MASTER THESIS

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DOES THE PILL IMPEDE UNLEARNING FEAR?

THE IMPACT OF ORAL CONTRACEPTION USE ON FEAR EXTINCTION IN AN ONLINE CONDITIONING TASK

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Abstract

Research on psychological effects of oral contraception (OC) use has suggested negative effects. Specifically, OC use has been linked to fear extinction deficits. This study examined the effect of OC use and OC pill phase on fear extinction. 251 participants completed an online experiment including a fear conditioning task and multiple questionnaires. In the first phase of the conditioning task, an image was conditioned to evoke fear in participants. In the second phase participants received extinction training to 'unlearn' previously acquired fear. Fearfulness ratings to the conditioned stimulus over the experiment were analysed and compared between groups of OC users and natural cyclers (NCs) and between OC users in different pill phases. Firstly data-driven latent growth modelling (LCGA) identified three distinct fear conditioning trajectories in participants, indicating their success of extinguishing fear. Contrary to the expectations, OC users and OC users in an active pill phase were not overrepresented in the class of poor extinguishers (N = 114). Also, no effects in the classes of normal conditioners (N = 77) and low fearful conditioners (N = 60) were found. Secondly, final fear scores of participants after extinction training were compared. OC users and OC users in an active pill phase did not show more fear compared to NCs and OC users in a pill-free week. Results of this study do not indicate disadvantageous effects of OC use and do not show an acute effect of hormones from the pill. Both hypotheses were rejected, but more research is needed to definitively rule out any unshown effect. Samples were small, resulting in low meaningful comparison of class subgroups containing under 10 OC users. Future studies should contain more OC users and more NCs. A distinction of NCs in different menstrual phases is recommendable for disentangling effects of endogenous and exogenous hormones. The approach presented in this study can be used in future research.

Introduction

Worldwide, more than 100 million women use oral contraception (OC), or: 'the pill' (Montoya & Bos, 2017). In the Netherlands alone, 1.1 million women take OC, making it the fourth most used medicine in the country – destined for only half the population (Stichting Farmaceutische Kengetallen, 2020). Despite broad use of the contraception method, many people are worried about unknown side effects of OC and do not feel well-informed about them (Schouten, 2021). Research examining psychological effects is limited but does show some concerning potential consequences (Lewis et al., 2019; Montoya & Bos, 2017; Schouten, 2021). Previous findings suggest hormonal alterations due to OC use can affect socio-emotional behaviour and underlying brain functions (Lewis et al., 2019; Montoya & Bos, 2017). Negative effects of OC use have been associated with stress and emotion regulation (Lewis et al., 2019; Montoya & Bos, 2017; Petersen et al., 2014; Roche et al., 2013; Kirschbaum et al., 1995; Kirschbaum et al., 1996; Kirschbaum et al., 1999; Bonen et al., 1991; Meulenberg et al., 1987;

Meulenberg & Hofman, 1990; Bouma et al., 2009; Crewther et al., 2015; Rohleder et al., 2003; Cohoon & Lovallo, 2013; Hofman, 1990). More specifically, research has demonstrated negative effects of OC use for fear regulation processes as well (Raeder et al., 2019; Graham et al., 2018; Merz et al., 2012; Lisofsky et al., 2016; Petersen et al., 2014). Not only can OC use lead to fear-related structure and activity alterations in the brain, but it may also impede recovery from anxiety disorders with exposure therapy treatments (Merz et al., 2012; Lisofsky et al., 2016; Raeder et al., 2019; Graham et al., 2018). Even though research findings are not univocal, and effects often do not manifest in subjectively measured stress and fear, evidence suggests OC interferes with natural emotion and fear regulation processes. As emotion regulation problems have been linked to anxiety and depression, OC use could lead to the risk of developing mood symptoms (Gross & Levenson, 1997; Joormann & Gotlib, 2010; Cisler et al., 2010; Campbell-Sills & Barlow, 2007; Schäfer et al., 2017). Anxiety disorders already belong to the most common mental diseases, especially amongst women (Kessler et al., 2005; Gustavsson et al., 2011; Alonso et al., 2004). They moreover appear to be relatively chronic, with patients often suffering from relapses (Gustavsson et al., 2011; Alonso et al., 2004; Vervliet et al., 2013). As exposure therapy is one of the most effective strategies to treat anxiety disorders, potential impeding effects of OC use for exposure treatment effectivity could be very problematic from this recovery perspective too (Hofmann, 2008). It is important to clarify the exact impact of OC for fear regulation in order to adequately educate women about this. That way, (potential) OC users will be well-informed, but not unnecessarily aversive to the pill out of uncertainty. Currently, there appears to be a trend of 'pill tiredness' such that OC use among (young) women is decreasing rapidly (Van Sprundel, 2019). Clearer understanding of and education about OC could prevent mass hysteria that may be contributing to this trend. Thus, the aim of this study is to contribute to such a clearer understanding by investigating the role of OC use in a fear extinction task. This study investigates whether women using OC show fear extinction deficits when compared to non-users. As the experiment will be conducted online, hopefully a large sample can be gathered that includes subgroups which are often underrepresented in related lab studies. Here for instance, we hope to gather a large enough group of naturally cycling women to use as a control group. By knowing whether OC use really impairs fear extinction processes not only education can be improved, but hopefully also fear and anxiety treatments can be optimised for the suffering group, and potentially prevented for women who may run a higher risk of mood and anxiety side effects of the pill.

Theoretical framework

Fear

Fear, along with anger, is considered one of the most prototypical human emotions (Shiota & Kalat, 2012). It is defined as *'the response to a perceived danger, either to oneself or to a loved one'* and can appear without previous experience of threat (Shiota & Kalat, 2012, p. 158; Marks & Tobena, 1990). Fear usually subsides quickly when the threat is gone (Shiota & Kalat, 2012). Unlike fear, *anxiety* is not an acute emotional response but rather a constant anticipation of impending doom and a continuous fear of low intensity (English & English, 1958, p. 34-35). Even though people experience fear as an unpleasant and disturbing emotion, it is very useful as it enables us to adapt to our environment. Fear readies the body for quick, vigorous action to deal with dangerous stimuli and threatening situations (Shiota & Kalat, 2012). It is characterized by the activation of the sympathetic nervous system and increased physical arousal in de form of an increased heart rate, heightened blood pressure and sweat production. This allows vigorous action, and a potentially life-saving reaction, also known as the 'fight' or 'flight' response (Shiota & Kalat, 2012). Eventually, by protecting us from harm, fear serves the evolutionary goal to help us live longer (Shiota & Kalat, 2012; Marks & Tobena, 1990).

Learning fear

With the exception of the fear for sudden loud noises that appears to be present from birth, the majority of people's fears are learned (Shiota & Kalat, 2012). However, we may be predisposed to learn to fear certain events and objects, especially if they are unpredictable and uncontrollable – like snakes (Shiota & Kalat, 2012; Marks & Tobena, 1990). Fear learning happens in different forms; one of which is elementary associative learning (Marks & Tobena, 1990). The term associative learning refers to people's ability to learn about relationships between events, such that a link is formed between them (Mitchell et al., 2009). Specifically: in emotional associative learning people may link a certain stimulus to an emotion that they experienced during exposure to that stimulus (Shiota & Kalat, 2012). In case of threat, a negative emotion can be linked to the situation or stimulus, whereafter this is perceived as dangerous. After this fear learning and the formation of an emotional memory, the relevant situation or stimulus has become conditioned and can by itself evoke the emotional, fearful response – even in the absence of actual threat (Pace-Schott et al., 2015).

Fear extinction

In the same way people learn to fear stimuli and situations if they link them to a negative emotion, positive or neutral experience with these stimuli can make them less frightening, stimulating the so-called 'extinction' of fear (Shiota & Kalat, 2012). *Fear extinction* is a crucial and normal part of life, as it enables organisms to adapt their behaviour to a changing environment and is an important aspect of emotion regulation (Bouton, 2004; Pace-Schott et al., 2015). It is argued that rather than unlearning previously learned fear, fear extinction involves the formation of a new memory trace without

affecting previously learned fear memories (Bouton, 2004; Pace-Schott et al., 2015). The new memory formed after fear extinction is thought to competitively inhibit the memory of the associated situation and previously learned emotion, and the outcome is thought to be context-dependent (Bouton, 2004; Pace-Schott et al., 2015). In fact, fear extinction processes appear to be somewhat fragile. Over time, the extinction memory traces may become weaker. Eventually, they can become dominated by previously learned fear (Vervliet et al., 2013). Accordingly, laboratory studies have demonstrated that although conditioned fears are easy to extinguish, they recover easily (Vervliet et al., 2013).

Studying fear

Fear conditioning

In fear experiments researchers often use the method of *fear conditioning* to purposefully make participants acquire fear. The method was based on classical conditioning experiments by the Russian physiologist Ivan Pavlov (Bouton, 2004; Shin & Liberzon, 2010; Mitchell et al., 2009). Fear conditioning is a procedure in which participants learn in the acquisition phase that a new, previously neutral conditioned stimulus (CS), like a tone, image or colour, predicts an aversive unconditioned stimulus (US), like an electrical shock or other aversive element (Shiota & Kalat, 2012, p. 118; Watson & Rayner, 1920; Marks & Tobena, 1990; Shin & Liberzon, 2010; Pace-Schott et al., 2015). The pairing with a shock or aversive stimulus induces the association that the tone, image, or colour – here regarded to as 'CS+' – is threatening as it leads to a negative consequence. This results in the expression of fear. After repeatedly pairing the CS to the US during the acquisition phase of fear conditioning, fear for the conditioned stimulus remains, even when it is presented without its initial aversive element (Shin & Liberzon, 2010; Pace-Schott et al., 2015). A second neutral stimulus that is also presented during the acquisition phase, but never paired with the US, is oftentimes used as a control stimulus and is labelled 'CS-'. In the extinction phase following acquisition, repeated presentation of the CS+ without the US typically leads to the extinction of the previously learned fear response in participants (Leen et al., 2021; Bouton, 2004; Pace-Schott et al., 2015). In fear conditioning experiments extinction learning abilities of participants can be investigated by monitoring their emotional fear responses to the CS at different times in the process of fear learning.

Measuring fear

Generally, three aspects of human emotions can be measured: subjective emotional state, physiology and behaviour or actions (Shiota & Kalat, 2012). To measure subjective emotional fear, self-reports of fear can be elicited by asking participants how scared they feel. Here, a scale-rating usually represents the amount of fear participants subjectively experience. Fear can also be measured physiologically,

which allows a more objective assessment. With physiological measures, fear is typically indicated by increased heart rate and blood pressure, rapid and irregular breathing, and increased skin conductance (Shiota & Kalat, 2012; Pace-Schott et al., 2015). Behavioural measures of fear include monitoring fearful facial expressions (which for instance are characterised by lifted inner and outer eyebrows, eyebrows that are pulled together, widened eyes, contracted muscles below the corners of the lips and pulled down skin of the lower cheeks (Ekman et al., 1987)), the startle reflex, freezing behaviour, avoidance behaviour and attentional behaviour (directed towards distracting, threatening content) (Shiota & Kalat, 2012; Shin & Liberzon, 2010; Pace-Schott et al., 2015).

Fear and the brain

Fear can also be investigated with neuroscientific measures, using methods like electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). The brain regulates the autonomic nervous system and drives bodily changes of an emotional (fear) reaction via integrated reflexes through the brainstem to the spinal cord and organs (Shiota & Kalat, 2012).

The amygdala, a subcortical brain structure located in the temporal lobe, is very commonly examined in fear learning research. The amygdala receives neural inputs that are associated with vision, hearing, other senses, and pain, and is involved in associating stimuli with outcomes that follow them (Uwano, et al., 1995). Even though the exact role of the amygdala for the experience of subjective fear is yet unclear, it has been demonstrated to play a prominent role in driving fear responses. Animals and humans with damage to the amygdala show less behavioural and physiological signs of fear. For example, in fear conditioning damaged rats have repeatedly shown smaller increases in blood pressure and weaker freezing responses after hearing a shock-conditioned tone compared to healthy controls (Antoniadis et al., 2007; Wilensky et al., 2006). Furthermore, animals and humans with amygdala damage show fear-absent tendencies like putting disgusting substances into their mouths and fearlessly approaching other – possibly threatening – humans or animals (Shiota & Kalat, 2012). FMRI analysis has moreover shown that the amygdala becomes more active during fear conditioning and when viewing fearful or angry faces (Kubota et al., 2000; Vuilleumier et al., 2001; Sato et al., 2004). At first glance, amygdala activation analysis thus seems like a good way to measure fear. However, the method does not seem to account for a complete measure. The amygdala might not be necessary for all aspects of fear, like the subjective experience of a fearful feeling. For example, people with amygdala damage have reported to continue feeling fear, even when physiological signs of fear might be lacking (Anderson & Phelps, 2002). Other neural structures have been identified that are involved in emotional (fear) experience, physiology, and behaviour. These include the hypothalamus – which is responsible for regulating the overall internal environment of the body –, the insular cortex, that is mainly involved in the experience of disgust

(Shiota & Kalat, 2012), and different regions of the prefrontal cortex. The dorsal anterior cingulate cortex (dACC) and medial prefrontal cortex (mPFC) are said to mediate or modulate (negative) fear expression in humans (Milad et al., 2007; Etkin et al., 2010). Ventral-rostral portions of the anterior cingulate (ACC) and medial prefrontal (mPFC) cortices are thought to have a regulatory role with respect to limbic regions involved in generating emotional responses (Etkin et al., 2010). Lastly, the ventromedial prefrontal cortex (vmPFC) has been shown necessary for the recall of extinction learning after a long delay (Quirk et al., 2000). Several brain areas thus are involved in generating emotions and fear specifically, but it remains the question how exactly these are in interplay and whether they make a complete fear reaction together. This makes it difficult to get a clear measurement of fear when using neuropsychological methods alone. In practice, as emotions are generally defined by their bodily ('arousal') and experience effect, we might only be able to speak of actual fear being present when at least a certain behavioural, physiological, or subjective fear reaction is detected.

Anxiety disorders and exposure therapy: the role of fear extinction

Even though fear is a very useful and effective emotion, too much of it is harmful (Shiota & Kalat, 2012). Excessive fear does not only influence someone's nervousness and likelihood of developing phobias, but it also is a key component of anxiety disorders (Shiota & Kalat, 2012; Shin & Liberzon, 2010). Anxiety disorders are characterised by recurring fears or concerns and belong to the most frequent mental disorders with a lifetime prevalence estimated on 11.6 percent (Kazdin, 2000; Baxter et al., 2012). Besides their emotional impact, anxiety disorders have a big financial impact as well, as they are associated with enormous costs (Gustavsson et al., 2011).

One of the most effective strategies to treat anxiety disorders such as phobias, panic disorders and post-traumatic stress disorder (PTSD) is by exposure therapy treatments (Hofmann, 2008; Raeder et al., 2020). Exposure therapy is aimed to help patients overcome their anxiety by creating a safe environment, in which they are repeatedly and systematically exposed to feared or avoided scenarios, leading to decreases of fear (Raeder et al., 2020). Fear extinction can be used to model the central mechanism underlying the main process of exposure therapy (Raeder et al., 2020; Pace-Schott et al., 2015; Leen et al., 2021). The assumption is that exposure therapy activates the fear extinction mechanism, and that scientific findings on fear extinction predict exposure treatment effectiveness (Berry et al., 2009; Hermans et al., 2006; Raeder et al., 2019; Graham et al., 2018; Forcadell et al., 2017). Unfortunately, exposure therapy is not as effective for everyone (Graham et al., 2018; Arch & Craske, 2009). Earlier it was already mentioned that fear extinction processes are fragile and that their effects are temporarily and context dependent. This could contribute to relapses among treated patients (Vervliet, 2013; Craske & Mystkowski, 2006). Moreover, given the role that fear extinction plays in the beneficial effect of exposure therapy, poorer treatment outcome may also be caused by

impaired extinction abilities of certain individuals (Craske et al., 2008; Lissek et al., 2005). Differences in individuals' extinction abilities are indeed thought to affect exposure treatment effectiveness, such that people with good fear extinction learning abilities benefit more from those therapies (Forcadell et al., 2017; Ball et al., 2017).

Besides in anxiety disorder treatment, failure to extinguish fear may also play a role in the development of excessive fear and in the aetiology of anxiety disorders themselves (Craske et al., 2014; Indovina et al., 2011; Jovanovic et al., 2010; Milad et al., 2009; Milad et al., 2013; Rougemont-Bucking et al., 2011). With this, extinction deficits do not only stand in the way of recovering from anxiety disorders, but they might be part of the very reason people are suffering in the first place. Fear extinction and the factors impeding that process thus are a research topic of great importance. Knowledge about hindering factors could help improving anxiety treatment or predicting whether treatment will (not) be effective for certain individuals. Moreover, by investigating what variables are associated with fear extinction impediment, it can be determined which people might have an increased risk for anxiety disorders, enabling more personalized education and advice. That is why in this study fear extinction is the central subject of interest.

The oral contraceptive pill (OC)

Over 100 million women worldwide use OC (Montoya & Bos, 2017). Research suggests that OC use might cause unintended psychological symptoms. Even though findings are limited and complicated, there are strong indications that OC use affects socio-emotional behaviour and underlying brain functions (Lewis et al., 2019; Montoya & Bos, 2017). Effects for emotion regulation have been found and OC has specifically been shown to interfere with natural processes of fear extinction.

Functioning of the pill

OC is a form of hormonal contraception (HC): it uses hormones to prevent women from getting pregnant (Evans & Sutton, 2015). The most commonly used pill contains two hormones: a synthetic estrogen (an estradiol) and progesterone (a progestin) (Evans & Sutton, 2015; Lewis et al., 2019). This so-called combined oral contraceptive (COC) pill thus consists of artificial forms of the female sex hormones estradiol (the major estrogen hormone) and progesterone, which are responsible for functioning of the female reproductive system (Hobeika et al., 2020).

OC use interferes with women's natural hormone production. Whereas the natural menstrual cycle is characterised by hormonal fluctuations (with estradiol peeking right before ovulation and progesterone peeking before menstruation), OC flattens levels of estradiol, progesterone and testosterone throughout the cycle. Endogenous hormone levels are suppressed during the entire cycle (Lewis et al., 2019). This applies for testosterone levels too, which are suppressed by up to 70

percent (Zimmerman et al., 2014). Endogenous hormone levels are replaced by constant levels of exogenous estradiol and progesterone from the pill (Lewis et al., 2019; Montoya & Bos, 2017).

Like natural fluctuations, hormonal alterations due to OC use could have an effect on the brain and on mood (Lewis et al., 2019; Montoya & Bos, 2017). For instance, it has been demonstrated that OC use could contribute to depressive symptoms and a decreased libido (Skovlund et al., 2016; Skovlund et al., 2018; Eldar et al., 2016; Burrows et al., 2012). Moreover, negative effects of OC use have been found for stress and emotion regulation generally (Lewis et al., 2019; Montoya & Bos, 2017; Petersen et al., 2014; Roche et al., 2013; Kirschbaum et al., 1995; Kirschbaum et al., 1996; Kirschbaum et al., 1999; Bonen et al., 1991; Meulenberg et al., 1987; Meulenberg & Hofman, 1990; Bouma et al., 2009; Crewther et al., 2015; Rohleder et al., 2003; Cohoon & Lovallo, 2013; Hofman, 1990). More specifically, worrying effects for fear regulation processes have been demonstrated (Raeder et al., 2019; Graham et al., 2018; Merz et al., 2012; Lisofsky et al., 2016; Petersen et al., 2014).

The pill and fear

It is plausible that the suppression of endogenous hormone levels caused by OC use could result in dysregulation of fear- and stress-related mechanisms (Montoya & Bos, 2017). This has indeed been supported by various studies.

Just like for animals on OC, fear extinction processes in the brain have been shown to be impaired for OC using women, indicating altered neural mechanisms by the pill (Graham & Milad, 2013; Merz et al., 2012). Research by Merz et al. (2012) showed that during fear extinction, OC users displayed higher activation in amygdala, thalamus, anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC) compared to men and naturally cycling women in their luteal phase. OC users' brains thus kept showing more fear for a previously conditioned stimulus after the extinction task, compared to the control groups (Merz et al., 2012). Moreover, women on OC showed slower habituating skin conductance responses (SCRs), suggesting impaired fear extinction (Merz et al., 2012). As administration of estradiol has turned out to be beneficial for fear extinction processes of both animals and naturally cycling women, this points to hormone suppression as a potential cause of impeded extinction (Graham & Milad, 2013). Comparable to the study of Merz et al. (2012), Lisofsky et al. (2016) found brain structural alterations in OC users as well. After three months of OC use, grey matter volume in the left amygdala/anterior parahippocampal gyrus (PHG) decreased in 28 users as compared to a control group of 28 naturally cycling women. Here, the finding of grey matter reduction was actually accompanied by decrease in OC users' positive affect scores, indicating direct mood consequences as well, shortly after starting OC use.

Beside altered neural mechanisms for fear processing suggested by fear-related activity in the brain, women on OC have furthermore shown decreased resting state functional connectivity of brain

regions that are important for cognitive and affective control and crucial in coping with fear and stress - and the executive network (Petersen et al., 2014). Altogether, the effects indicate that neural structures involved in fear regulation might be altered as a consequence of OC use.

Beside at this neurological level, fear effects of OC have been found at the clinical level as well. It has been demonstrated that women who take OC benefit less from exposure therapy treatments (Raeder et al., 2019; Graham et al., 2018). Raeder et al. (2019) and Graham et al. (2018) investigated the impact of contraception use on exposure treatment effectiveness, specifically for women with spider-phobia. Graham et al. (2018) looked at general hormonal contraception use and found behavioural differences expressed in BAT (Behavioural Approach Test) scores. HC users showed more behavioural avoidance after extinction training compared to non-users (Graham et al., 2018). Raeder et al. (2019) focused on the oral contraceptive pill and found differences in subjective fear measurements of three questionnaires about spider fear. However, this latter subjective fear effect was only found six weeks after the extinction experiment and not immediately after. Besides, both the effect and sample (n = 54) were small, underscoring the importance of further investigation (Raeder et al., 2019).

The exact role of OC is still under discussion and clearer education about it is needed. The mentioned findings, combined with the fact that women are twice as like as men to be affected by anxiety disorders (Gustavsson et al., 2011; Alonso et al., 2004; Regier et al., 1990; Kessler et al., 2005; Bandelow and Domschke, 2015) and show a greater illness burden (McLean et al., 2011), emphasize the need to further investigate the role of OC in fear regulation.

Pill phase

In addition to general OC use, pill phase could be a variable affecting fear extinction processes. OC users usually only take a pill during three out of four weeks in the OC cycle. Therefore, effects of OC for fear extinction abilities might differ between active and inactive pill weeks. Should extinction impediment be acutely caused by the administration of hormones from the pill, fear extinction should mainly be impaired during active pill weeks and not (or to a lesser extent) during pill-free weeks. In that case, negative effects might be reversible on the short term. Alternatively, if no pill phase effect exists, side effects might be more difficult to reverse.

Although the direct effect of pill phase for fear extinction has not been examined yet, research of Petersen et al. (2014) has demonstrated an effect (of not only OC use generally, but also) of pill phase for resting state functional connectivity. Here, women in an inactive pill phase showed greater coherence between the left middle frontal gyrus (MFG) and the executive control network (ECN) than did women in an active pill phase (Petersen et al., 2014). As the MFG and ECN play an important role in cognitive and emotional processing, reduced functional connectivity between the two could cause

emotion regulation problems. The study of Peterson et al. (2014) thus indicates potential emotion regulation problems during weeks in which a pill is taken. Hence, an impairing effect for fear extinction processes of active pill phase compared to inactive pill phase is plausible.

Control group of naturally cycling women

Studies investigating effects of OC use often use a control group of naturally cycling women. An important methodological consideration is the menstrual phase of those natural cyclers (NCs) (Montoya & Bos, 2017). As hormone levels of NCs fluctuate strongly over the cycle and estradiol levels are thought to affect fear extinction, comparison of OC users to NCs in different menstrual phases might yield different effects. During the early and mid-follicular menstrual phase NCs show a low endogenous hormone profile. This profile is comparable to that of OC users, whose endogenous hormone levels are constantly suppressed (Montoya & Bos, 2017). In contrast, during the luteal phase NCs' hormone levels are high, peeking before menstruation. As it is assumed that estradiol levels mediate fear extinction processes, OC users and NCs in their follicular phase might perform similarly in fear conditioning experiments. Therefore, if an effect between these groups is found nevertheless, this could indicate an exogenous hormone effect (Montoya & Bos, 2017; Merz et al., 2012; Gingnell et al., 2003). However, literature investigating the role of estradiol suggests a fear effect between OC users and NCs in their luteal phase is more plausible. As comparing OC users to NCs during the follicular phase of their cycle is informative for disentangling the effects of exogenous hormones, a menstrual phase distinction could be very useful (Merz et al., 2012; Gingnell et al., 2003). However, sample sizes do not always allow for such a distinction to be made.

This study

This study investigates whether OC use impairs fear extinction processes and whether pill phase has an effect. An online fear conditioning experiment will be deployed to simulate fear learning and fear extinction in respondents, for a certain stimulus. Subjective fearfulness ratings of the conditioned stimulus at different times during the experiment will provide insight in how fear develops in participants and in their success of extinguishing fear. Individual fear learning trajectories of participants will be examined using Latent Class Growth Analysis (LCGA). Based on their courses of fear over the experiment participants are classified into distinct extinction classes. Aim is to distinguish participants who fail to extinguish fear from those succeeding and from those who do not acquire much fear in the first place. Classes reflect participants' assumed fear extinction abilities. Besides, final levels of fear after extinction training will be investigated. This study examines whether OC users and NCs differ in their abilities to extinguish fear and in their final fear levels. Furthermore, this study researches a difference between pill users in an active and those in an inactive pill phase.

By investigating the effect of OC use and pill phase on fear extinction and final fear levels, this study contributes to a clearer understanding of the psychological functioning of the pill and attempts to broaden this underexposed field of research.

An answer is sought to the following research questions:

> RESEARCH QUESTION 1:
>
> ***How does oral contraception use affect subjectively measured fear extinction and final fear of participants in an online fear conditioning task?***

> RESEARCH QUESTION 2:
>
> ***How does oral contraception pill phase affect subjectively measured fear extinction and final fear of participants in an online fear conditioning task?***

Firstly, this study examines how OC use generally affects fear extinction by comparing OC users and a control group of NCs. By measuring self-reported fear, the approach is similar to that of Raeder et al. (2019) and Graham et al. (2018). However, whereas Raeder et al. (2019) and Graham et al. (2018) investigated the effect of HC and OC use for fear of spiders, this study investigates fear more generally as it does not focus on a specific phobia. Like Raeder et al. (2019) and Graham et al. (2018) this study analyses average group fear scores. These final fear scores will be compared between OC users and NCs. By considering individual fear learning trajectories as well, this study adds to Raeder et al. (2019) and Graham et al. (2018) and takes into account within-group variability in fear acquisition and extinction. By comparing into which extinction classes OC users and NCs are classified mostly, a potential difference in fear extinction abilities can be exposed. This study was planned with a bigger and more diverse sample than Raeder et al. (2019) were able to when investigating the effect of the pill. The experiment alterations implemented here might enable finding a clearer fear extinction effect of pill use than Raeder et al. (2019) did, directly after the experiment.

Previous research has indicated impeding effects of OC on fear extinction processes in the brain and exposure therapy effectiveness. In line with this literature, OC users in this study are expected to be overrepresented in groups of poor fear extinction and to end up with higher final fear scores compared to NCs.

> HYPOTHESIS 1:
>
> ***Oral contraception users will be overrepresented in groups of poor fear extinction and will end up with higher final fear scores as compared to natural cyclers.***

Secondly, this study investigates whether OC users in an active and those in an inactive pill phase differ in their abilities to extinguish fear. As the effect of pill phase for fear extinction abilities has not yet been examined, this research should provide new insights regarding the subject. Again, both the classification into fear extinction classes and final fear scores will be analysed.

Research on the effect of OC for functional connectivity has linked the active pill phase to potential emotion regulation problems. In accordance with this literature, it is hypothesised that in this study OC users in an active pill phase will be overrepresented in groups of poor fear extinction and end up with higher final fear scores as compared to OC users in their pill-free week.

> HYPOTHESIS 2:
>
> ***Oral contraception users in an active pill phase will be overrepresented in groups of poor fear extinction and will end up with higher final fear scores as compared to OC users in an inactive pill phase.***

Implications

Should OC use indeed show to affect extinction training effectiveness in this experiment, this should be seen as a contraindication to start using the pill. After all, this would make OC use a risk factor for developing anxiety disorders. It would substantiate the need for more education about this psychological disadvantage and for a more individual approach in prescribing OC. Furthermore, a difference between OC users in different pill phases would indicate an acute pill effect that might be reversible on the short term.

The original fear conditioning task used here was validated in a physically conducted experiment (Leen et al., 2021). However, various pilots have demonstrated that characterising people with LCGA also seems possible and valid using online data. This experiment will be performed online, so that participant recruitment can be done regardless of the consequences of the Corona virus that currently has a grip on the world. Advantage of this online testing is that a bigger and more diverse sample can be gathered. Unfortunately, this also complicates controlling the experiment and

ensuring participants go through the experiment as they are supposed to. In the method section the measures taken to ensure a dataset as reliable as possible will be explained.

Materials & Method

Participant recruitment

After the experiment was approved by the Ethics Review Board (FETC) of the Faculty of Social and Behavioural Sciences of Utrecht University, participants were recruited in three ways. Firstly, participation could take place via a link on the website of Prolific, a platform for online participant recruitment for surveys and market research. Participants registered here have expressed their interest in participating in studies and receiving compensation for their contributions. Participation in this study was rewarded with a €4 compensation via Prolific. Secondly, participants were recruited via the SONA system. Through this platform, psychology students at Utrecht University could be granted 0.5 participation credit after completing the experiment. Lastly, participants were recruited via online and social media platforms using a Qualtrics link. Participation through this route was rewarded with a Yesty gift voucher of €4. The Qualtrics link was shared in various public Facebook and LinkedIn research groups, and in the personal networks of the researchers.

Experimental design, stimuli, and measures

Two types of data were collected. Central to the experiment was a fear conditioning task simulating fear learning and fear extinction in participants. At different times during the fear conditioning task participants were asked to indicate the amount of fear they felt for the presented stimuli and the expectancy that a scream would be presented alongside each stimulus. As a result, CS fearfulness and US expectancy levels could be determined. Secondly, participants completed multiple behavioural measures that have been previously associated with fear extinction processes.

Fear conditioning task

For this study, the fear conditioning task employed by Leen et al. (2021) was modified into an online version in Gorilla. Two female faces with a neutral expression were used as the conditioned stimulus (CS) images, one face coloured blue and one coloured green. The sound of a loud female scream with a duration of 1 second functioned as the unconditioned stimulus (US). The loudness of the scream was determined in advance by participants. They had to adjust it to their equipment and set it to a very loud volume.

The fear conditioning task had nine separate blocks. In each block participants had to focus on a white fixation cross that was presented in the middle of a black screen for a duration of 2 seconds. After the fixation cross, one of the facial images was presented on that same position for a duration of 4 seconds. Thus, each block consisted of eight fixation crosses and female faces, with both coloured faces being shown 4 times each. The nine fear conditioning blocks were divided over three phases: a pre-conditioning phase, a fear acquisition phase, and a fear extinction phase. Fear acquisition and fear extinction both consisted of two parts: an instructed part and an uninstructed part. Pre-conditioning, which consisted of one block, was intended to have participants acquainted with the stimuli and procedure. During fear acquisition one of the two female faces (CS+) was presented along with the scream (US) at a 75% reinforcement rate. The other face was not coupled with the scream at all (CS-). The scream was presented and delivered 2.5 seconds after the presentation of the CS+. In the two blocks of uninstructed acquisition participants perceived the CS and US without being instructed. In the two successive instructed acquisition blocks participants did receive an instruction about the fact that a scream would be presented, and about which face would be coupled to the scream. During the fear extinction phase neither face was coupled with the scream. In the first two blocks of extinction (uninstructed extinction), participants received no instructions, whereas in the final two blocks (instructed extinction) participants were instructed about the absence of the US.

After each block, participants indicated levels of fearfulness and US expectancy of both CSs on a slider scale (0-100). To assess CS fearfulness levels, participants were asked "How scared/nervous are you when seeing this picture?" on a scale from "Absolutely not scared/nervous" to "Really scared/nervous". US expectancy was measured by the question 'How likely is it that you hear the scream when seeing this picture?'. This rating was measured on a scale of 'Very unlikely' to 'Very likely'. Finally, the question 'How confident are you about your answers?' provided insights into the certainty with which participants had answered the previous fearfulness and US expectancy questions. Scale for this question ranged from 'Not confident at all' to 'Really confident'. Additionally, after the four acquisition phase blocks in which a scream was presented, a question measuring the aversiveness of the US was included. It stated, 'How would you rate the scream?'. The scale ranged from 'Not aversive' to 'Very aversive'. Fearfulness and US expectancy of both facial images were measured after each block. Fear scores after the first block of pre-conditioning served as a baseline measurement.

The condition to which participants were assigned determined which face would function as CS+ and which one would be CS-, and what the (fixed) order was of presenting CS+ and CS-. Hence, there were 4 conditions in total. An overview of the task is given in **Figure 1** below. In total, the computer task had a duration of approximately 15 minutes.

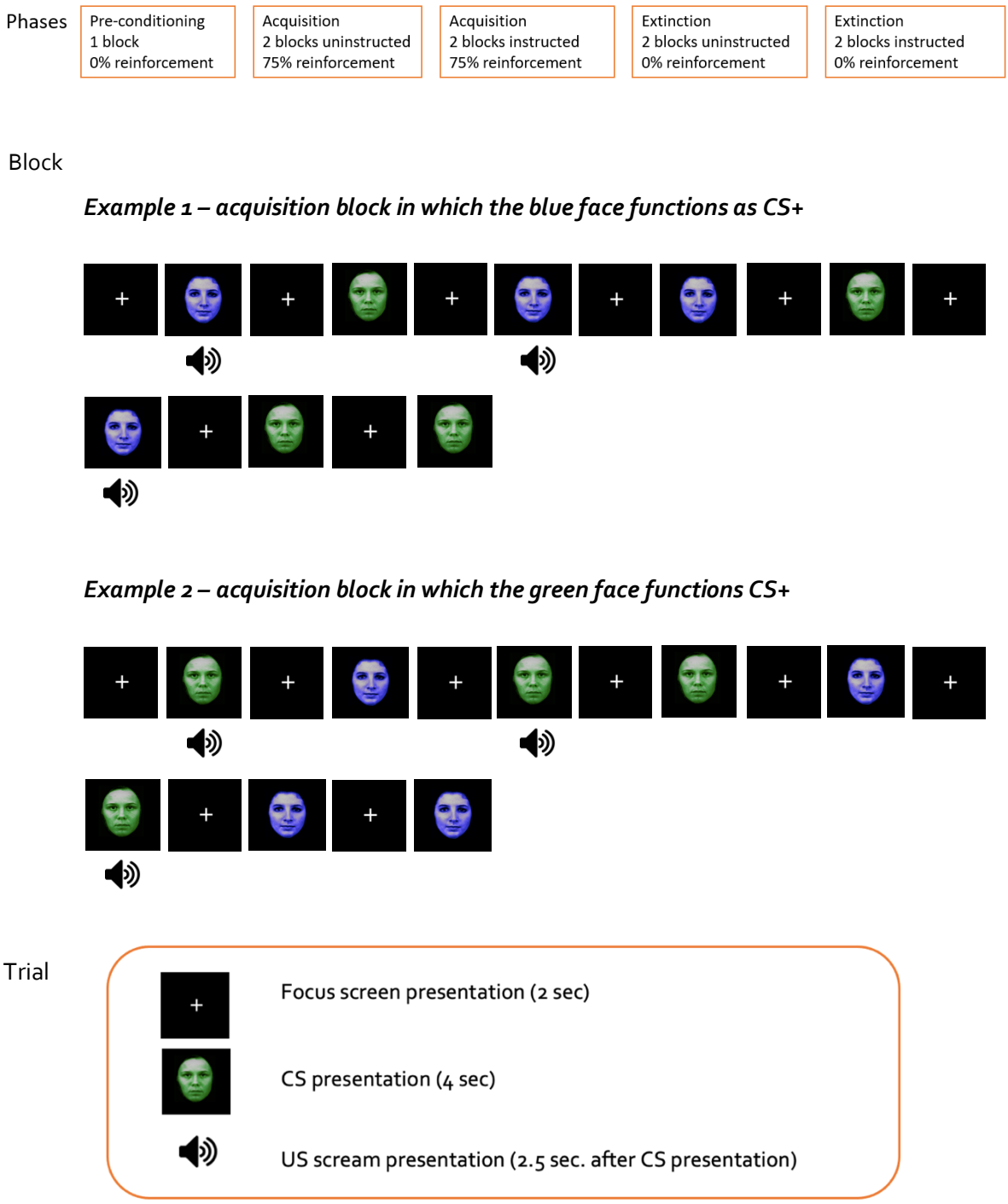

Figure 1: Overview of the fear conditioning task. Adapted from Leen et al. (2021).

Additionally, to ensure valid and reliable data was recorded, measures (catch trials) were taken to assess participant's attention during the task. Two types of catch trials were used: beep and number trials. During the beep trial, 2-5 short and soft beeps were played, and participants had to enter the

number of beeps. The number trials consisted of a number quickly presented on the screen and the participants had to enter the shown number. Four beep trials and four number trials were included. Beeps were presented at a volume set in advance, only just hearable for participants and depending on their equipment. Numbers were initially shown for a duration of 250 milliseconds and in a later version of the experiment for a duration of 500 milliseconds. Beeps and numbers were presented after the third block in the uninstructed acquisition phase, after the fifth block in the instructed acquisition phase, after the seventh block in the uninstructed extinction phase and after the nineth block in the instructed extinction phase. The catch trials were (with the intervention of a text screen closing off the block) immediately followed by the questions regarding the spotted number and the presented number of beeps. Catch trials had to assure participants did not take off their headphones, tune down their volume or look away from the screen. Participant data were excluded from analysis when less than three beep or visual catch trials had been answered correctly.

Questionnaires

In the experiment a total of 6 questionnaires were included to provide insights in various personality traits of participants. The first questionnaire covered the topic of Intolerance of Uncertainty (IoU), measuring how well different participants deal with uncertain or uncontrollable situations. Here, a short version of the original IoU questionnaire was used that was validated in Carleton et al. (2007) and Helssen et al. (2013) (for the Dutch version) and consists of 12 items. Secondly, the original 20-item State-Trait Anxiety Inventory (STAI) trait questionnaire from Defares et al. (1980) was included. This questionnaire measured participants' general state feeling of anxiety or calmness. Thirdly, a questionnaire was included related to the Corona epidemic, assessing participants thoughts and concerns regarding the virus. It consisted of 8 items and was developed by Mertens et al. (2020). The fourth questionnaire was a short version of the 53-item Brief Symptom Inventory (BSI) questionnaire from Derogatis & Spencer (1993). The shorter version from Derogatis (2001) consisted of 18 items and covered aspects of physical as well as the mental well-being of participants. Fifthly, a second Corona-related questionnaire and accompanying task were included, which were piloted by a bachelor psychology student group of Utrecht University in the fall of 2020. This Corona Avoidance task and questionnaire included questions measuring participants' tendency to avoid 8 different real-life situations during the pandemic. Lastly, a questionnaire was included regarding menstrual cycle and hormonal status of female participants: the Menstrual Cycle Questionnaire (MCQ). The original MCQ of Merz et al. (2012) was adapted into a longer version consisting of 13 questions for this study.

In this study, only data from the **Menstrual Cycle Questionnaire** and no other questionnaires were analysed. Just specific items of the MCQ were included in data analysis. Other data will be analysed

in future research. Participants were asked about their HC use in a question stating: *'Are you currently using any form of contraception (with the exception of condoms and other barrier methods)?'*. In a follow-up question, if participants answered yes, they were to specify which form of contraception they used. Participants could choose one of the options in the drop-down menu: the contraceptive pill, hormone IUD, copper IUD, contraceptive patch, contraceptive ring, contraceptive implant, and contraceptive injection. Also, a specifiable *'other'* option was included. To determine pill phase of OC users, another question stated: *'If you are using the contraceptive pill, are you taking a pill on the day of the examination (today) or are you in the pill-free week?'*. Participants could choose between the options *'not applicable'*, *'I took a pill today'* and *'I'm in my stop week (or haven't taken the first pill of a new strip yet)'*. To be able to determine menstrual cycle phase of naturally cycling women, a question was asked assessing the starting date of participants' last period. To determine whether the menstrual cycle of naturally cycling participants was regular, a question stated: *'If you don't use hormonal contraception, do you typically have a menstrual period about once per month (about every 22 – 28 days)?'*. Answer options were *'no'*, *'I do not know'*, *'I no longer menstruate'* or *'yes'*. In the latter case participants were asked to indicate the duration of their normal natural cycle in days. The complete MCQ can be found in **Appendix 1**.

Procedure

After entering the Gorilla environment via either Prolific, SONA or Qualtrics links participants were first asked to choose their language of preference for completing the experiment. The experiment could be run in Dutch and English and was titled *"Associations between profiles of extinction learning, variation in personality and response to the corona related threat"*. Next, participants read the information sheet, which contained information regarding the study's aim, the advantages and disadvantages of participation, voluntary participation, and data processing. A declaration of consent was signed by each participant. By continuing participants said to have read the provided information and have no further questions, gave permission for the use of their data, and declared to be 16 years or older. Participants were instructed about the required internet browser and equipment needed for eligible participation. They were instructed to turn on full-screen mode, turn off device notifications, and to ensure they would not be disturbed for the upcoming 35 minutes.

Prior to the fear conditioning task, participants had to complete the auditory titration task. The aim was to adjust volume settings of different participants to comparable and desired levels. Firstly, the volume had to be set at maximum. Participants had to indicate whether they were using headphones or in ear buds while running the experiment and were asked not to turn down their volume during the experiment. A sound test followed in which the volume of two types of sounds were tested and adjusted to the right level. Participants were required to indicate whether they could

hear the soft neutral beep, which was supposed to be just hearable. Participants could only proceed when they could hear the sound, albeit after adjusting their sound settings. This beep sound would be used in the catch trials during fear conditioning. Secondly, the US scream was tested. Participants were required to listen to the scream multiple times at different volume levels and indicate the loudness by answering the question 'How loud is this noise?'. Answer options were *'not at all'*, *'a bit'*, *'moderately loud'*, *'very loud'* and *'extremely loud'*. Participants were instructed that any rating up to a *'moderately loud'* sound meant it was easily tolerable, a *'very loud'* sound meant it had the maximum volume level they considered tolerable, and an *'extremely loud'* scream was intolerable. The US sound test and questions were repeated until participants indicated the scream was *'very loud'*. The US would be set at this volume during fear conditioning.

After the auditory titration participants were instructed about the catch trials included in the experiment to ensure their full attention. Thereafter, participants started the questionnaires. Firstly, demographic information was collected, including sex and age of participants, some pubertal development information, participants' history with mood disorders and their medicine use. Three questionnaires were presented before the fear conditioning task. Firstly, participants had to complete the Intolerance of Uncertainty (IoU) questionnaire, then they moved to the State-Trait Anxiety Inventory (STAI) trait questionnaire and thirdly they had to complete the Corona concernedness questionnaire. For the fear conditioning task participants were automatically assigned to one of the four conditions. After finishing the fear conditioning task, the three other questionnaires followed: Firstly, the short Brief Symptom Inventory (BSI) questionnaire, then the Corona Avoidance questionnaire and task and finally the Menstrual Cycle Questionnaire were presented. The MCQ was only presented to female participants.

Next, participants completed one more task that assessed the extent of fear generalisation. Here, the two different facial images were presented again, and combinations of the two images blended together were shown. Participants had to give fearfulness ratings (but no US expectancy ratings) like they did in the fear conditioning task.

In the debrief participants were informed that the experiment had ended, and they were thanked for their participation. A short explanation of the context, aim and measures of the experiment was given, and contact information was provided in case participants wanted to express any complaints. Moreover, it was advised participants visited a general practitioner in case they experienced fear symptoms because of the coronavirus. Subsequently, information was given about a follow-up EEG study that participants could express their interest in by providing their contact information. Finally, depending on participants' way of entering the experiment, information was given about how to collect compensation.

Figure 2: Procedure overview.

Data analysis

Dependent variables

Two types of dependent variables were used representing fear of participants. Firstly, **fear extinction learning trajectories** were examined using Latent Class Growth Analysis (LCGA). LCGA is a data-driven approach to investigate latent homogenous classes within a larger heterogenous sample (Leen et al., 2021). With this, respondents are classified into classes based upon similar patterns of fear over time (Jung & Wickrama, 2008; Berlin, Parra, & Williams, 2013). This way of analysing patterns has been proposed as a powerful next step to take into account patients' within-group variability in fear acquisition and extinction (Lonsdorf & Richter, 2017; Duits et al., 2021). Extinction classes were determined based on fearfulness ratings to the CS+. All nine fearfulness ratings participants provided over the course of the experiment were used. As examining fear generalisation data exceeded the scope of this study, CS- scores were disregarded. LCGA was conducted in MPlus (Version 8). Similar to Leen et al. (2021) and Duits et al. (2021) model fit was compared between models with 1–6 trajectories. Likewise, model selection was based on three criteria that were based on results from Galatzer-Levy et al. (2017): apparent drops in Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC), a large entropy score and the smallest number of classes that would still be theoretically meaningful. Participants were assigned to the extinction class for which they had obtained the highest probability according to the best fitting model. Classes of poor extinction were the focus of this study, as these indicate fear extinction deficits.

The second dependent variable of this study was a **final fear** score after extinction training. Like the extinction classes, participants' final fear scores were determined using fearfulness ratings to the CS+. The two scores in the instructed part of the extinction phase were averaged. As final fear scores do not take into account the time courses of fear development across the experiment, and do not allow distinction between people who never showed fear to begin with (low fearful class, see below) and those with normal extinction, they do not strictly isolate fear extinction patterns. However, these final scores allowed for analyses to be conducted with a greater power than those determining representation over extinction classes. By providing insights in levels of fear participants were 'left with' after extinction training, they therefore were a good addition.

Independent variables

The independent variables of this study were extracted from responses to the MCQ. Firstly, **OC use** of participants was determined to distinguish OC users and NCs. Female participants answering *'no'* to the question *'Are you currently using any form of contraception (with the exception of condoms and other barrier methods)?'*, were labelled part of the control group of NCs. If they answered *'yes'* and specified their HC use comprised of using OC, participants were designated OC users. Data of all participants that were not classified into these two categories were disregarded.

Secondly, **pill phase** of OC users was determined using answers to the MCQ question *'If you are using the contraceptive pill, are you taking a pill on the day of the examination (today) or are you in the pill-free week?'*. Participants who answered *'I took a pill today'* were identified as OC users in an active pill phase. Participants that chose the option *'I'm in my stop week (or haven't taken the first pill of a new strip yet)'*, were labelled OC users in an inactive pill phase.

It could not be predicted how participants would be divided over the different groups. Based on the broad use of hormonal contraception methods, a large group of OC users in the sample was to be expected. Therefore, comparing a subdivision of OC users in different pill phases appeared doable in this study. However, numbers of natural cyclers were expected to be much smaller. Therefore, a comparison of OC users and NCs in different menstrual phases seemed less feasible and statistically comparing OC users and the general group of NCs was the alternative.

Still, in this study a distinction of NCs in different **menstrual phases** was made exploratively and reported descriptively. This was done based on the MCQ questions about regularly cycling women's last menstruation start date, the date at which they completed the experiment, and cycle length. A distinction was made based on Schmalenberger et al. (2020), who differentiated four menstrual phase groups. In this study, the method of Schmalenberger et al. (2020) was used to identify women in two of those phases: the mid-follicular and mid-luteal phase respectively. Only these phases were distinguished, as they are characterised by relatively stable low (follicular) and high (luteal) hormone levels for a duration of multiple days (Schmalenberger et al., 2020). According to Schmalenberger et al. (2020) the mid-follicular phase runs from day 4 to day 7 of the menstrual cycle and mid-luteal phase runs from 9 to 5 days before menstruation. Using this strict phase definition would only allow for a very limited number of naturally cycling women to be classified into phase groups. Therefore, a more useful liberal approach would be to extend the mid-follicular phase of Schmalenberger et al. (2020) to an early/mid follicular phase running from day 3 to 10 of the menstrual cycle, and use a mid-luteal phase running from 11 to 3 days before menstruation. This way, the follicular and luteal phase do not overlap with the other two phases of Schmalenberger et al. (2020)

and are still characterised by relatively stable hormone levels (although initiating fluctuations cannot be ruled out). Here, the liberal approach was used such that in practice, counting forward from the start date of participants' last menstruation, participants had to have completed the experiment 3 to 10 days later to be classified into the follicular category. Counting backwards from participants' expected next menstruation start date, they had to have completed the experiment 11 to 3 days earlier to be classified into the luteal category. Next menstruation start date was determined by adding participants' reported cycle length to the start date of their last menstruation. In accordance with Schmalenberger et al. (2020), only of NCs that reported a cycle length of 21 to 35 days the menstrual phase was determined.

Statistical analysis

Statistical analysis was performed in the statistical programme SPSS (Version 25). Firstly, the general effect of OC use (irrespective of menstrual / pill phase) was investigated. Chi-square analyses were used to compare the representation of OC users and NCs over fear extinction classes. Comparing final fear scores of OC users and NCs was done using Independent-Samples T Tests. Secondly, the pill phase effect was examined. Again, Chi-square analyses were used to compare representation of the two pill phase groups over fear extinction classes. Comparing final fear scores of OC users in an active pill phase and those in an inactive pill phase was done using Independent-Samples T Tests. No statistical analysis was performed to compare NCs in different menstrual phases.

Results

Sample description

In the period of May 16th to June 28th, 2021, a total of 310 participants completed the experiment. Due to bad performance on catch trials, data of 90 participants were excluded. From July 6th to July 8th another 39 female participants completed a slightly different version of the experiment, in which presentation duration of catch trial numbers was increased to 500 milliseconds, because a shorter duration may have made exclusion on these trials too stringent. Data of 8 of these participants were excluded. Of the 251 participants in the final sample 139 were female (55.4%), 108 were male (43.0%), and 4 (1.6%) did not report their sex. Among women, 59 (42.4%) used hormonal contraception, of whom 46 (78.0%) specifically used the oral contraceptive pill. Of OC users, 34 (73.9%) were in an active pill phase and 12 (26.1%) were in a pill-free week. 76 women (54.7%) reported to cycle naturally. Of the NCs, 56 (73.7%) reported to have a menstrual period about every 22 to 28 days. Of 22 of these participants the menstrual phase could be determined: 8 (36.4%) were in their follicular phase and 5 (22.7%) were in their luteal phase at the day they completed the experiment. The remaining 9

participants were in different menstrual phases or in between phases. Menstrual phase of 34 NCs could not accurately be determined, because they reported a varying cycle length (N = 7), did not report a (correct) last period date (N = 6), or reported a cycle length of under 21 or over 35 days (N = 21). 19 NCs actually reported a cycle length of under 8 days, indicating a potential misinterpretation of the question. The remaining 4 women that were not classified HC user or NC did not report their hormonal status or were post-menopausal. Ages ranged from 18 to 59 years, with an average of 23.5 years. In Table 1 an overview of sample characteristics is given.

Characteristic	**Frequency (N)**
Total sample	251
Group	
Men	108
Other gender	4
OC using women	46
Active pill phase	*34*
Inactive pill phase	*12*
Naturally cycling women	76
Follicular menstrual phase	*8*
Luteal menstrual phase	*5*
Different menstrual phase	*9*
Undefined menstrual phase	*34*
Women using different HC	13
Missing hormonal status / post-menopausal women	4
Age (in years)	
Mean age	*23.5*
Age minimum	*18*
Age maximum	*59*

Table 1: Sample description, after excluding participant data based on catch trial performance (N = 251).

No age difference existed between the groups of OC users (M = 22.7, SD = 2.7) and NCs (M = 24.0, SD = 6.3) (t (120) = 1.39, p = .17). Ages of OC users in an active (M = 22.7, SD = 2.6) and inactive pill phase (M = 22.8, SD = 3.0) did not differ either (t (44) = 0.11, p = .91).

Latent Class Growth Analysis

Latent Class Growth Analysis was performed over data of all 251 included participants to ensure a meaningful classification. LCGA showed three distinct classes on CS+ fearfulness rating. The 3-class model was selected, as BIC and AIC scores showed substantial drops from the 2nd to the 3rd class model (398 and 363 resp.), the entropy score was high (0.928), and this was in congruency with previous studies. Model fit indices are presented in Table 2 and best fitting model values are displayed in bold. The smallest class was labelled 'low fearful conditioners' (23.9%, N = 60) and reported low fearfulness scores over all conditioning phases. The second class labelled 'normal extinguishers' was characterized by an increase in fearfulness scores during acquisition and a strong decrease during extinction phases (30.7%, N = 77). The largest class was that of 'poor extinguishers' (45.4%, N = 114) and was characterized by a sustained fearfulness to the CS+ during extinction phases. In figure 3 fearfulness rating courses of different classes over the experiment are shown.

No. of classes	**AIC**	**BIC**	**Entropy**	**Sample size per class based on most likely class membership** (N = 251)
1	21637	21701	NA	251
2	−1265	−1230	.972	73/178
3	**−398**	**−363**	**.928**	**60/77/114**
4	−216	−181	.928	39/44/76/92
5	−149	−114	.947	28/40/30/61/92
6	−123	−87	.924	40/28/55/28/59/41

Table 2: Fit indices for 1-6 class Latent Growth Models, based on CS+ fear ratings (AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; NA = Not Applicable. AIC and BIC values for 2 classes and up are expressed as reduction with respect to the previous model.)

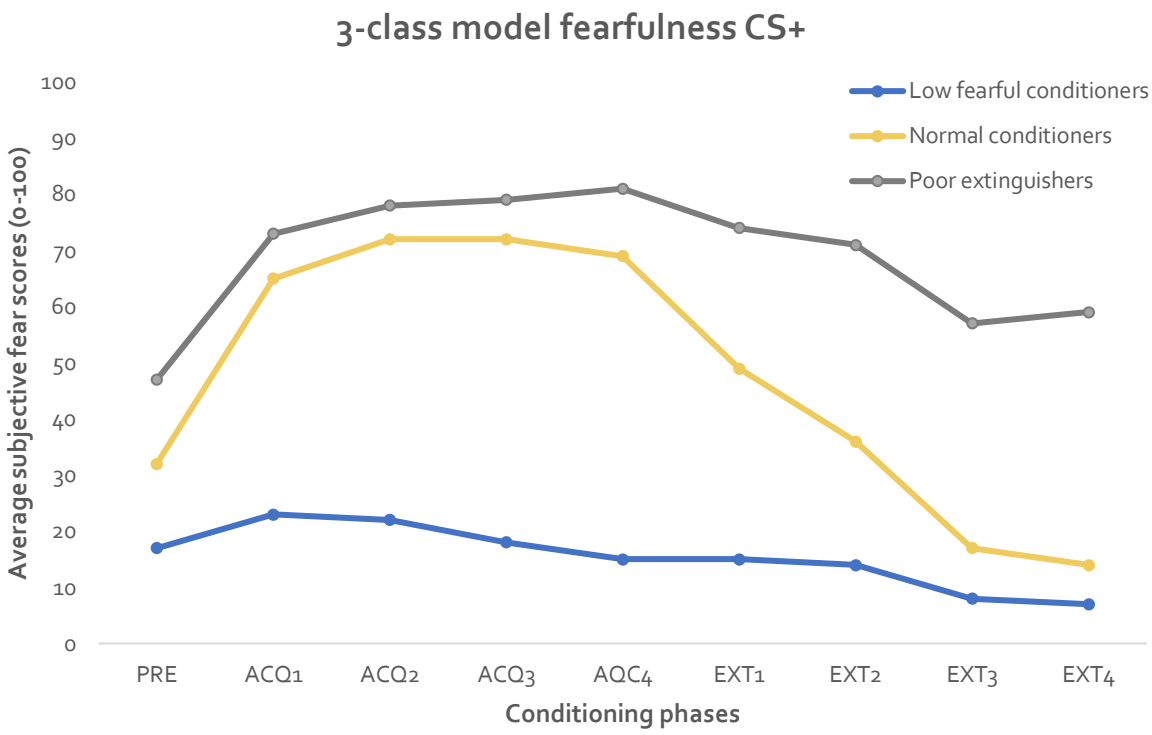

Figure 3: Estimated means of fearfulness ratings to the CS+ in the selected 3-class model, during the different preconditioning (PRE), acquisition (ACQ), and extinction (EXT) blocks.

In Table 3 below an overview is given of group frequencies and mean ages for the groups defined based on gender and hormonal status as a function of the three fear extinction classes. In the largest class of poor extinguishers also highest group frequencies were found. Classifying groups of OC users in different pill phases and NCs in different menstrual cycle phases into distinct fear extinction classes resulted in very small subgroups of under 10 participants.

	Low fearful conditioners (N = 60)	**Normal conditioners** (N = 77)	**Poor extinguishers** (N = 114)	**Overall N**
Men	33 (31%)	34 (31%)	41 (38%)	108
Other gender	1 (25%)	1 (25%)	2 (50%)	4
Women	26 (19%)	42 (30%)	71 (51%)	139
OC users	11 (24%)	11 (24%)	24 (52%)	46
Active pill phase	7 (21%)	8 (24%)	19 (56%)	34
Inactive pill phase	4 (33%)	3 (25%)	5 (42%)	12

Natural cyclers	11 (14%)	28 (37%)	37 (49%)	76
Follicular phase	1 (13%)	2 (25%)	5 (63%)	8
Luteal phase	2 (40%)	1 (20%)	2 (40%)	5
Different menstrual phase / in between phases	1 (11%)	6 (67%)	2 (22%)	9
Age (M(SD))	24.5 (5.4)	24.2 (6.6)	23.1 (3.0)	

Table 3: Frequency and age distribution of groups of men, other gender, women, OC users, and NCs over three fear extinction classes, including percentages per hormonal status group.

Oral contraception use effect

Extinction class distribution

Chi-square analysis demonstrated no significant effect in the distribution of OC users and NCs over fear extinction classes (X^2 (2) = 2.98, p = .23). OC users were not more often categorized as poor extinguishers (52.2%) than NCs (48.7%), rejecting Hypothesis 1. In Table 4 results are presented. No cells had an expected count of less than 5.

	Low fearful conditioners (N = 60)	**Normal conditioners** (N = 77)	**Poor extinguishers** (N = 114)	
OC users (N = 46)	11	11	24	
Group proportion (%)	(23.9%)	(23.9%)	(52.2%)	
Adjusted residual	*1.3*	*-1.5*	*0.4*	
Natural cyclers (N = 76)	11	28	37	
Group proportion (%)	(14.5%)	(36.8%)	(48.7%)	
Adjusted residual	*-1.3*	*1.5*	*-0.4*	X^2 (2) = 2.98, p = .23

Table 4: Chi-square analysis results of distribution of OC users and NCs over the three fear extinction classes.

Final fear ratings

An Independent-Samples T-test comparing final fear scores of OC users and NCs, did not yield a significant effect (t (120) = 1.11, p = .27). OC users did not show more fear after extinction training (M = 29.0) than NCs (M = 34.6), see Table 5. Hence, Hypothesis 1 was rejected.

	Final fear (M (SD))
OC users (N = 46)	29.0 (25.2)
Natural cyclers (N = 76)	34.6 (28.2)
	t (120) = 1.11, p = .27

Table 5: Means and standard deviations of final fear scores of OC users and naturally cycling women.

Pill phase effect

Extinction class distribution

When comparing OC users in an active and those in an inactive pill phase, Chi-square analysis demonstrated no significant difference in their distribution over fear extinction classes (X^2 (2) = 0.954, p = .62). Although group proportions were in the expected direction, OC users in an active pill phase were not more often categorized as poor extinguishers (55.9%) than OC users in an inactive pill phase (41.7%), rejecting Hypothesis 2. In Table 6 results are presented. Two cells (33.3%) had an expected count of less than 5.

	Low fearful conditioners (N = 60)	**Normal conditioners** (N = 77)	**Poor extinguishers** (N = 114)	
OC users in active pill phase (N = 34)	7	8	19	
Proportion (%)	(20.6%)	(23.5%)	(55.9%)	
Adjusted residual	*-0.9*	*-0.1*	*0.8*	
OC users in inactive pill phase (N = 12)	4	3	5	
Proportion (%)	(33.3%)	(25.0%)	(41.7%)	
Adjusted residual	*0.9*	*0.1*	*-0.8*	X^2 (2) = 0.954, p = .62

Table 6: Chi-square results of distribution of OC users in an active pill phase and OC users in an inactive pill phase over the three fear extinction classes.

Final fear ratings

An Independent-Samples T Test comparing CS+ final fear scores of OC users in an active those in an inactive pill phase did not yield a significant effect (t (44) = -0.74, p = .47). Even though ratings were in the expected direction, OC users in an active pill phase did not show significantly more fear after extinction training (M = 30.6) than OC users in an inactive pill phase (M = 24.4), see Table 7. Thus, Hypothesis 2 was rejected.

	Final fear (M (SD))
OC users in active pill phase (N = 34)	30.6 (24.8)
OC users in inactive pill phase (N = 12)	24.4 (26.9)
	t (44) = -0.74, p = .47

Table 7: Means and standard deviations of final fear scores of OC users in an active pill phase and OC users in an inactive pill phase.

Discussion

The current study investigated an effect of oral contraception use and OC pill phase on fear extinction abilities. Participants completed an online experiment including a fear conditioning task and multiple questionnaires. In the first phase of the conditioning task, an image was conditioned to evoke fear in participants. In the second phase participants received extinction training to 'unlearn' previously acquired fear. Fearfulness ratings to the conditioned stimulus over the experiment were analysed and compared between groups of OC users and natural cyclers and between OC users in different pill phases. Data of 251 participants were included in the final sample.

Firstly, data-driven latent growth modelling (LCGA) was applied to identify distinct fear conditioning trajectories in participants and determine their success of extinguishing fear. In accordance with previous research, analyses proposed a 3-class model that classified participants into groups of low fearful conditioners, normal conditioners, and poor extinguishers. So far, patterns found in previous studies were replicated (Leen et al.2021, Duits et al., 2021). In this study, 60 low fearful conditioners showed low fearfulness scores to the conditioned stimulus over the course of the entire fear conditioning experiment. The class of normal conditioners, which contained 77 participants, was characterized by increased fearfulness scores in the acquisition phase that decreased in the extinction phase. This group was most successful extinguishing previously acquired fear. The group of poor extinguishers was largest, consisting of 114 participants that showed sustained fearfulness scores to the CS during extinction. This distribution of participants over

extinction classes differed greatly from previous studies. In those studies, not the poor extinguishers class, but the normal conditioners class was repeatedly shown largest (Leen et al.2021, Duits et al., 2021). In contrast, here, almost 50% of participants was assigned to the poor extinguishers class, indicating worse overall performance on fear extinction. Implications for success of conditioning fear online will be discussed at the end of this section. Classification into the group of poor extinguishers suggested fear extinction deficits. Overrepresentation of OC users and OC users in an active pill phase in this class was examined. Secondly, final fear scores of participants after extinction were analysed, by comparing fearfulness ratings to the CS at the end of the extinction phase between OC users and NCs, and between pill users in an active and inactive pill phase.

Contrary to the expectations, no difference was found in the distribution of OC users and NCs over fear extinction classes. OC users were not overrepresented in the group of poor extinguishers. Also, no effect of OC use was found on participants' final fear scores: these did not differ significantly between OC users and NCs. Therefore, the first hypothesis of this study that stated *'oral contraception users will be overrepresented in groups of poor fear extinction and will end up with higher final fear scores as compared to natural cyclers'* was rejected. The mentioned findings are in contrast with previous research, suggesting impeding effects of the pill for fear extinction processes. Previous literature has suggested altered neural structures involved in fear regulation as a consequence of OC use (Graham & Milad, 2013; Merz et al., 2012; Lisofsky et al., 2016; Petersen et al., 2014). More specifically, research has demonstrated an impeding effect of hormonal contraception use and the pill for extinction training-like exposure treatment effectiveness in spider phobic women (Raeder et al., 2019; Graham et al., 2018). The impact of OC use on fear extinction was not confirmed in this study, addressing more general fear, and using additional fear trajectory analyses and a larger sample than Raeder et al. (2019) and Graham et al. (2018). Findings of this study thus implicate no disadvantageous effect of OC use for women's fear extinction abilities. It must be noted, however, that due to an unexpectedly limited number of OC users in the sample (N = 46), class distribution comparison had to be performed using small subgroups in distinct fear extinction classes. The smallest class subgroup contained 11 participants. Research using a sample size ensuring subgroups of at least 20 participants in each class could provide more certainty with respect to the role of OC use in fear extinction. Taking into account that 18.3% of participants was identified OC user in this study, and 23.9% was classified into the smallest extinction class, a sample size of at least 455 participants is expected to be needed to meet this criterion. Future studies using larger samples are needed to definitively rule out any OC effect.

An effect of pill phase was not found either. Contrary to the expectations, OC users in an active pill phase were not overrepresented in the poor fear extinction class and final fear scores of OC users in an active pill phase did not differ significantly from those of OC users in a pill-free week. Hypothesis 2 stating *'oral contraception users in an active pill phase will be overrepresented in groups of*

poor fear extinction and will end up with higher final fear scores as compared to OC users in an inactive pill phase' thus had to be rejected as well. However, like the analyses concerning the OC effect, pill phase analyses had to be performed comparing very small subgroups of participants in different classes. The smallest group of active pill phase OC users in the normal conditioners class consisted of as few as 3 participants. Results thus must be interpreted with big caution, if they can be interpreted at all. Future research using samples with more OC users is needed to be sure of any unshown effects by this study. A pill phase effect is still plausible. Though an effect of pill phase on fear extinction abilities was not examined before, previous research investigating resting state connectivity has related active pill phase to potential emotion regulation problems (Petersen et al., 2014). Both distribution results and final fear scores here were in the expected direction: a larger proportion of OC users in active pill phase was in the poor extinction group, and final fear scores of active OC users were slightly higher compared to the inactive pill phase group. Based on the subgroup proportions of this study, future research should investigate the effect again using sample sizes of at least 1.667 participants in order to make a meaningful class comparison. Though, the advised number is actually higher, as the last 31 participants added to the used sample of this study were just females.

The online conditioning experiment of this study offers great possibilities for future data collection. As opposed to experiments conducted in the lab a much larger and diverse sample can be gathered using an online conditioning task. Not only does it take less time gathering data online as participants can complete the experiment simultaneously, but it furthermore takes away the need for surveillance by researchers. Though this study was performed over a limited first sample, possibilities for future analyses look promising, as new data can be – and currently are – gathered every day. Unfortunately, online testing also comes with difficulty ensuring good quality data. In this study, almost half of participants was classified poor extinguisher, indicating relatively bad overall fear extinction performance compared to earlier studies. This indicates that although fear conditioning in the acquisition phase was successful, fear extinction training might not have been as effective here. One possible explanation for this would be that attention of participants decreased over the course of the experiment. Whereas attention deficits can be detected by the researchers in lab studies, behaviour of participants cannot be observed experimenting online. It is crucial that the right measures are taken to ensure full attention of participants unto the end of the experiment in the future. Catch trials were already included to test attention of participants and these suggested no attention deficits towards the ending of the experiment. However, though participants might not have looked away from their screens, motivation might have been a problem due to the experiment duration. Perhaps other measures might increase attention and motivation later on in the experiment, leading to increased extinction training effectivity. For instance, participants can be

asked to complete the experiment in a darkened room, or a quest element can be included with a chance of winning a prize.

Recommendations for future research

Future studies should use larger samples. Not only will a larger number of OC users in the sample enable more meaningful analysis and provide more clarity about the effects tested here; a larger number of NCs will furthermore make it possible to compare participants in different menstrual phases. In this study, a comparison of OC users and NCs in different menstrual phases was not analysed due to sample size feasibility. However, this would be an important addition to existing research.

Comparing OC users (and other HC users) to NCs in their follicular and luteal phase provides more clarity than comparing OC users and NCs generally. Firstly, an effect of OC use would be easier to reveal, as potentially diverging performances of NC groups in different phases are not averaged. As endogenous hormone levels of OC users and NCs in their follicular phase are comparably low, and fear extinction is thought to be positively affected by estradiol levels, these two groups are expected to perform similarly on fear extinction tasks. However, as hormone levels of NCs in their luteal phase are higher, an underperformance effect of OC users is more plausible when these groups are compared. Secondly, comparing OC users to NCs in different menstrual phases enables disentangling the effects of endogenous and exogenous hormones. An effect between OC users and luteal phase women would point to suppressed estradiol levels as the impeding factor of OC use – supporting previous research. However, if an effect is found between OC users and follicular phase NCs with similar endogenous hormone levels, this indicates artificial hormones in the pill might be the problem.

A similar approach to distinguishing NCs presented in this study is advisable in related research. Firstly, even though Schmalenberger et al. (2020) distinguished 4 menstrual phases, here, only participants in follicular and luteal phase were distinguished. The follicular and luteal phase are characterised by relatively stable low (follicular) and high (luteal) hormone levels for a duration of multiple days. Therefore, hormone levels of participants in these phases can be assumed with relative certainty. As other menstrual phases are characterised by hormone fluctuations that can occur within a short period of time and at specific moments, hormone levels of participants in these phases are uncertain, especially using rough self-reports about the cycle. Comparison of NCs in 4 different menstrual phases would only be possible when hormone levels are more accurately (physiologically) measured. Secondly, as using the standard of Schmalenberger et al. (2020) only allows for a very limited number of NCs to be assigned to one of the two phases, here a more liberal approach was

proposed. Instead of assigning participants to the follicular phase when they were at day 4 to day 7 of their menstrual cycle at the time of the experiment, a timespan running from day 3 to day 10 was used. Furthermore, whereas Schmalenberger et al. (2020) used a luteal phase running from 9 to 5 days before menstruation, here it ran from 11 to 3 days before menstruation. Even though the strict criteria of Schmalenberger et al. (2020) allow for a more precise distinction, the more liberal approach still does not allow overlap of phases and ensures a larger usable sample. In view of feasibility with regards to sample size, the liberal approach may be more practical, although initiating hormone fluctuations of participants in these phases cannot be ruled out.

Beside the distinction between NCs, other additions can be made to improve research on the role of OC in fear extinction in the future. Research on psychological effects of OC use has suggested potential mediating effects of various factors. For example, in studies linking OC use to depression, a young age, traumatic experiences, and increased psychosocial and interpersonal stress in participants might have played a role (Skovlund et al. 2016; 2018; Hertel et al., 2017). Also, family history of depression or comorbid medical or substance abuse problems could be confounding factors for negative effects of OC use (Robakis et al., 2019). Controlling for these intra-group differences could be valuable in fear extinction research too. Furthermore, recency of initiating OC use has been shown a mediating factor of OC fear effects, as fear related brain alterations were found in participants shortly after initiating HC but were not in similar studies that did not account for the variable (Lisofsky et al., 2016). When the mentioned variables are taken into account, effects of OC use on fear extinction can be determined with more certainty.

The current experiment can be used for future investigation of OC effects on fear extinction. Two aspects need improving. Firstly, presentation duration of the catch trial numbers should be increased, like was already done in this study for the last 31 participants. In this study, initially data of 90 participants were excluded based on poor catch trial performance. For the sake of clarity, we assumed participants that answered less than 3 visual or beep trials correctly did not pay enough attention, indicating that their data should not be used. However, given the high exclusion rate and responses of some participants, catch trials might have been too difficult. Mainly the number trials appeared difficult and resulted in participants commenting that 'there was something wrong with the numbers' or that they blinked and missed them. In the future the increase of number presentation duration hopefully leads to the use of more good quality data. Secondly, the MCQ question regarding cycle length of participants should be more carefully formulated. Here, many participants reported a cycle length of under 8 days, indicating they might have misinterpreted the question for the duration of their menstruation. This remained the case even after the question was reformulated in a second version of the experiment for the last 31 participants. Of 56 participants reporting a regular menstrual cycle, in total 21 participants reported a cycle length of under 8 days. Better explanation of the

menstrual cycle length should enable determining the menstrual cycle phase of more NCs in future research.

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Appendices

Appendix 1

Complete Menstrual Cycle Questionnaire (MCQ)

If you are female, we would like to ask you some questions regarding your menstrual cycle. Please fill in if this is applicable to your situation.

"Instructions menstrual cycle questionnaire"
The following questions will be about your menstrual cycle and the use of hormonal contraception (for example, the pill or IUD).

1. At what age did you first get your period?

2. Are you currently using any form of contraception (with the exception of condoms and other barrier methods)?
- Yes
- No.

3. If your answer was yes to the previous question, what form of contraception are you using?
- Does not apply
- Birth control pill
- Hormone IUD
- Copper IUD
- Contraceptive patch
- Contraceptive ring
- Contraception implant
- Injection pill
- Other, namely: ** to be entered by the participant **

4. If you use the contraceptive pill, which one are you currently on?
- Microgynon30
- Microgynon50
- Lovette
- Diane
- Yasmin
- Stediril30
- Marvelon
- Trigynon
- Mercilon
- Femodeen
- Zoely
- Unbranded pill: ethinylestradiol, levenorgestrel
- Unbranded 3 phase pill: ethinylestradiol, levenorgestrel
- Unbranded pill: ethinylestradiol, drospirenon
- Unbranded pill: ethinylestradiol, desogestrel

- Unbranded pill: nomegestrol, estradiol
- Unbranded pill: ethinylestradiol, cyproteron(acetaat)
- Different: *** to be entered by the participant ***
- I don't know / unknown

5. If you are using the contraceptive pill, at what age were you first prescribed?

*** to be entered by the participant ***

6. How long (in years) have you been using the pill?

*** to be entered by the participant ***** years

7. If you are using the contraceptive pill, are you taking a pill on the day of the examination (today), or are you in the pill-free week?

- Does not apply
- Today I take the pill
- I'm in the stop week (or haven't taken the first pill of a new pill strip yet)

→ If you are in the stop week, when did you take your last pill?

*** to be entered by the participant ***** day(s) ago

8. What was your main reason to start using your current contraception method?

- Preventing pregnancy
- Regulating menstruation
- As a treatment for acne
- Decreasing physical symptoms due to my natural menstrual cycle (f.e. stomach cramps)
- Decreasing psychological symptoms due to my natural menstrual cycle (f.e. premenstrual mood symptoms)
- Different: *** to be entered by the participant ***

9. Which of the following symptoms do/did you experience before or during your menstruation in your natural cycle (without contraception)?

- Physical symptoms (f.e. stomach cramps, headache, breast pain or sensitiveness, nausea, irregular bleedings, acne)
- Psychological / mood symptoms (f.e. premenstrual mood symptoms)
- Sex-related symptoms
- I don't'/didn't experience any symptoms

10. Which of the following side effects of your current contraceptive do you experience?

- Physical symptoms (f.e. headache, nausea, stomach-ache, breast pain or sensitiveness, weight gain)
- Psychological symptoms (f.e. mood symptoms)
- Sex-related symptoms (f.e. lowered libido / sexual desire)
- I don't experience any symptoms
- Other, namely:
- I don't use contraceptives (except from condoms and other barrier methods)

11. If you don't use hormonal contraception, when was the first day (date) of your last period? (DD/MM/YYYY. Example: 21/12/2020)

- Not applicable
- The first day of my last period is:

_____

12. If you don't use hormonal contraception, what week of your cycle are you in?

- First week (I'm menstruating this week)
- Second week from menstruation
- Third week from menstruation
- Fourth week from menstruation
- I don't know
- Not applicable

13. If you don't use hormonal contraception, do you typically have a menstrual period about once per month (about every 22 – 28 days)?

- Yes
    - If yes, how long does your cycle normally last?
      *** to be entered by the participant ***** days
- No.
- I do not know
- I no longer have my period