (4-7), and similar (8-10). Frequency of participants in these three similarity-rate groups based on each discernment group can be inspected on Table 1. A three-way cross-tabulations analysis was conducted to assess the interaction between gum similarity, confidence level, and discernment. Results revealed that amongst participants with high confidence level, there was an association between similarity (or dissimilarity) of the gum and discernment, χ^2 (2) = 15, $p < .001$.

Table 1

Frequency of participants who discerned and did not discern the drug treatment and participants' similarity rates according to discernment groups.

Discernment	Placebo	Nicotine	Total
Discerned	13	12	25
Rated "dissimilar"	7	3	10
Rated "not sure"	6	7	13
Rated "similar"	0	2	2
Did not discern	2	3	5
Rated "dissimilar"	0	1	1

Rated "not sure"	1	2	3
Rated "similar"	1	0	1

Note. $N = 30$ (female = 20). Participants were asked whether the gum they took in the *second* session contained or did not contain an active ingredient.

Pupillary and Behavioural Responses

Participants' pupillary responses, eye blinks, response accuracy, and response times were analysed using separate 2 (Drug: placebo vs. 2 mg nicotine) x 3 (Load: two vs. three vs. four) repeated-measures analysis of variance (ANOVA). The Huynh-Feldt sphericity correction was used for the omnibus effects and the Bonferroni correction was used for the post-hoc test(s) of significant effects. Figures in this section show only statistically significant results and the baseline-corrected pupil trace.

Pupillary responses. First, the repeated-measures ANOVA with Drug and Load as within-participants factors was run to examine tonic pupillary responses between the placebo and nicotine conditions. Results of the analysis confirmed that there was an effect of Drug on tonic pupils, $F(1, 29) = 6.24$, $p = .018$, $\eta_p^2 = 0.177$ (Figure 4a). Specifically, tonic pupils were more constricted in the nicotine condition than in the placebo condition ($M_{difference}$ (henceforth M_d) = 0.11, $SE = 0.05$, $p_{Bonferroni\text{-}adjusted}$ (henceforth p_{Bonf}) = .018, Cohen's $d = 0.456$). Furthermore, results also showed a main effect of Load on tonic pupils, $F(2, 58) = 9.03$, $p < .001$, $\eta_p^2 = 0.237$, whereby tonic pupils were smaller in the two-target load than in the three-target ($M_d = -0.02$, $SE = 0.01$, $p_{Bonf} = .013$, Cohen's $d = -0.564$) and four-target loads ($M_d = -0.034$, $SE = 0.01$, $p_{Bonf} < .001$, Cohen's $d = -0.816$). There was no significant interaction between Drug and Load on baseline pupil size, $F(2, 58) = 2.55$, $p = .087$, $\eta_p^2 = 0.081$.

Second, the baseline-corrected pupil size was analysed to assess the changes in pupil sizes during the tracking interval (Figure 4b). This analysis revealed that phasic pupils in the nicotine condition did not evidently differ from the placebo condition, $F(1, 29) = 0.06$, $p = .815$, $\eta_p^2 = 0.002$. However, there was a significant main effect of Load on phasic pupils, $F(2, 58) = 11.94$, $p < .001$, $\eta_p^2 = 0.292$ (Figure 4c). Specifically, the phasic pupils in the two-target load were smaller than in the three-target ($M_d = -0.04$, $SE = 0.01$, $p_{Bonf} = .004$, Cohen's $d = -3.523$) and four-target loads ($M_d = -0.05$, $SE = 0.01$, $p_{Bonf} < .001$, Cohen's $d = -4.712$). The phasic pupils between the three-target and four-target loads were not significantly different ($M_d = -0.02$, $SE = 0.01$, $p_{Bonf} = .603$, Cohen's $d = -1.308$). An interaction between Drug and Load was not significant, $F(2, 58) = 0.51$, $p = .579$, $\eta_p^2 = 0.017$.

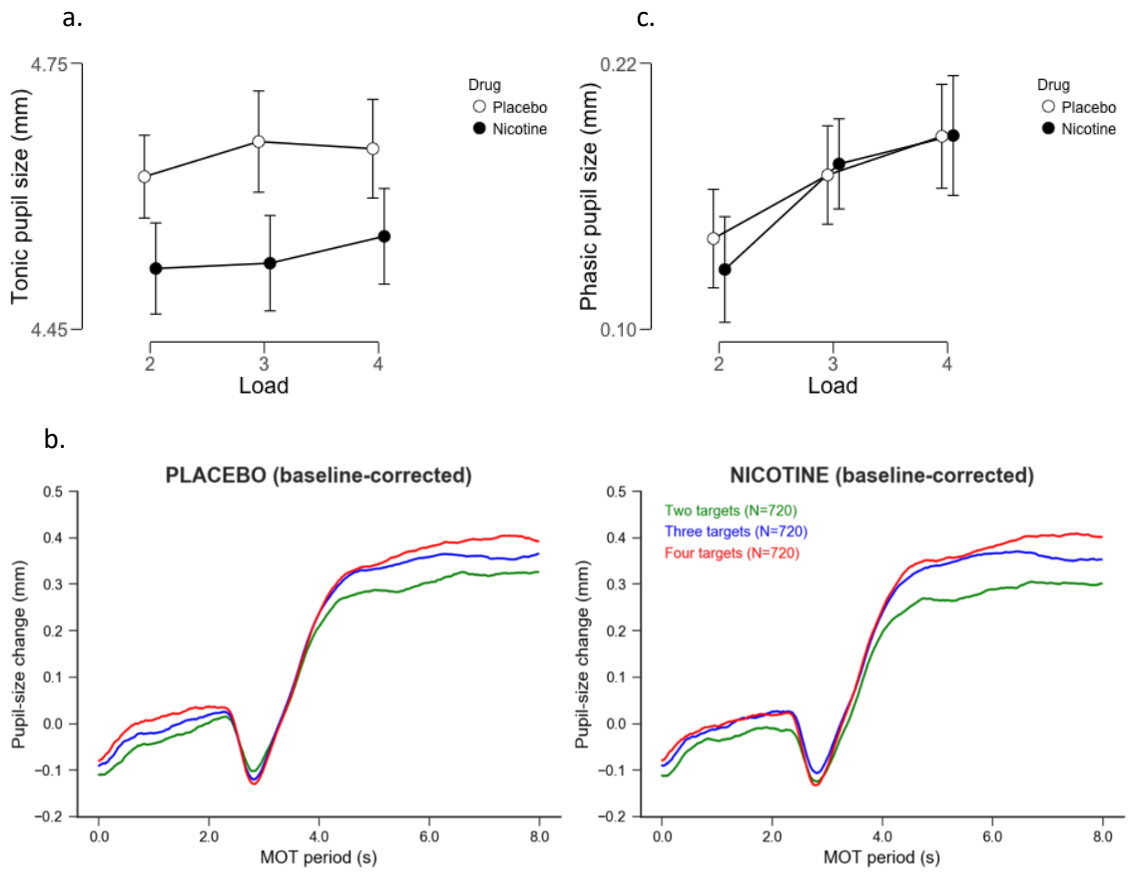

Figure 4. A significant main effect of Drug on tonic pupil size (a); averaged pupil traces during tracking interval in placebo and nicotine conditions after baseline correction (b); a significant main effect of Load on phasic pupil size (c). Error bars are confidence intervals of 95%.

Eye blink rates. The eye blink rates during the whole trial (10,000 ms) were analysed. Results revealed that there was a significant interactive effect between Drug and Load on eye blink rates, $F(2, 58) = 3.82$, $p = .028$, $\eta_p^2 = 0.116$ (Figure 5a). A follow-up test of simple main effects showed that in the nicotine condition, eye blink rates were higher in the two-target load than in the three-target ($M_d = 0.23$, $SE = 0.06$, $p_{Bonf} = .002$) and four-target loads ($M_d = 0.23$, $SE = 0.05$, $p_{Bonf} = .001$).

Response accuracy. Results demonstrated that there was a significant effect of Load, $F(2, 58) = 6.17$, $p = .004$, $\eta_p^2 = 0.175$, on response accuracy (Figure 5b). Response accuracy in the two-target load was higher than response accuracy in the three-target ($M_d = 3.12$, $SE = 0.96$, $p_{Bonf} = .006$, Cohen's $d = 0.592$) and four-target loads ($M_d = 3.61$, $SE = 1.08$, $p_{Bonf} = .004$, Cohen's $d = 0.611$). There was no difference in the response accuracy between the three-target and four-target loads ($M_d = 0.49$, $SE = 0.99$, $p_{Bonf} = 1.000$, Cohen's $d = 0.090$). No main effect of Drug, $F(1, 29) = 0.88$, $p = .356$, $\eta_p^2 = 0.029$, and interaction between Drug and Load, $F(2, 58) = 1.71$, $p = .190$, $\eta_p^2 = 0.056$, were found.

Response latency. Results revealed that there were no main effects of Drug, $F(1, 29) = 0.46$, $p = .505$, $\eta_p^2 = 0.015$, and Load, $F(2, 58) = 1.39$, $p = .257$, $\eta_p^2 = 0.046$, on response latency. The interactive effect between Drug and Load was also not significant, $F(2, 58) = 0.47$, $p = .627$, $\eta_p^2 = 0.016$.

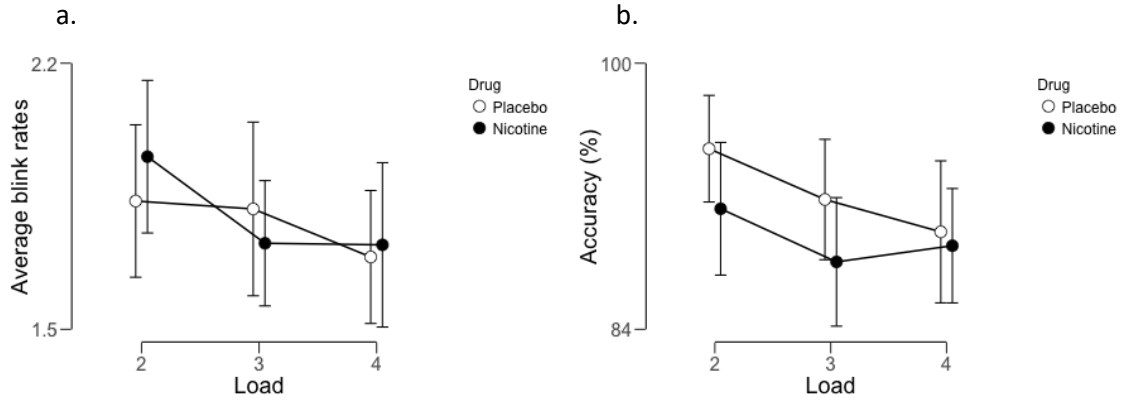

Figure 5. A significant interactive effect of Drug and Load on eye blink rates during the 10,000-ms epochs (a) and a significant main effect of Load on response accuracy (b). Error bars are confidence intervals of 95%.

Discernment of Drug Treatment

Additional 2 (Drug: placebo vs. nicotine) x 3 (Load: two vs. three vs. four) repeated-measures ANOVAs were executed separately for participants who discerned and did not discern the nicotine treatment. The analyses were done for tonic pupil size, phasic pupil size during the tracking interval, eye blink rates, response accuracy, and response latency. Figures are provided only for statistically significant results.

Participants who discerned. There were 25 out of 30 participants (83.33%) who discerned the drug treatment. Amongst this sample, there was a significant main effect of Load, $F(2, 48) = 6.74$, $p = .003$, $\eta_p^2 = 0.219$, on tonic pupils (Figure 6a). Specifically, tonic pupils were more constricted in the two-target load than in the three-target ($M_d = -0.02$, $SE = 0.01$, $p_{Bonf} = 0.044$, Cohen's $d = 0.526$) and four-target loads ($M_d = -0.03$, $SE = 0.01$, $p_{Bonf} = 0.003$, Cohen's $d = 0.760$). There were no significant main effect of Drug, $F(1, 24) = 3.69$, $p = .067$, $\eta_p^2 = 0.133$, and interactive effect of Drug and Load, $F(2, 48) = 1.99$, $p = .148$, $\eta_p^2 = 0.077$, on tonic pupils in this sample.

Analysis of phasic pupils during the tracking interval showed that the effect of Load was significant, $F(2, 48) = 8.96$, $p < .001$, $\eta_p^2 = 0.272$ (Figure 6b). Pupils were more constricted in the two-target load than the three-target ($M_d = -0.45$, $SE = 0.01$, $p_{Bonf} = .012$, Cohen's $d = -0.633$) and four-target loads ($M_d = -0.05$, $SE = 0.01$, $p_{Bonf} < .001$, Cohen's $d =$ -

0.837). No effect of Drug, $F(1, 24) = 0.03$, $p = .861$, $\eta_p^2 = 0.001$, and interactive effect between Drug and Load, $F(2, 48) = 0.86$, $p = .430$, $\eta_p^2 = 0.035$, on phasic pupils were found.

Results also revealed that there was a significant interaction between Drug and Load on blink rates, $F(2, 48) = 3.63$, $p = .034$, $\eta_p^2 = 0.131$ (Figure 6c). A follow-up test of simple main effects indicated that in the nicotine condition, eye blink rates were higher in the two-target load than in the three-target ($M_d = 0.27$, $SE = 0.07$, $p_{Bonf} = .001$) and four-target loads ($M_d = 0.28$, $SE = 0.06$, $p_{Bonf} < .001$).

On response accuracy, there was a significant effect of Load, $F(2, 48) = 4.3$, $p = .019$, $\eta_p^2 = 0.152$ (Figure 6d). Response accuracy was higher in the two-target than the three-target load ($M_d = 3.17$, $SE = 1.17$, $p_{Bonf} = .038$, Cohen's $d = 0.540$). No significant effect of Drug, $F(1, 24) = 1.05$, $p = .316$, $\eta_p^2 = 0.042$, and interaction between Drug and Load, $F(2, 48) = 2.14$, $p = .128$, $\eta_p^2 = 0.082$, were reported.

Results revealed that there were no main effects of Drug, $F(1, 24) = 0.02$, $p = .883$, $\eta_p^2 = 0.001$, and Load, $F(2, 48) = 0.52$, $p = .598$, $\eta_p^2 = 0.021$, on response latency. The interactive effect between Drug and Load was also not significant, $F(2, 48) = 0.64$, $p = .530$, $\eta_p^2 = 0.026$.

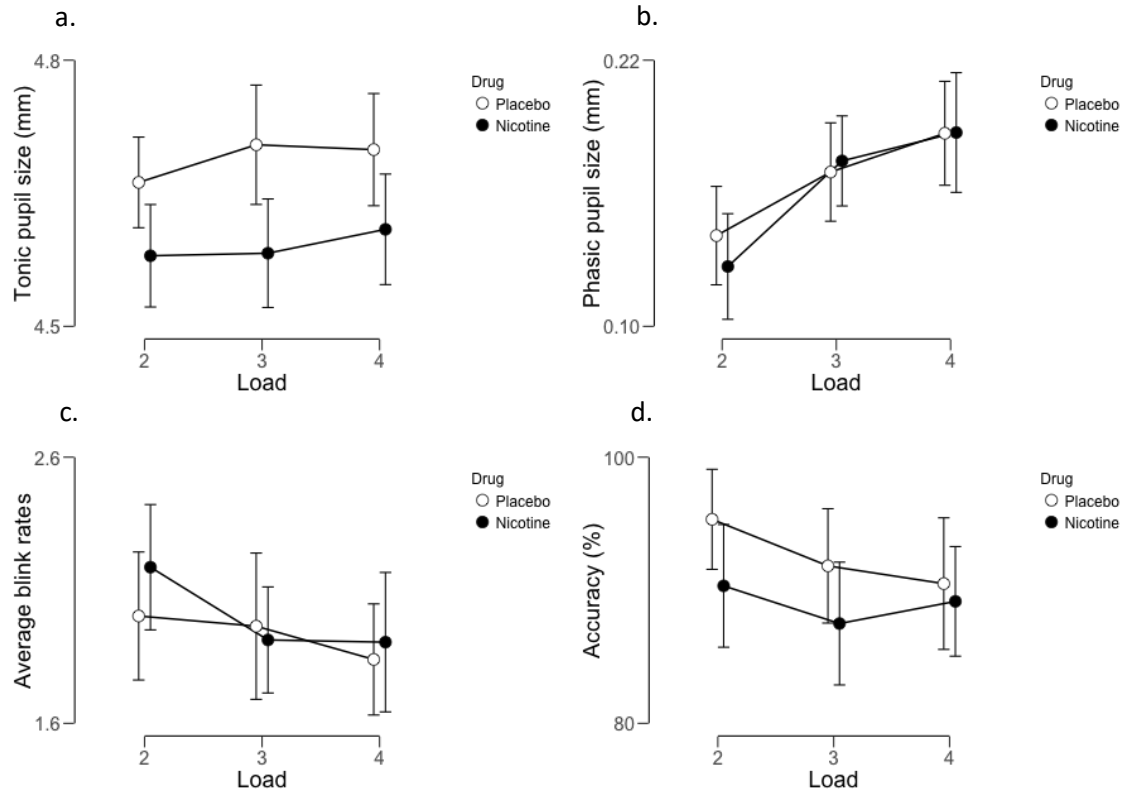

Figure 6. All figures depict statistical differences in the group who discerned the drug treatment. Significant main effects of Load on tonic pupil size (a), phasic pupil size (b), and response accuracy (d); a significant interaction between Drug and Load on blink rates (c). Error bars are confidence intervals of 95%.

Participants who did not discern. There were 5 out of 30 participants (16.67%) who did not discern the nicotine treatment. Statistical analyses yielded no significant effects of Drug nor Load on tonic pupil size, phasic pupil size, and response accuracy. However, there was a significant effect of Drug on eye blink rates, $F(1, 4) = 15.36$, $p = .017$, $\eta_p^2 = 0.793$, such that eye blink rates were higher in the placebo than in the nicotine condition, $M_d = 0.21$, $SE = 0.05$, $p_{Bonf} = .017$, Cohen's $d = 1.753$ (Figure 7a). Furthermore, results revealed a significant main effect of Load on response latency, $F(2, 8) = 9.19$, $p = .008$, $\eta_p^2 = 0.697$, whereby response latency was faster in the two-target load than in the four-target load, $M_d = -147.27$, $SE = 23.64$, $p_{Bonf} = .010$, Cohen's $d = -2.786$ (Figure 7b).

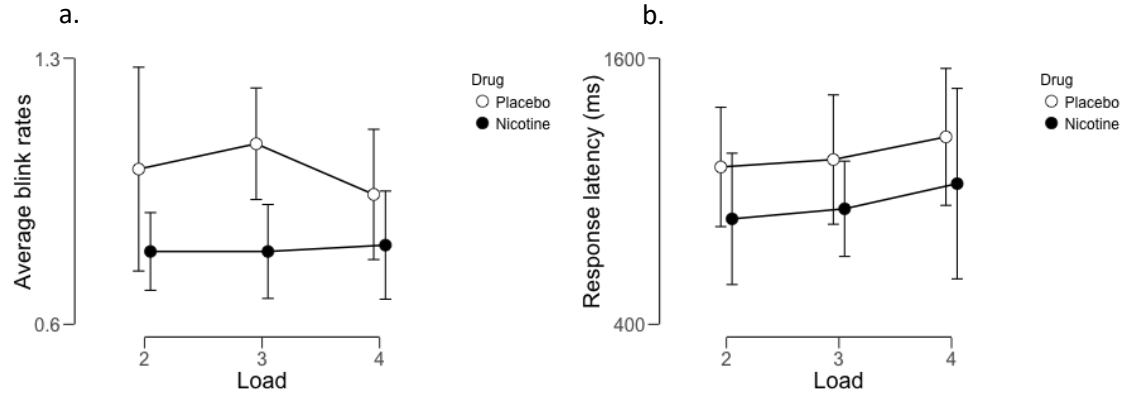

Figure 7. Significant main effects of Drug on eye blink rates (a) and Load on response latency (b). Error bars are confidence intervals of 95%.

Discussion

The current study aimed to investigate the baseline effects of nicotine on pupillary responses and cognitive performance. Participants were all non-nicotine users without pre-existing attentional deficits. Experiment with this type of samples helped eliminate withdrawal and tolerance effects. Nicotine withdrawal was associated with negative affect (Benowitz, 2009), which in turn could influence performance. Drug intake was manipulated using low-dose nicotine (2 mg) and taste-matched placebo (0 mg of nicotine) chewing gums. Participants' pupil size, eye blink rates, response accuracy, and response latency were measured during performance on a multiple-object-tracking (MOT) task wherein they were allowed to move their eyes during the tracking interval.

As expected, a small amount of nicotine was sufficient to change tonic pupil size. Tonic pupils were remarkably more constricted in the nicotine condition than in the placebo condition. Using pupillometry, our study showed that nicotine excited the cholinergic system and interacted with the nicotinic acetylcholine receptors in the parasympathetic nervous

system. Pupillary constriction was evidence of the iris sphincter muscles innervation that was caused by the release of acetylcholine in the iris. Our finding supported the findings of Lie and Domino (1999) as well as Erdem and colleagues (2015) showing pupillary constriction after smoking cigarettes containing relatively low nicotine, both in smokers and non-smokers. Additionally, our finding confirmed the notion that arousal was more likely to influence tonic pupillary responses (Beatty, 1982).

In our study, we varied task loads to enable us to compare differences in phasic pupil size between loads. Stimuli were made isoluminant to eliminate the constriction or dilation caused by the pupil light reflex (PLR) when looking at different elements in the displays. In line with the hypothesis, phasic pupil size increased proportionally with the task load: higher tracking load was found to cause larger pupil dilation. This was consistent with previous studies presenting evidence of task-evoked pupillary responses (TEPRs). Higher demands of the cognitive task were related with higher attentional effort (Espeseth et al., 2010). The attentional effort exerted by the individuals was reflected, amongst others, in their pupil size (e.g., Geng et al., 2015; Laeng et al., 2011; van Steenbergen & Band, 2013). The current study strengthened the previous findings showing that increasing task load accounted for increasing attentional effort and that pupil dilation could reliably index the fluctuations of attentional effort (e.g., Kahneman & Beatty, 1966; Kang et al., 2014; Smallwood et al., 2011).

With respect to eye blink rates, we found that eye blink rates were high when the task load was low and, conversely, eye blink rates declined as the size of the task load grew. This was in line with our hypothesis and a previous finding suggesting that a higher cognitive load was associated with inhibition of eye blinks (Siegle et al., 2008). This occurred potentially because higher attentional effort that was exerted during a higher cognitive load involved mental engagement, which in turn inhibited eye blinks. Furthermore, we found that the effect of task load on eye blink rates was moderated by nicotine. It was shown that eye blinks were more frequent in the nicotine condition than in the placebo condition only when the task load was low. When the task load grew to medium and high, there were no differences in eye blink rates between the nicotine and placebo conditions. Quite contrary to our hypothesis, we did not find an effect of nicotine alone on eye blink rates compared to the placebo condition. As demonstrated by eye blink rates, a small amount of nicotine that was administered one time might not be sufficient to mediate the release of dopamine. As a consequence, there was no enhancement in the dopamine-related cognitive performance. In literature, dopamine had been shown to facilitate cognitive performance, such as attention and sensorimotor integration, and lesion in dopaminergic system was consistently associated with impairment

in the aforementioned area (see Nieoullon, 2002). Due to the importance of dopamine, several treatments to augment dopamine release had been done by using, for example, nicotine (Imperato et al., 1986; Levin et al., 2006). However, augmented dopamine release by nicotine was argued to occur only amongst chronic smokers due to reduced brain monoamine oxidase A and B (Benowitz, 2009). Despite being in contrast to our hypothesis, it appeared that our finding supported Benowitz's argument. Our participants were all healthy non-nicotine users and most likely did not have lesion in dopaminergic system or reduced neural functions mediated by chronic nicotine consumption just as found in chronic smokers. Thus, presumably there had to be repeated or long-term exposure to nicotine in non-nicotine users for nicotine to augment dopamine release.

As expected, response accuracy was higher when cognitive load was low compared to medium and high cognitive loads. We found no difference in the selection accuracy between the medium and high cognitive loads. It was likely that the medium and high cognitive loads were deemed as equally difficult, thus accuracy was comparable between both conditions. Results also revealed that the task load, or the inherent difficulty level of the task, appeared to have a stronger effect on response accuracy. We did not find a reliable effect of nicotine on selection accuracy. If anything, selection accuracy in the nicotine condition appeared to be lower than selection accuracy in the placebo condition. One explanation of this finding could be that a dose of nicotine as small as 2 mg did not have a direct effect on facilitating task performance in the multiple-object-tracking paradigm. Nevertheless, this argument was not supported by a previous study finding that performance was improved amongst individuals who were induced with 1 mg of nicotine (Kumari et al., 2003). The difference between the current study and that of Kumari and colleagues was that nicotine was administered through subcutaneous injection in the latter. This led to another explanation as to why the low dose of nicotine in our study did not show a performance-enhancing effect. Nicotine administration through oral consumption might not be an effective manipulation because participants might be able to distinguish the difference despite both types of the chewing gum had been taste-matched. Albeit no reports of dizziness and nausea, the possible unpleasant side effects of nicotine in the mouth or throat might be too distinct or too strong for non-nicotine users, such that it distracted them from performing efficiently on the task. (The problem of the distinguishable taste is addressed separately below).

In contrast to our hypothesis, neither nicotine nor task load had an effect on response latency. Our finding did not replicate other findings (e.g., AhnAllen et al., 2015; Levin et al., 1996a, 1996b; White & Levin, 1999) demonstrating shorter response latency in the nicotine-

induced condition. However, most of the previous studies found an effect of nicotine on response latency amongst participants with pre-existing attentional deficits, such as patients with Alzheimer's, ADHD, and schizophrenia. In this type of samples, nicotine might have a restorative effect. For instance, Levin and colleagues (1996b) demonstrated that nicotine attenuated the adverse effects of the antipsychotic drug haloperidol and improved response latency. The restorative effect of nicotine was also found in smokers. It had been shown that smokers who were deprived of nicotine performed poorly on a cognitive task, but performance improved after nicotine administration (e.g., Ernst et al., 2001). Detrimental effects of nicotine withdrawal were also reflected in the reduced P3a and P3b amplitudes (Evans et al., 2013). From this evidence, we surmised that perhaps there had to be a low baseline response latency for nicotine to act as a stimulant.

Albeit the result on response latency was not in line with our hypothesis, this finding supported previous studies suggesting that a single nicotine administration to healthy non-nicotine users did not improve cognitive performance or enhance P300 amplitudes (e.g., Ernst et al., 2001; Evans et al., 2014). Nevertheless, there were also some methodological challenges in our study. First, the MOT task that we used might not be sensitive to examining response latency. Although participants were instructed to make a selection as fast as possible, we did not set a time limit at the probing phase. The next trial started at participant's will, thus allowing participants to take time to identify the probe. Second, at the probing phase, one object was superimposed and participants had to indicate whether the superimposed object was a target or a non-target. This method was one of the standard variants of MOT task (Pylyshyn, 2004) to assess object indexing in attention research (Pylyshyn & Storm, 1988). This was normally used to measure selection accuracy in multiple and parallel tracking (Sears & Pylyshyn, 2000). Studies using this method consistently found that accuracy landed above 85% (Pylyshyn, 2004; Sears & Pylyshyn, 2000). Therefore, even though we also found that selection accuracy was above 85% in placebo and nicotine conditions, the MOT task was more sensitive in measuring focal and peripheral attention than speeded or slowed responses. Several plausible solutions to these challenges were to execute analyses that were compatible with the method. For instance, it might be more useful to analyse response time variability at each task load across trials. It might also be revelatory to analyse and compare response latency for targets and non-targets.

A serious limitation of the present study is that, despite the double-blind procedure, the self-reports showed that a large proportion of the participants (83.33%) were able to distinguish which of the two types of the chewing gum they had taken (i.e., whether the

chewing gum had an active ingredient or not). This indicated that discernment of nicotine treatment was more than just guessing (i.e., above 50%). It would be quite intuitive to assume that if participants discerned a drug treatment, then performance would improve due to a motivational-related effect of stimulant (Ilieva & Farah, 2013). However, we noted that our results showed there was no improvement in task performance while participants were induced with nicotine. Based on this finding, we surmised that participants who discerned the drug treatment might have had a negative expectation (i.e., that the drug might deteriorate performance). This expectation could be linked to possible felt negative side effects caused by the oral consumption of nicotine, such as tingling sensations and a sore throat. However, there was a possibility, too, that participants attempted to balance performance in both sessions due to the discernment and the expectation. Meanwhile for the rest of the participants who did not discern the drug treatment (16.67%), nicotine and task load did not have effects on either tonic or phasic pupil size and task performance. In contrast, eye blink rates were remarkably less frequent in the nicotine condition and response latency was faster in the lower task load. Nevertheless, note the sample size was too small to make any statistical relevant conclusion (Button et al., 2013).

A plausible explanation to how a large portion of participants could discern the nicotine treatment was the taste of each chewing gum. We administered nicotine using a 2-mg nicotine chewing gum (Nicorette) and placebo using a taste-matched chewing gum (Extra peppermint) to non-nicotine users. Albeit the placebo chewing gums were taste-matched, distinct taste and side effects caused by the nicotine chewing gum could be more salient amongst non-nicotine users. In self-reports, participants in the nicotine condition reported bitter taste and a tingling sensation during and after chewing the gums. Some participants also reported a sore throat post-administration. This might explain discernment of nicotine treatment and performance expectation (either negative or positive). Although a previous study had also used chewing gums to administer drug manipulation (Ernst et al., 2001), they did not collect self-reports on participants' subjective assessment of the chewing gums. Thus, it was unclear whether participants in their study also experienced the effects that our participants had. Future studies are strongly encouraged to consider carefully the method of administering drug manipulation. Subcutaneous or even intravenous injection may be more effective than oral administration using chewing gums.

Self-reports could also be useful in analysing participants' mood states, concentration levels, anxiety traits, and side effects. This could help identify some confounding factors that existed. For example, perhaps participants performed better in the nicotine condition because

they felt happier than when they performed in the placebo condition. We did collect information about participants' subjective mood states and concentration levels using a questionnaire. Nevertheless, we developed our own scales, which were not validated. In the future studies, we could use validated and reliable scales such as Profile of Mood States (POMS; McNair et al., 1981; cited in Levin et al., 1998), Adult Temperament Questionnaire-Attentional Control Scale (ATQ-ACS; Evans & Rothbart, 2007), Cognitive Failures Questionnaire (CFQ; Broadbent et al., 1982), State Trait Anxiety Index (STAI; Spielberger et al., 1970; cited in Ernst et al., 2001), and Subjective Treatment Emergent-Symptoms Scale (STESS; Guy, 1976). Information from these scales could help provide a well-rounded explanation as to why a treatment was effective or not.

Mainly due to ethical consideration and time limitation, our study only compared a small-dose nicotine with a placebo. If we were able to vary the nicotine doses, we could have assessed the effects of low-, medium-, and high-dose nicotine against each other and a placebo. This assessment could have enabled us to determine the threshold at which healthy participants might benefit from nicotine administration in a cognitive task. Levin and colleagues (1996b) varied the nicotine doses in their study, but their participants were schizophrenic smokers, so it would not be directly comparable to healthy participants with no pre-existing attentional deficits. It may be arguable that a small dose of nicotine is not sufficient to improve cognitive performance, as shown in our study. However, there is still equivocal findings surrounding this issue. Kumari and colleagues (2003) used 1 mg of nicotine and placebo that were injected subcutaneously, and they found brain activation and behavioural improvement in the nicotine condition. In contrast, Evans and colleagues (2014) used a higher dose of nicotine (7 mg) and found no enhanced P300 amplitudes and behavioural performance in the nicotine condition relative to the placebo condition. Therefore, it is suggested that future studies vary the nicotine doses and employ a placebo to establish the baseline effect of nicotine on nervous system activation and cognitive performance in healthy participants with no pre-existing attentional deficits.

Another limitation in our study includes the number of trials, the randomisation of load-level sequence, and the data analysis. First, we employed only 72 trials per participant in each condition. The trials were presumably too short to measure artefact-free pupillary and behavioural responses. Consequently, this could lead to low signal-to-noise ratio (i.e., the noise outweighs the signal). One of the noise sources could come from participants not being alert and engaged in the task. This could also be one of the reasons why no effects of nicotine were observed on selection accuracy and response latency. It might take more trials for a

small-dose nicotine administered orally to act on motoric and central nervous systems, and a few trials would not allow us to measure the effect of nicotine. Second, the 72 trials were broken down into four blocks and each block had the same order of trial. Each block also always begun with gradual load levels, meaning that it begun with two-target trial followed by three-target, then four-target trials. This sequence of stimulus and trial types could potentially cause a preparatory response from participants even before imperative stimuli were presented (Woodman, 2010). Preparatory responses could contaminate the phasic pupillary waveforms because then modulation had happened during the baseline period and was not evoked by the stimulus. Consequently, data would be obscure and the interpretation of the data could be misleading. Third, due to time restraint, data analyses in our studies were limited to two-way analysis of variance on averaged pupil size, eye blink rates, accuracy, and response latency. It could be more informative if we analysed the data on a participant or trial level. For example, we could analyse the correlation between absolute pupil size per participant and task performance (either response accuracy or latency) in each condition separately. By doing this, we would be able to see whether participants with larger pupil size had a better performance or not. The correlation between pupil size and task performance could also be done based on each trial to examine if pupillary response prior to the presentation of the critical stimuli differed between trial types. If it was shown that there were differences in the pupillary waveforms between trial types before the critical stimuli appeared, then we should be cautious about noise in our data. Therefore, future studies are highly encouraged to have longer trials, fully randomise the trial sequence, and conduct additional necessary analyses that are informative about the data.

In our study, since it was based on a rather small sample, we did not control for biological individual differences (e.g., ethnic origin, sex, and genotype) in nicotine reception. It has been shown that Asians have lower intake and slower nicotine metabolism compared to Caucasians (Benowitz et al., 1999; 2002). Furthermore, unlike men, the effectiveness of nicotine administration amongst women is highly correlated with genotype (Yudkin et al., 2004). This demonstrates that genetic differences interact with sexes in nicotine reception. Future studies should take into consideration factors such as ethnic origin and sex to be able to elucidate nicotine-related variability in performance, brain activations, and pupillary responses. Furthermore, there is also a possibility of running exploratory data analysis and permutation test in this area to generate robust and directive findings.

Conclusion

Overall, the present study shows that pupillary responses can provide a reliable measure for tonic physiological changes caused by a psychopharmacological stimulant like nicotine. It also confirms, as in previous studies, that pupillary changes are a reliable index of cognitive processing and, in particular, for attentional load. Additionally, results of eye blink rates are consistent with behavioural results. Eye blink rates show that a single administration of a small-dose nicotine in healthy participants does not stimulate dopaminergic system. This helps explain why task performance does not improve after nicotine administration. We find that task load has a stronger influence on pupillary changes, eye blink rates, and selection accuracy.

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Appendices

Appendix 1

UiO : University of Oslo
Faculty of Social Sciences – Departement of Psychology

Intan Kusuma Wardhani
intankw@student.sv.uio.no

CC: Bruno Laeng

Ref.number: **1730636**
Date: 11 May 2017

Ethical evaluation of research project

Your project, "Effect of nicotine on divided attention" has been ethically evaluated by the Department of Psychology's internal research ethics committee based on received information.

Your project, "Effect of nicotine on divided attention" has been exempt from full review and thus approved by the Department of Psychology's Research Ethics Committee. Remember to register the project at the department through net form: https://nettskjema.uio.no/answer/51849.html.

Sincerely yours, on behalf of the Committee,

Erik Stänicke and Mona-Iren Hauge
Members of the Department of Psychology's Research Ethics Committee

Appendix 2

UNIVERSITY OF OSLO
FACULTY OF SOCIAL SCIENCE
Department of Psychology

PARTICIPANT INFORMATION SHEET
Divided attention

The purpose of the study
The purpose of this study is to investigate participants' capacity for divided attention. This study is conducted by Intan K. Wardhani (intankw@student.sv.uio.no) under the supervision of Professor Bruno Laeng (bruno.laeng@psykologi.uio.no) .

What is involved
You are first asked to fill out a consent form, consume a gum (either with or without active ingredient in each session) for 30 minutes, and then complete an attention-demanding task involving the presentation of circles moving randomly on the computer screen. Your eye movements will be tracked and recorded during the session. The completion of the task will take approximately 60 minutes.

Participation and withdrawal
Participation in this study is completely voluntary and you are free to withdraw from this study at any time without prejudice or penalty. If you wish to withdraw, simply stop undertaking the exercises. If you do withdraw from the study, the materials that you have completed to that point will be deleted and will not be included in the study.

Risks
Participation in this study should involve no physical or mental discomfort, and no risks beyond those of everyday living. Should you find any question or procedure to be invasive or offensive, you are free to omit answering or participating in that aspect of the study.

Confidentiality and security of data
All data collected in this study will be stored confidentially. Only members of the research team will have access to the identified data. All data will be coded in an unidentifiable manner and subsequently analysed and reported in such a way that responses will not be able to be linked to any individual. The data you provide will only be used for the specific research purposes of this study.

Ethics clearance and contacts
This study has been cleared in accordance with the ethical review processes of the University of Oslo.

If you would like to learn the outcome of this study in which you are participating, you can contact us at the e-mail address above after completion of the study and we will send you the Abstract of the study and findings.

Thank you for your participation in this study.

IKW (Project leader)

Appendix 3

Consent Form

I, hereby, agree that I will participate voluntarily in the research project “Divided Attention”.

My signature indicates that I have received written and oral information about the project.
This consent includes my understanding that I will be requested to take an oral tablet (a gum) for 30 minutes and then perform a computerised task for about 60 minutes. I am also aware that my eye movements will be monitored throughout the task.

I may at any time resign from the project, without stating reasons, requiring that all data related to my investigation will be deleted. Only project staff at the Department of Psychology will be able to gain access to identifiable data regarding my participation in the survey. Information about me will, in unidentified form, be retained for project completion.

_____	_____	____/____/____
Signature and name	Place	Date
(Participant)		
	_____	____/____/____
	Place	Date

I confirm that I have given information about the study:

_____	_____	____/____/____
Signature and name	Place	Date
(Project leader)		
	_____	____/____/____
	Place	Date

Appendix 4

Please circle a number and/or write down your answer that you find very relevant with your situation.

SESSION 1

Q1 - From 1 (*very distracted*) to 10 (*very focussed*), how focussed were you during the experiment in session 1?

1 2 3 4 5 6 7 8 9 10

Q2 - What did you feel during the first 30 minutes while chewing, doing the first task, and reading the catalogues?

_____

SESSION 2

Q3 - From 1 (*very distracted*) to 10 (*very focussed*), how focussed were you during the experiment in session 2?

1 2 3 4 5 6 7 8 9 10

Q4 - What did you feel during the first 30 minutes while chewing, doing the first task, and reading the catalogues?

Q5 - Which type of gum do you think you took in today's session?

WITH active ingredient WITHOUT active ingredient

Q6 - From 1 (*very doubtful*) to 10 (*very confident*), how confident are you in answering Q5?

1 2 3 4 5 6 7 8 9 10

Q7 - Try to remember: From 1 (*very dissimilar*) to 10 (*very similar*), how similar was the taste of the two gums?

1 2 3 4 5 6 7 8 9 10

Appendix 5

A group of distractors and targets in the form of orange disks will appear on the screen.
The *distractors* will be highlighted to green and the *targets* will remain orange.

Then, the targets will flicker to green before all of the disks become orange again
and move randomly on the screen.
Pay attention to the movement of the target disks.

At the end of each trial, one disk will be circled in red colour (O).
Your task is to decide, **as fast and accurate as possible**,
if the circled disk was one of the targets assigned at the beginning.

Press the yellow key if YES; press the red key if NO.
After your response, press the space-bar to move on to the next trial.

Please keep your eyes open (no blinking) during the animation.

Press spacebar to continue

Appendix 6

https://osf.io/hk5uq/?view_only=60b5f8dba05a4dc0b0e41ce77ad5235e