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Measuring Oxytocin in Everyday Life: Why, When, and How (often)?

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Content

List of Included Publications

I. **Schneider, E.**, Müller, L. E., Ditzen, B., Herpertz & Bertsch, K. (2021). Oxytocin and social anxiety: Interactions with sex hormones. *Psychoneuroendocrinology*, 128, 1-7. doi.org/10.1016/j.psyneuen.2021.105224

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III. **Schneider, E.**, Hopf, D., Eckstein, M., Scheele, D., Aguilar-Raab, C., Herpertz, S. C., Grinevich, V. & Ditzen, B. (2023a). Stress during the COVID-19 Pandemic Moderates Pain Perception and Momentary Oxytocin Levels. *Journal of Clinical Medicine*, 12(6), 1-12. doi.org/10.3390/jcm12062333

Note: For individual author contributions, see Appendices I to III.

Summary

Research spanning over a hundred years has highlighted the importance and complexity of the oxytocinergic system. Oxytocin - a neuropeptide and hormone - has a wide-ranging impact on both the body and brain. A tremendous number of studies have focused on the role of oxytocin in social behavior and social cognition, with intranasal administration of the hormone being the prevalent method in human research due to its potential use for treatment. Only in the recent past assessments of endogenous oxytocin levels have increased and gained popularity. Despite the increasing number of papers with peripheral oxytocin measures, current research indicates that there are still uncertainties about the value of measuring oxytocin in bodily fluids and debates about the reliability and validity of peripheral oxytocin measures. Therefore, my dissertation aims to achieve two goals. Firstly, to highlight the importance of studying endogenous oxytocin levels by reviewing previously published research and presenting my own empirical work. Secondly, to outline recommendations on when and how to include oxytocin measures in future human research.

My empirical work presented in this dissertation focuses on exploring the role of endogenous oxytocin levels within anxiety, social bonding, and pain, which are fields widely studied in animal oxytocin research. The first paper of my dissertation aims to analyze the associations of plasma oxytocin with self-reported anxiety levels in high and low-socially anxious individuals by including sex hormones as a moderator (Paper I). The results indicate that high levels of plasma oxytocin and estradiol predict lower levels of social anxiety in women, especially in highly anxious individuals. Additionally, basal levels of oxytocin were significantly different in women using hormonal contraception compared to naturally cycling women. Paper II explores how salivary oxytocin relates to affectionate touch during the first lockdown of the COVID-19 pandemic, and Paper III examines the link between salivary oxytocin and emotional and physical pain during the second lockdown one year later. Both Papers (II-III) present data from Ecological Momentary Assessment within the general population. Statistical analyses presented in Paper II revealed that momentary salivary oxytocin levels increased with the intensity of experienced affectionate touch. On the contrary, in Paper III, the results indicate that individuals' oxytocin levels were lower when they reported higher intensity of emotional pain. Additionally, individuals' stress levels moderated the link between physical pain and salivary oxytocin, suggesting that oxytocin levels were the lowest when participants reported higher stress and more bodily pain.

Taken together, these results highlight the importance of investigating endogenous oxytocin measures, as they demonstrate significant links to various aspects of human

(social) life, including health-related outcomes. Furthermore, I addressed the second aim of my dissertation by synthesizing the data gathered from the presented projects and providing recommendations and directions for upcoming research accentuating on diurnal endogenous oxytocin measures.

1 Background of Oxytocin Research: Milestones, Development, and Challenges

Oxytocin is an ancient peptide that has been conserved throughout evolution over millions of years and can be found in a diverse range of species, including mammals, birds, and invertebrates. Over time, oxytocin's functions have been adapted by organisms to suit their changing environments and to meet the specific needs of different species. In mammals, oxytocin regulates social behavior, lactation, and reproduction (Feldman et al., 2016).

In recent years, researchers have discovered many additional functions of oxytocin, which led to an increase in interest in and fascination with this peptide. This chapter provides an overview of the background of the oxytocinergic system, the evolution of oxytocin research, and the methodological challenges researchers face in this field.

1.1 Physiology of the Oxytocinergic System

Oxytocin is a nonapeptide composed of nine amino acids. It is synthesized mainly in the magnocellular neurosecretory cells of the paraventricular nucleus (PVN), supraoptic nucleus (SON), and the accessory nucleus (AN) in the hypothalamus, with a smaller amount produced in the parvocellular neurosecretory cells (Grinevich & Neumann, 2021). Oxytocin-containing neurons release oxytocin directly from the soma and dendrites (Ludwig & Leng, 2006), and they also project to numerous parts of the central nervous system, including the hippocampus, amygdala, cerebral cortex, brainstem, and spinal cord. Additionally, their axons project to the posterior pituitary, where oxytocin is released into the bloodstream. Besides central synthesis, oxytocin can also be produced in various peripheral tissues, including the uterus, mammary gland, testes, heart, and skin (Chaves et al., 2013).

Oxytocin is packaged and transported in large secretory vesicles. It travels down the axon along with its precursor and carrier protein neurophysin-1 and is released at the nerve terminal via exocytosis (Carter, 2017). Oxytocin-producing neurons exhibit the capacity for pulsatile release of the peptide due to their morphological and functional plasticity (Carter et al., 2020). In the bloodstream, most of the oxytocin is bound to proteins and is rapidly metabolized by the enzyme cystinyl aminopeptidase (also known as oxytocinase) (Brandtzaeg et al., 2016). Oxytocin has a relatively short half-life of a couple of minutes in the bloodstream compared to its half-life in the brain, which is approximately 20 minutes (Ludwig & Leng, 2006). Upon reaching its target organs, oxytocin can affect their functions by binding to specific oxytocin receptors (Carter, 2017).

The oxytocin receptors are coupled to G-proteins and can activate various signaling pathways, resulting in similar or opposing effects. On the one hand, activating G-proteins by the oxytocin receptors can lead to both inhibitory and excitatory consequences within the same cell, depending, among other things, on the type of protein it is bound to. On the other hand, activating multiple signaling pathways by oxytocin receptors in different cell systems can work together to produce a synergistic effect, such as the contraction in myometrial cells (Busnelli et al., 2012). Moreover, oxytocin receptors can form both homomers (pair of oxytocin receptors) and heteromers (pair of oxytocin receptors with a different receptor), potentially increasing the range of their activities and the complexity of oxytocin response (Agnati et al., 2010; Fuxe et al., 2012). The expression and distribution of oxytocin receptors are dynamic and change throughout development. For instance, the increase of the expression of oxytocin receptors throughout the brain begins prenatally and peaks in early childhood and late adulthood, suggesting that there are critical periods when the expression peaks occur. There are numerous other factors, including the specific brain region and biological sex of the individual, affecting the levels and location of oxytocin receptors (Rokicki et al., 2022).

The synthesis and expression of oxytocin and oxytocin receptors are genetically regulated by the oxytocin gene located in chromosome 20 and the oxytocin receptor gene located in chromosome 3 (Carter, 2017). Notably, the functions of the oxytocinergic system can be influenced by epigenetic modifications resulting from an individual's experiences, such as exposure to stress and trauma (Carter et al., 2020). Furthermore, several other factors, including other hormones and neurotransmitters, can also influence and regulate the oxytocin system. For example, projections from serotonergic, GABAergic, and catecholaminergic neurons to the paraventricular nucleus have been reported to have regulatory functions on the oxytocinergic system (Banerjee et al., 2017). Steroid hormones, particularly estrogens, regulate oxytocin synthesis and oxytocin receptor expression (Gabor et al., 2012). Oxytocin and vasopressin, a related peptide, share several similarities, including structure, synthesis, release, and receptor system. Both peptides can bind to some of each other's receptors, thereby regulating each other's effects (Carter, 2017). Moreover, oxytocin can autoregulate its release through a positive feedback mechanism in which stimulation of the oxytocin system triggers the release of even more oxytocin, leading to increased concentration. Interestingly, oxytocin's precursor hormone, the extended form of oxytocin, as well as its fragments, also appear to be biologically active. However, their specific roles of actions are not yet well understood. Besides the regulation within the central nervous system, peripheral signals such as suckling also have regulatory effects on the

oxytocin system by inducing the release of oxytocin. (Carter et al., 2020; Ludwig & Leng, 2006).

The scope and the complexity of the physiological and functional specifics of the oxytocinergic system are far more extensive and complex, going beyond what is presented in this overview. A long history of research and dedication from numerous research teams was required to acquire the current knowledge of the oxytocinergic system.

1.2 Milestones and Evolution of Oxytocin Research

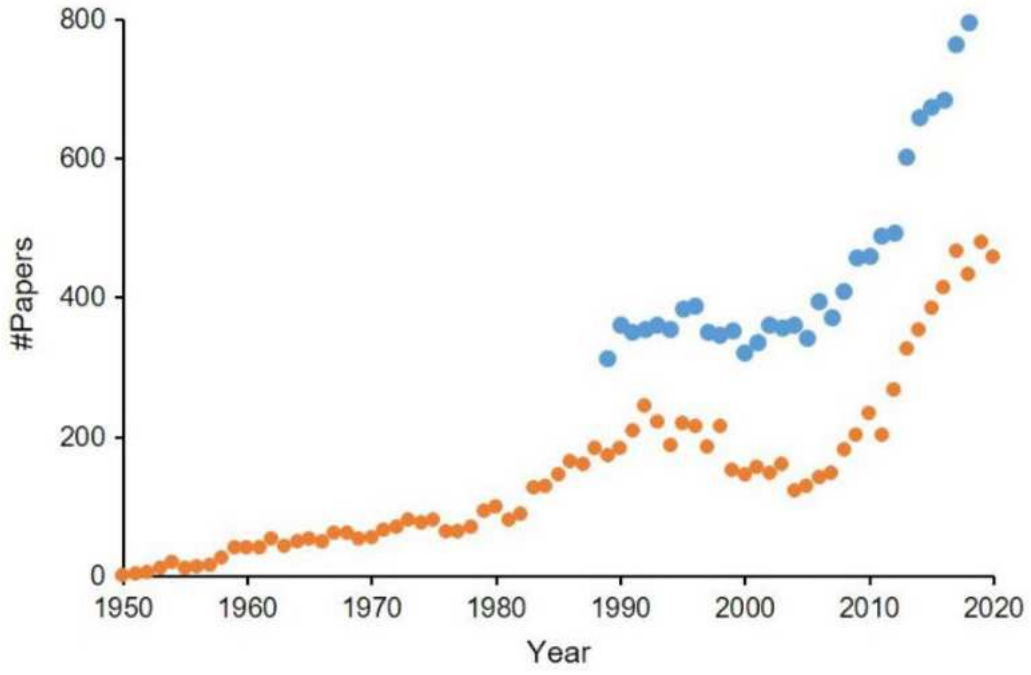
The history of oxytocin dates back to its discovery by Henry Dale in 1905, who reported about a neurohypophysical substance that caused uterine contractions (Dale, 1906). A few years later, Mackenzie reported that a pituitary extract can stimulate milk ejection (Mackenzie, 1911). About 40 years later, Vincent du Vigneaud described the structure of oxytocin, synthetically produced it for the first time (Du Vigneaud et al., 1954), and received a Nobel Prize for his work. The discovery and synthesis of oxytocin set a massive milestone in oxytocin research, and synthetic oxytocin has been used since the 1950s to induce labor in pregnant women.

Besides its physiological effects during parturition and lactation, in the 1970s, oxytocin was reported to influence maternal behavior. Pedersen and Prange demonstrated that oxytocin administration into a virgin rat's brain can induce maternal behavior, including building a nest for both their own and foster pups (Pedersen & Prange, 1979). Although some of the replication studies have failed to reproduce the results (Bolwerk & Swanson, 1984; Rubin et al., 1983), other studies confirmed the effects of oxytocin on maternal behavior in rats (Pedersen et al., 1982) and sheep (Kendrick et al., 1987). In the same decade, some literature suggested oxytocin's involvement in sexual behavior, reporting oxytocin administration inducing lordosis, which facilitates sexual behavior in female rats (Caldwell et al., 1986) and endogenous oxytocin increase during orgasm in humans (Carmichael et al., 1987).

The growing interest in oxytocin had its first publication peak in the 1990s, about the time when the research group of Sue Carter discovered that oxytocin played one of the critical roles in social bonding in prairie voles, the species that are known for their monogamous bonding and mating behavior (see Figure 1, retrieved from Leng & Leng, 2021). The researchers were able to show that centrally, but not peripherally, administered oxytocin affected partner preference (Williams et al., 1994) and that prairie voles have a different oxytocin receptor distribution in the brain compared to other species, concluding that these differences might mirror the differences in partner bonding and other social

behavior (Witt et al., 1991). This pioneering research demonstrated oxytocin's involvement in forming and maintaining social bonds and affiliative and social behavior in general, thus setting the beginning of the understanding of oxytocin as a social peptide.

Figure 1. Publication process over time.



Note: The graph depicts the number of papers (reviews and articles) published each year between 1950 and 2020. Orange dots represent papers with oxytocin in the title; blue dots represent papers with oxytocin as a topic but not in the title. Source of the graph:(Leng & Leng, 2021).

During the same timeframe, the first reports on oxytocin's role in other, nowadays highly investigated fields related to pain, stress, and anxiety behavior were published. Some of the first papers demonstrated that oxytocin levels were elevated in response to different types of stress, including forced swimming, restraint, and ether (Gibbs, 1984; Lang et al., 1983). Oxytocin administration before stress exposure has been shown to dampen the stress-induced corticosterone response in rats. Additionally, these rats showed more explorative and less anxious behavior when exposed to stress (Windle et al., 1997). Regarding pain perception, early studies provided the first evidence of the analgesic effects of oxytocin. For example, exogenously administered oxytocin reduced thoracic pain in cancer patients (Madrazo et al., 1987), showed antinociceptive effects in rats and mice

(Arletti et al., 1993; Lundeberg et al., 1994), and increased the threshold for pain perception in mice (Uvnäs-Moberg et al., 1992). However, despite these early reports of the analgesic effects of oxytocin in the 1980s, it is only in the last decade that interest in this area of research has increased dramatically, as the number of publications suggests (Leng & Leng, 2021).

In the early 2000s, another big surge of interest in oxytocin research emerged after studies were conducted on the role of oxytocin in trusting behavior. Kosfeld and his colleagues reported that intranasally administered oxytocin increases trust in humans (Kosfeld et al., 2005). Although this hypothesis was later discarded due to insufficient replicability of these effects (Nave et al., 2015), the study by Kosfeld et al. was one of the first contributing to the high visibility of oxytocin in general media and its reputation as a "love hormone," "intimacy elixir," or "trust molecule" (e.g., Wilhelm, 2008; Zak, 2011). Another influential study at that time found that participants who received oxytocin better recognized affective states from images of other people's eye regions than participants in the placebo condition, suggesting that oxytocin improves "mind reading" (Domes et al., 2007). Based on these findings, among other studies, it was concluded that oxytocin may be used as a drug for therapeutic purposes. For example, researchers considered its potential use as an intervention in couples therapy (Ditzen et al., 2009), as well as for treating neurodevelopmental and psychiatric disorders that are characterized by social dysfunction, such as autism (Domes et al., 2007), anxiety disorders (Heinrichs et al., 2009) and schizophrenia (Pedersen et al., 2011).

However, this initial hype was followed by disenchantment with oxytocin and studies questioning oxytocin's potential for several reasons (Alvares et al., 2017). Some studies were not able to replicate previously reported positive effects of oxytocin (e.g., Nave et al., 2015), while other studies reported null findings in clinical trials (e.g., Cacciotti-Saija et al., 2015; Dadds et al., 2014; Einfeld et al., 2014). Moreover, opposing results were reported regarding the effects of oxytocin on social behavior, which contradicts the previously accepted hypothesis that oxytocin is primarily associated with social cognition and prosocial and affiliative behavior. For example, oxytocin administration was associated with increased negative feelings such as envy and gloating (Shamay-Tsoory et al., 2009) and decreased cooperative behavior with out-group members (De Dreu et al., 2011). These mixed and conflicting reports suggested that the effects of oxytocin are not as straightforward as initially hoped. Instead, research indicates that the effects may be influenced by contextual factors and individual factors such as gender, personality, and genetics (Bartz et al., 2011). Furthermore, methodological differences in these studies seem to be at least partially responsible for the conflicting results (Alvares et al., 2017).

The increase in reported discrepancies in oxytocin research has led to a growing number of publications analyzing methodological differences and focusing on improving the methods used in oxytocin research over the past decade.

1.3 Methodological Advancements and Challenges

A body of reported evidence for the role of oxytocin in social behavior comes from animal studies using a variety of methodological approaches. For example, researchers can observe changes in social behavior after knocking out the oxytocin (receptor) genes in animals or experimentally manipulating oxytocin levels in specific brain regions. This involves stimulating oxytocin expression by virus injections or intracerebral injections of synthetic oxytocin (e.g., Neumann & Slattery, 2016).

Human studies have more limited methodological options than animal research due to technical limitations and ethical considerations. For example, it is impossible to measure oxytocin in the living human brain or genetically manipulate the oxytocin system. Thus, so far, most human studies rely primarily on indirect measures of oxytocin levels, e.g., in plasma and saliva or the exogenous administration of oxytocin (Quintana et al., 2021).

Most human studies with exogenous administration have used 24 International Units (IU) of oxytocin, although reported doses vary between 8 and 48 IU. For a long time, decisions to use 24 IU were frequently based on precedent rather than systematic research (Quintana et al., 2015a). Although several dose-response studies have been performed, no consensus has yet been reached on the most efficacious dosage of intranasal oxytocin. For example, Quintana and colleagues reported that low doses of oxytocin (8IU) most effectively affect the amygdala response to emotional stimuli (Quintana et al., 2016). In this study, researchers used an advanced nasal delivery method called a “Breath Powered device,” designed to optimize medication delivery through the nasal cavity (Djupesland, 2013). Other studies suggest that higher doses of oxytocin may be better when using conventional nasal sprays. While Spengler and colleagues showed that a moderate, commonly used dose of oxytocin (24 IU) was the most effective in affecting amygdala functioning in men (Spengler et al., 2017), a recent study suggests that an even higher dose of oxytocin (40 IU) may be the most effective (Martins et al., 2020a).

Many uncertainties have been discussed in the literature regarding the underlying mechanisms of how and where exogenous oxytocin reaches and affects the brain. Although not fully understood, recent research suggests that intranasally administered oxytocin reaches the brain directly, primarily through olfactory and trigeminal nerve fibers (Quintana et al., 2021). Indeed, increased oxytocin levels in cerebrospinal fluid (CSF) have been

reported after intranasal application (Striepens et al., 2013). However, in non-human studies, only small changes in oxytocin concentrations have been found, suggesting that no more than 0.005% of exogenously administered oxytocin was measurable in the CSF, possibly because oxytocin is degraded in brain tissues and only a small amount of oxytocin (approximately 5% in rats) reaches the CSF in general (Leng & Ludwig, 2016). Importantly, there is also an indirect pathway to the brain through the periphery. Intranasally applied oxytocin enters systemic circulation through intranasal blood vessels and can act in the periphery as oxytocin receptors are located in several organs (Quintana et al., 2015a). Several studies demonstrated a significant increase of peripheral oxytocin levels after intranasal administration as measured, e.g., in plasma (Striepens et al., 2013) and saliva samples even after a few hours (Daughters et al., 2015; Huffmeijer et al., 2012). However, because oxytocin is a large molecule, only a minimal amount of the molecule can cross the blood-brain barrier and reach the brain. Thus, most research on oxytocin and social behavior focused on the more direct delivery pathway using the intranasal application of oxytocin (Quintana et al., 2015a). Indeed, some research indicates that intranasal administration appears to be more effective in affecting amygdala and social cognition than peripheral (intravenous) administration (Quintana et al., 2016; Quintana et al., 2015b). Noteworthy, few studies reported similar effects after intravenous oxytocin administration, indicating that peripherally administered oxytocin can affect the processing of social information (Hollander et al., 2007) as well as specific parts of the brain such as the amygdala and anterior cingulate cortex (Martins et al., 2020a).

Not only peripheral administration but also measuring oxytocin has not always enjoyed popularity in research despite its importance. There are two most common methods for measuring oxytocin: radioimmunoassay (RIA), developed in the 1980s, followed by enzyme-linked immunoassay (ELISA), developed later in the 1990s. RIA and ELISA are similar in principle as they rely on binding the hormones to antibodies. Therefore, oxytocin present in the sample competes with added radiolabeled or enzyme-linked oxytocin for binding to the antibody. The higher the concentration of oxytocin in the sample, the less labeled oxytocin will bind. The quantity of labeled oxytocin is then measured, and the amount of oxytocin from the sample is calculated based on a standard curve generated from known concentrations (Leng & Sabatier, 2016). Despite the similarities of the techniques, different immunoassays use antibodies with different epitopes, which can cause variability in the results (MacLean et al., 2019). For example, comparing samples analyzed with RIA vs. ELISA showed that the concentrations did not correlate (Lefevre et al., 2017), indicating that comparisons between studies using different methods are critical.

The precision of the analyses is not solely dependent on the method used but also on the type of samples utilized (Tate & Ward, 2004). Especially when analyzing oxytocin in plasma samples, there are various possible sources of interference. Substances, such as hormones other than oxytocin, present in the sample can interfere with antibodies and affect the concentration value (Leng & Sabatier, 2016). Therefore, pre-analytical sample preparation, such as extraction, has been proposed to eliminate interfering matrix components. In fact, Szeto and colleagues reviewed that oxytocin without extraction was up to 100-fold higher than extracted plasma samples, and there was a small correlation between extracted and unextracted samples measured with ELISA (Szeto et al., 2011). While sample extraction before the analyses is currently predominantly recommended (Tabak et al., 2022), some concerns have been expressed that extraction might also influence the concentration values. For example, it may result in the partial discard of oxytocin bound to itself (in dimers or trimers), other proteins, or lipids, as well as insufficient recovery of the analyte or degradation of oxytocin through exposure to high temperatures and oxidation (MacLean et al., 2019). Moreover, MacLean and colleagues argue that possibly not only free oxytocin, measured in extracted samples, but also bound oxytocin might be a valid measure with biological relevance (MacLean et al., 2019).

Measuring oxytocin in saliva samples has recently become more popular due to its non-invasive nature. Although this approach was initially criticized and remains a topic of debate to date, it is now used more frequently because saliva has relatively low protein levels, resulting in fewer matrix interference issues during measurement without extraction (MacLean et al., 2019). Indeed, previous reports indicate that oxytocin concentrations in extracted and non-extracted samples are highly correlated when measured with ELISA (MacLean et al., 2018). A new pre-processing method for saliva samples has been introduced to avoid the time-consuming extraction procedure involving lyophilization or evaporation of the sample followed by reconstitution in assay buffer (de Jong et al., 2015). Although this method is less time-consuming, evaporation of the sample may lead to other interference issues, such as undissolved residues during reconstitution and alterations in pH and ionic strength, which can affect the antibody binding of the assay (Tabak et al., 2022).

Taking together, research on the human oxytocin system has encountered several methodological challenges, specifically regarding central administration as well as measurements of peripheral oxytocin. Even though endogenous oxytocin has been measured since the 1980s, it remains underrepresented and is only now starting to gain more frequent use. Current research indicates that there are still many debates and uncertainties regarding the purpose of oxytocin measurements and even more regarding what is the best way to measure oxytocin (Tabak et al., 2022). Although there have been

many improvements in recent years, as reviewed above, further methodological research and development of assays are still needed.

1.4 Aims of the Present Dissertation

To follow these ongoing debates surrounding the research on endogenous oxytocin measurements, the present dissertation aims to address some of the open questions mentioned above. The first aim of my dissertation is to emphasize the importance of measuring endogenous oxytocin and its value in social bonding, anxiety, and pain. I specially focus on these research domains as oxytocin animal research traditionally focuses on these. The studies I report in this dissertation particularly involve measures of endogenous oxytocin levels using different methodological approaches. The second aim of this dissertation is to synthesize my findings with previous research and outline recommendations on when and how often oxytocin should be measured in saliva.

2 Why Measuring Oxytocin?

Studies conducted on the impact of oxytocin on social behavior have mainly involved intranasal administration of the hormone due to its potential therapeutic benefits. Nevertheless, focusing on this approach has resulted in neglecting other oxytocin research methods, such as measuring oxytocin levels, partially because of concerns about the accuracy of peripheral oxytocin measures in indicating central concentration levels (Leng & Ludwig, 2016). The correlation between central and peripheral oxytocin levels remains uncertain due to contradictory findings. Some studies have found no link (e.g., Altemus et al., 2004; Kagerbauer et al., 2013)), while others have reported a positive correlation (e.g., Carson et al., 2015; Wang et al., 2013). Lefevre et al. demonstrated that differences in methodology could account at least for some of the reported discrepancies. The authors reported a significant correlation between plasma and CSF oxytocin levels when measured with ELISA but not with RIA (Lefevre et al., 2017). In the same year, a meta-analysis by Valstad and colleagues was published revealing a positive association between central and plasma oxytocin measures; however, only when intranasal oxytocin was administered or experimentally induced stress was present, but not under basal conditions (Valstad et al., 2017). These results illustrate that peripheral oxytocin levels could serve as a marker of central levels under specific conditions. Indeed, non-human research suggests that oxytocin can be released centrally and peripherally, simultaneously, or independently (Tabak et al., 2022). For example, rodent studies demonstrated coordinated release of central and

peripheral oxytocin in response to pain (Eliava et al., 2016) as well as after social touch (Tang et al., 2020). Therefore, analyzing peripheral oxytocin levels in humans can provide valuable insights into the contextual factors of these associations.

One of the methods to study contextual effects is ecological momentary assessment (EMA). Over the last two decades, EMA has become more widely used in psychological and related research, making it possible to capture psychological phenomena of interest in their natural environment. EMA involves frequent assessments in daily life, increasing ecological validity and accounting for within-subject changes (Wrzus & Neubauer, 2023). Regarding psychobiological measures in everyday life, studies with diurnal salivary cortisol measures have become increasingly popular recently. However, to our knowledge, there are no studies on salivary diurnal oxytocin regarding socioemotional functioning, partially due to the ongoing methodological debates described above. Nevertheless, studying diurnal oxytocin has several advantages. For example, following the evidence that peripheral oxytocin is responsive in specific contexts and can reflect central oxytocin levels, it seems essential to study the associations under natural circumstances. Since blood collection is more invasive and thus can cause stress, measuring diurnal salivary oxytocin might be more suitable. Interestingly, a recent study found that the positive correlation between oxytocin levels in saliva and CSF was even stronger than the correlation with concentrations in plasma samples (Chen et al., 2022).

Moreover, some early administration studies showed that oxytocin could affect the brain and social cognition not only through the central pathway but also through the peripheral pathway, possibly by crossing the blood-brain barrier or by feedback mechanisms from receptors located within the peripheral organs (Quintana et al., 2015a). Improving our understanding of the peripheral oxytocin system can enable us to understand administration studies better and identify people for whom oxytocin administration might be most beneficial (Tabak et al., 2022).

Thus, in the following, the value of measuring peripheral oxytocin concentrations will be exemplified in the popular fields of oxytocin research using different methodological approaches.

2.1 The Role of Endogenous Oxytocin in Social Anxiety (Paper I)

Social anxiety is a type of anxiety that involves persistent fear and avoidance of social or performance situations, causing impairments for individuals and significant economic costs (Dams et al., 2017). A variety of techniques have been applied to address social anxiety disorders, such as various forms of psychotherapy and medical treatments.

Nevertheless, these treatments are only successful in 60-70% of cases. With the sighting of oxytocin's involvement in social behavior, there has been increased interest in exploring its effects, as it has been suggested as a potential treatment option (Jones et al., 2017). Indeed, a body of research has studied the stress-reducing and anxiolytic effects of oxytocin to date. Administration studies indicate that oxytocin can reduce social fear (Zoicas et al., 2014) and avoidance behavior (Lukas et al., 2011) in rodents and affect fear-related neurocircuitry in humans, e.g., during fear processing (Kirsch et al., 2005) or fear extinction (Eckstein et al., 2015). However, evidence on the relationship between social anxiety symptoms and peripheral oxytocin levels is conflicting. While some studies report positive associations (Hoge et al., 2008; Oh et al., 2018), other studies report negative correlations (Carson et al., 2015; Scantamburlo et al., 2007). These inconsistencies could be due to differences in study methodologies, populations, and biological sex. In fact, numerous animal studies demonstrated that oxytocin interacts with other hormones and neurotransmitters rather than acting alone. For instance, estrogens seem to directly influence oxytocin's production and expression (Dellovade et al., 1999). They also affect oxytocin's effects on social recognition (Choleris et al., 2004) and maternal behavior (Pedersen & Prange, 1979) and can even boost oxytocin's anxiolytic effects (McCarthy et al., 1996). However, research on the interactions between oxytocin and sex hormones regarding social anxiety in humans is still lacking. Thus, our study aimed to address this gap by focusing on how endogenous oxytocin and sex hormones relate to self-reported anxiety levels. We hypothesized that sex hormones may affect the link between oxytocin and social anxiety and possibly shed light on the contradicting associations between oxytocin and social anxiety. Specifically, we expected that elevated levels of sex hormones, such as estradiol in females and testosterone in males, along with increased levels of oxytocin, would be associated with lower levels of social anxiety.

To test our hypotheses, 99 highly socially anxious individuals (n = 51 women) and 100 individuals with low social anxiety (n = 50 women) were invited to the laboratory. The age range of the participants was between 18 and 43, with a mean age of 23.98 (SD = 4.74). Female participants were scheduled during their follicular phase of the menstrual cycle, and 68.3% reported using hormonal contraception. Participants provided blood and saliva samples, which were subsequently analyzed biochemically. Oxytocin analyses of extracted plasma samples were conducted at the RIAgnosis laboratory in Munich, Germany, using RIA. In the female subsample, plasma estradiol and progesterone concentrations were analyzed using chemiluminescence immunoassay, while ELISA was used to assess salivary testosterone levels in all participants. Additionally, participants completed the Liebowitz

Social Anxiety Scale (LSAS) (Heimberg et al., 1999) to rate their fear and avoidance in various situations, including social interaction or performance situations.

We found significant hormonal variations in the female subsample depending on whether they used hormonal contraception. Women who used hormonal contraceptives had higher levels of oxytocin but lower levels of estradiol and progesterone in their blood plasma compared to women who did not use them and had a natural menstrual cycle. The results of regression analyses showed a significant interaction of oxytocin and estradiol concentrations predicting social anxiety scores in women. More specifically, these results indicate that women with high levels of oxytocin and estradiol experienced less anxiety compared to those with lower levels of these hormones. Further analysis revealed that this relationship was particularly significant among women with high social anxiety levels. However, no significant correlation was found between testosterone and oxytocin with anxiety in men.

It is important to note that in this study, only oxytocin and testosterone levels were analyzed in men, not estradiol concentrations. Therefore, it is unclear if the significant association between oxytocin and estradiol with anxiety levels found in women also applies to men. However, the results of this paper imply that it is essential to include sex hormone assessments, especially estradiol levels, when analyzing the role of oxytocin in social anxiety, as they seem to interact with endogenous oxytocin levels.

2.2 The Role of Endogenous Oxytocin in Affectionate Touch (Paper II)

Throughout evolution, social bonding has played a vital role in reproductive success, brain development, and emotional well-being (Carter, 2014). It is characterized by positive and affiliative behavior, including emotional attachment and physical contact (Carter, 2005). Gentle physical contact, also known as affectionate or social touch, stimulates unmyelinated sensory neurons called C-fibers and is believed to be particularly important in activating different pathways, including the reward system, and promoting bonding (Carozza & Leong, 2021).

Social touch has been conceptualized as a social safety signal (Eckstein et al., 2020) as it has been shown to have stress and pain-reducing effects (Ditzen et al., 2007; Kreuder et al., 2019). Various experimental studies have demonstrated that social touch, such as massages, can reduce subjective feelings of anxiety and stress (Kirschner & Kirschner, 2019), as well as lower cortisol levels (Maratos et al., 2017). Oxytocin is believed to play a role in these calming effects of social touch as it has been associated with promoting social closeness and reducing social anxiety in rodents (Campbell, 2008). Several studies

demonstrated that physical contact during parent-infant interactions results in the endogenous increase of oxytocin levels in both parents and infants (Scatliffe et al., 2019). Additionally, researchers have found that foot massage induces a significant increase in plasma oxytocin levels, and higher levels of oxytocin are associated with greater liking of social touch (Li et al., 2019; Morhenn et al., 2012). On the other hand, touch deprivation has been suggested to affect individuals' mental state and well-being negatively, increasing psychological distress and the risk for psychological disorders (Banerjee et al., 2021). However, exogenously administered oxytocin has been shown to reduce stress response after acute social isolation in monkeys (Parker et al., 2005).

With the outbreak of the COVID-19 pandemic, the world has faced an unprecedented situation where various measures, including physical contact restrictions, were necessary measures to reduce the spread of the virus. These restrictions were particularly challenging during the first lockdown, and studies have shown they were associated with increased loneliness and mental distress (Chandola et al., 2020; Fancourt et al., 2021; Pierce et al., 2020). At the same time, as the restrictions continued, self-reported desire and perceived pleasantness of social touch increased (Meijer et al., 2022). Moreover, longing for intimate touch was linked to higher self-reported loneliness (Von Mohr et al., 2021) and psychological distress (Burleson et al., 2022).

To better understand the role of oxytocin in response to affectionate touch during the pandemic, we conducted a cross-sectional study during the first lockdown in Germany between April and August 2020. The first part of the study comprised a large online survey assessment including over 1,000 participants; however, this data will not be presented here (for more details, see Appendix II). In the second part of the study, interested participants were invited to participate in a 2-day ecological momentary assessment (EMA). This involved collecting data on their momentary mental state, the occurrence and intensity of affectionate touch, and saliva samples at six time points per day. Saliva samples were stored frozen until analyzed at the Institute for Medical Psychology laboratory in Heidelberg, Germany, using ELISA for cortisol and oxytocin without prior extraction. We hypothesized that experiencing affectionate touch in everyday life would be linked to higher ratings of individuals' well-being, higher oxytocin levels, and lower levels of cortisol. Based on previous literature, we further expected that oxytocin would mediate the link between affectionate touch and individuals' well-being.

In total, 247 (n = 173 women; n = 74 men) individuals participated in the EMA study, with an average age of 32.02 (SD = 13.12). The results of hierarchical linear models revealed that the occurence of affectionate touch was linked to lower self-reported stress, cortisol levels, and higher happiness. We also found that the more intense the affectionate touch

was rated, the lower the subsequent levels of subjective anxiety, general burden, and stress were reported. Additionally, higher intensity of affectionate touch was associated with increased oxytocin levels and self-reported happiness. However, contrary to our hypothesis, individuals' momentary oxytocin levels did not mediate the positive effects of affectionate touch on individuals' distress. Nevertheless, these results indicate that specific behavior, such as social touch, can stimulate endogenous oxytocin release and improve individuals' well-being. This has implications for interventions aimed at vulnerable individuals during times of social isolation and prolonged stress.

2.3 The Role of Endogenous Oxytocin in Pain Perception (Paper III)

In addition to reported increased mental distress, there have been reports of increased pain perception during the pandemic. Patients suffering from chronic pain have reported significantly higher levels of pain (Nieto et al., 2020), especially when patients did not have sufficient access to treatment (Lynch et al., 2020). Psychological stress and negative thoughts, such as insecurity, worries about the future, loneliness, sadness, and fear of infection, have been identified as triggers for increased pain during lockdown (Amja et al., 2021; Nieto et al., 2020). This increase was not limited to chronic pain patients but also extended to the non-clinical population (Grech et al., 2022; Papalia et al., 2022).

Recently, research focusing on the analgesic effects of oxytocin has been increasing (Leng & Leng, 2021). A large number of animal studies demonstrated that both administering oxytocin and stimulating the endogenous release of oxytocin have analgesic effects in various animal pain models (Li et al., 2020). These effects were found in both central and peripheral (injection) oxytocin administration (Rash et al., 2014). Although human research is less conclusive, there is some evidence supporting this idea. For example, patients with chronic lower back pain showed a higher tolerance to experimentally induced pain after intranasal oxytocin administration. However, this effect did not extend to their spontaneously occurring pain (Boll et al., 2020). In another study using an infrared laser to selectively activate Ad- and C-fiber nerve endings with heat pulses, oxytocin was found to have analgesic effects but not for noxious thermode heat stimuli (Paloyelis et al., 2016). These findings suggest that the analgesic effects of oxytocin may be limited to certain types of pain rather than all types. Moreover, these effects seem to be sex[1] specific. For example, our previous work found that when oxytocin was repeatedly administered, it had a pain-reducing

[1] Following the recommendations of the American Psychological Association (7th edition), within this dissertation, when not further specified, the term *sex* is used as a biological distinction, whereas the term gender represents the social construct and social identity.

impact for men experiencing wound pain but not for women (Pfeifer et al., 2020). However, research on the associations between pain and peripheral oxytocin levels in humans is inconclusive. While some studies have shown that individuals with migraines have higher levels of oxytocin (You et al., 2017), other studies demonstrated that children with abdominal pain (Alfvén, 2004) and adults with low back pain have lower levels of oxytocin (Yang, 1994). Moreover, social factors, such as storytelling or a mother's voice, can reduce pain and increase oxytocin concentrations in children (Brockington et al., 2021; Filippa et al., 2021). Given that the outbreak of the COVID-19 pandemic was a global enduring stressor affecting individuals' well-being and pain perception (Amja et al., 2021; Mata et al., 2021; Nieto et al., 2020; Pierce et al., 2020), our study aimed to examine the relationship between physical and emotional pain perception and endogenous oxytocin concentrations in daily life during the pandemic. Based on previous research, we hypothesized that subjective pain rating would be positively associated with perceived stress but negatively associated with salivary oxytocin levels.

To test our hypotheses, we conducted a follow-up study based on our cross-sectional study reported in section 2.2. of this dissertation. Following the same methodological approach outlined in (Schneider et al., 2023), we conducted a second 2-day EMA study that included subjective emotional and physical pain ratings in our assessment. This follow-up study was conducted one year after the initial assessment. In total, we collected data from 247 individuals participating between April and August 2020 (t1) and from 254 participants (n = 196 individuals participating for the second time; n = 58 newly recruited) between April and August 2021 (t2). Biochemical quantification of oxytocin levels in unextracted saliva samples was conducted at the Institute of Medical Psychology in Heidelberg, Germany, using the same ELISA, however, with a different lot number.

The results of our hierarchical linear model analyses demonstrated that on the between-person level, self-reported stress has a significant impact on physical pain. Specifically, individuals who reported higher levels of stress as compared to the sample average experienced higher physical pain levels. This association was significant in both longitudinal and cross-sectional data. We additionally found that emotional pain was positively associated with self-reported stress levels on both between-person and within-person levels, indicating that when individuals experienced higher levels of emotional pain, they also reported higher stress levels. However, this association was significant only for cross-sectional data. Furthermore, oxytocin levels were significantly and negatively correlated with emotional pain ratings, whereas the negative association with physical pain did not reach statistical significance. Further analyses showed that stress significantly moderates the relationship between physical pain and oxytocin levels. Specifically,

individuals had lower oxytocin levels when they reported high levels of stress and physical pain. This suggests that stress can affect the pain-reducing effects of oxytocin. Therefore, finding ways to reduce stress might help to reduce subjective pain perception in the general population.

3 General Discussion

3.1 Summary and Interpretation of the Results

The purpose of my dissertation was to further explore the role of oxytocin as a social peptide and its associations with social-related behavior. Specifically, my research primarily focused on inquiring about the value of measuring endogenous oxytocin levels in relation to anxiety, social bonding, and pain. While oxytocin effects have been traditionally studied in animal models and administration studies within these research fields, there is limited research using peripheral oxytocin measures in humans. Therefore, the first aim of my dissertation was to address this issue and to build upon previous research emphasizing the importance of measuring endogenous oxytocin levels. In Paper I, I explored the associations between peripheral oxytocin levels, sex hormones, and social anxiety. In Paper II, I investigated the relationship between diurnal oxytocin levels and affectionate touch, which is one of the crucial aspects of social bonding. Lastly, Paper III focused on the link between salivary oxytocin levels, emotional and physical pain, and stress levels in the general population during the COVID-19 pandemic.

To examine how peripheral oxytocin is linked to social anxiety, one basal measurement of oxytocin was taken from the blood and analyzed using RIA. As there have been discussions in the literature about the sex-specific effects of oxytocin, I focused here on its interactions with gonadal hormones predicting social anxiety levels in high and low socially anxious individuals (Paper I). The study revealed that women with high levels of oxytocin and estradiol reported lower anxiety levels. Previous studies have shown that administering oxytocin can have anxiolytic and stress-reducing effects (Ditzen et al., 2009; Eckstein et al., 2015; Labuschagne et al., 2010). However, it is less clear how peripheral oxytocin levels relate to anxiety, especially social anxiety. Our results align with studies reporting a negative association between oxytocin and anxiety in (predominantly) female samples (Nagahashi-Araki et al., 2022; Scantamburlo et al., 2007) but contrast with studies that report a positive association (Hoge et al., 2008; Oh et al., 2018). Furthermore, in our study, the significant association between hormonal interaction and anxiety was particularly

strong in women with high social anxiety. This finding is in line with literature indicating that the impact of oxytocin on anxiety varies based on different moderating factors, including anxiety levels, type of anxiety, sex, and age, potentially explaining differing research findings (Labuschagne et al., 2010; Slattery & Neumann, 2010; Yoon & Kim, 2022). Animal research also suggests that other hormones, such as estradiol, can affect the oxytocin system by influencing oxytocin production, receptor binding (Dellovade et al., 1999; Young et al., 1998), and its effects on social behavior (Choleris et al., 2004). Moreover, McCarthy and colleagues have shown that oxytocin has stronger anxiolytic effects in animals that have been pretreated with estrogen (McCarthy et al., 1996; McCarthy et al., 1997). Although our data are correlational and do not prove causation, they do indirectly support the idea that oxytocin and estradiol may have potential benefits for managing social anxiety.

To investigate associations of endogenous oxytocin levels with affectionate touch, we analyzed data from our psychobiological EMA study with repeated salivary oxytocin assessments collected during the first COVID-19 lockdown (Paper II). The main findings of this big, mixed-method study indicate that individuals experiencing affectionate touch showed lower cortisol levels and reported lower stress and higher happiness levels. The intensity of touch was linked to higher oxytocin levels and self-reported happiness and was also associated with lower anxiety, stress, and general burden levels. These findings are in accordance with earlier research reporting elevated salivary oxytocin levels in response to different types of social touch, including standardized touch and self-touch, as well as massage (de Jong et al., 2015; Li et al., 2019; Moussa et al., 2021; Portnova et al., 2020). Recent animal research has found that in the brains of male mice, the density of oxytocin neurons increases after social isolation and that these neurons help regulate social craving (Musardo et al., 2022). Additionally, social touch can activate the central oxytocin system and stimulate oxytocin secretion into the periphery (Tang et al., 2020). Moreover, it has been proposed that oxytocin release in response to C-tactile (gentle stroking) stimulation might serve the purpose of calming and stress-reduction, increasing well-being (Walker et al., 2017). In our study, we did not find a significant mediation between individual well-being and affectionate touch by oxytocin levels. However, the results of our study indicate that affectionate touch can have a positive impact on individuals' emotional states, induce psychobiological stress reduction, as well as oxytocin release, potentially through similar mechanisms as seen in animals.

In our follow-up EMA study (Paper III), we found that subjective stress predicted higher levels of emotional and physical pain in individuals' everyday lives during the pandemic, cross-sectionally and longitudinally. Subjective stress was also found to moderate the negative relationship between physical pain and salivary oxytocin levels. More

specifically, the results indicate that oxytocin levels were the lowest when participants experienced higher stress and more intense pain. In addition, we found that lower levels of momentary oxytocin were linked to higher levels of emotional pain. The findings of this study support prior research that found lower levels of plasma oxytocin in patients with fibromyalgia syndrome who experienced high levels of stress and pain (Anderberg & Uvnäs-Moberg, 2000). Animal studies have demonstrated that oxytocin can directly reduce pain signaling to the spinal cord (Eliava et al., 2016; Espinosa De Los Monteros-Zúñiga et al., 2020) and has potential therapeutic implications for treating pain patients and stress-induced hyperalgesia (Li et al., 2020). However, our previous research found sex-specific effects and no general positive impact of oxytocin administration on pain perception. Men who received intranasal oxytocin reported reduced pain levels from skin wounds, while women reported less wound pain when assigned to instructed positive partner interaction (Pfeifer et al., 2020). Thus, further research is needed to better understand the conditional associations between oxytocin and pain.

Taking together, the papers presented above (Papers I-III) provide valuable human-data-based support for the link between endogenous oxytocin concentrations and social anxiety, affectionate touch, and pain perception. The results suggest that there is a (hypothesis-confirming) positive link between oxytocin levels and more intense affectionate touch (Paper II), reduced social anxiety (Paper I), and attenuated pain perception (Paper III) levels. However, it is important to note that specific conditions influence these associations with oxytocin. For example, Paper I suggests that the anxiolytic associations with endogenous oxytocin may depend on sex hormones. Additionally, Paper II shows that only intense affectionate touch (as opposed to affectionate touch in general) is associated with oxytocin increases. Furthermore, Paper III suggests that individual stress levels can modify the relationship between oxytocin and subjective pain perception. These studies highlight the importance of studying endogenous oxytocin concentrations in relation to these research domains, particularly paying attention to these conditional effects on a psychological (stress and anxiety) level and biological (interactions with sex hormones) level. Doing so can help to understand the relationship better and develop reliable paradigms for robust oxytocin responses in humans.

3.2 Strengths and Limitations

As outlined in the second chapter of my dissertation, research including endogenous oxytocin levels in humans is increasing, although the findings are still somewhat inconclusive

and debated. One of the main strengths of my dissertation is exploring endogenous oxytocin measures in various contexts using different methodological approaches.

The multi-method approach in my dissertation included collecting single and repeated measurements, with and without extraction techniques, before the analyses and using RIA and ELISA for oxytocin quantification. In Paper I, only one baseline plasma oxytocin measurement was assessed. However, we used a psychobiological EMA approach in Paper II and Paper III, which involved taking non-invasive, repeated salivary oxytocin measurements in everyday life. The EMA method has been shown to offer higher ecological validity and account for changes within individuals (Wrzus & Neubauer, 2023). To the best of my knowledge, using this approach to measure oxytocin levels is a novel concept that provides new insights into salivary diurnal oxytocin levels in a natural setting. We are not aware of other studies using the EMA method for this purpose to date. Regarding biochemical analyses of oxytocin, in Paper I, plasma samples were extracted before analysis with RIA, while in Paper II and Paper III, saliva samples were analyzed with ELISA without prior extraction. The use of these different methodological designs makes it more challenging to compare findings between the studies. As thoroughly discussed in the first chapter of my dissertation, this topic remains a subject of debate in the literature (e.g., Leng & Sabatier, 2016; MacLean et al., 2019; Tabak et al., 2022). Moreover, in each of the papers I presented, I focused on different aspects of social-related behavior, which also limits the comparability and generalizability of the results. Additionally, different sample characteristics make it even more difficult to generalize the findings. For example, in Paper I, individuals with high and low social anxiety scores were analyzed, which makes it difficult to generalize the findings to other burdened groups or the general population. In contrast, the results of Paper II and Paper III were obtained from the general population. However, due to the specific context of the COVID-19 pandemic, it remains unclear whether or to what extent these results can be generalized to pre- and post-pandemic everyday life.

Furthermore, my dissertation is solely based on an observational study design investigating the relationship between peripheral oxytocin levels and various outcomes. Notably, none of the parameters in my studies were experimentally manipulated. While observational studies can detect associations in a highly ecologically valid environment, they cannot prove causality and, thus, need to be interpreted cautiously.

4 When and How (often) Measuring Oxytocin?

In my dissertation, the second objective was to analyze the results we obtained from the studies presented and to provide conclusions and recommendations on the frequency

and timing of including endogenous oxytocin measures. Additionally, I aimed to outline the open questions that need to be addressed in future studies in this area.

The findings of our Paper I suggest that the association between oxytocin and anxiety depends on estradiol levels as well as basal social anxiety levels of tested women. The significant interaction of oxytocin and estradiol predicting anxiety levels was particularly significant in highly socially anxious individuals. These results support previous studies in rodents (Slattery & Neumann, 2010) and humans (Labuschagne et al., 2010) that have demonstrated the potential of oxytocin administration to reduce anxiety-related behavior and amygdala reactivity in highly anxious individuals. However, the reported effects were not seen in those not experiencing clinically relevant anxiety (healthy controls). Thus, our study highlights the importance of accounting for contextual factors when measuring peripheral oxytocin and suggests that high baseline levels of anxiety might play a role or be indicative of basal endogenous oxytocin levels. Future studies investigating, e.g., the role of endogenous oxytocin concentrations in (social) anxiety are required to address this issue and choose appropriate samples, as the associations might be specific to highly anxious individuals.

At this point in my dissertation, I want to address another critical issue involving women's basal hormonal concentrations. In our study, we found that women who used hormonal contraceptives had lower levels of estradiol and progesterone, but higher levels of plasma oxytocin compared to women who were naturally cycling (as outlined in Paper I). This finding is in accordance with previous studies reporting increased concentrations of oxytocin in contraceptive users (Amico et al., 1981; Silber et al., 1987). Additionally, it has been observed that oxytocin concentrations fluctuate in naturally cycling women throughout their menstrual cycle, with levels increasing during the early follicular phase and reaching the peak around ovulation (Engel et al., 2019). Given these observations, it is crucial to conduct more research that includes explicitly women and addresses the hormonal fluctuations caused by their menstrual cycle or use of hormonal contraceptives. This is especially important since research on biologically female individuals is still underrepresented compared to research on male individuals.

Research on the relationship between oxytocin levels and social behavior has not given enough attention to fluctuations in oxytocin concentrations in general. To the best of my knowledge, our EMA studies (Paper II-III) are the first to investigate salivary diurnal changes in everyday life. However, it is important to note that previous studies have addressed the question of intraindividual stability of oxytocin measures with mixed results. For instance, some studies reported that oxytocin levels remained stable across time in parents (Feldman et al., 2013) and couples (Schneiderman et al., 2012). However, another,

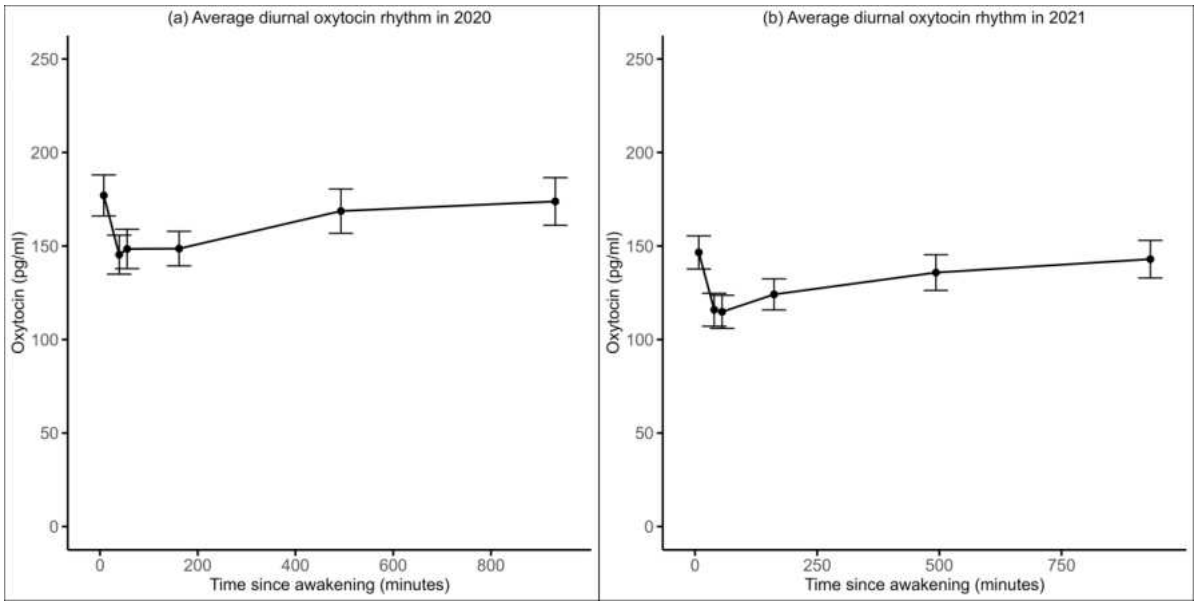
more recent study suggested that single oxytocin measures may not accurately represent baseline levels due to a lack of correlation between assessments (Martins et al., 2020b). In our EMA study, we observed fluctuations in oxytocin levels throughout the day and published the illustration and graphical comparison of diurnal oxytocin and cortisol changes. We further reported a significant positive correlation between individual mean oxytocin levels during the first lockdown on the two assessment days ($r(227) = 0.850$, $p<0.001$) (Schneider et al., 2023b).

To further analyze these fluctuations, I have conducted several statistical analyses using hierarchical linear modeling (unpublished data, in prep). More specifically, I examined whether momentary oxytocin levels were significantly varying across six measurement points over the day (directly after awakening, 30 min after, 45 min after, 2½ hours after, 8 hours after, and directly before going to sleep) separately for the year 2020 and 2021 (see Figure 2). For the analyses of the sample during the first lockdown in 2020, I included momentary oxytocin levels as outcome variable and measurement points as predictor, controlling for day, food and drink intake, caffeine, smoking, alcohol use, brushing teeth, and physical activity on Level I. To further control for Level II variables, I included sex, day, age, body mass index (BMI), and relationship status (single vs. in relationship) into the model.

On a within-person level (Level I), the results revealed significant associations with measurement point ($b = -.067$; $t(2254) = -5.896$; $p<.001$), suggesting that oxytocin levels decrease over the day. Furthermore, significant associations were found for food intake ($b = .197$; $t(2254) = 4.614$; $p<.001$), caffeine ($b = -.123$; $t(2254) = -3.825$; $p=.001$), brushing teeth ($b = .113$; $t(2254) = 4.002$; $p=.001$), and physical activity ($b = .097$; $t(2254) = 3.186$; $p=.002$). These results suggest that oxytocin levels were higher when participants reported food intake, were physically active, brushed their teeth, and were lower when they had coffee before the sample collection. On a between-person level (Level II), individuals' oxytocin levels were associated with age ($b = -.009$; $t(205) = -3.083$; $p=.002$), indicating that with increasing age individuals have lower average oxytocin levels.

I followed the same analytical approach to analyze the data from the second lockdown in 2021. The analyses show comparable results. On a within-person level, oxytocin levels increased when participants took a meal ($b = .268$; $t(2491) = 6.738$; $p<.001$) and brushed their teeth ($b = .113$; $t(2491) = 4.002$; $p<.001$) and decreased when drinking coffee ($b = -.179$; $t(2491) = -5.635$; $p<.001$) before sample collection. Similarly to the results in 2020, oxytocin levels significantly decreased over the day ($b = -.085$; $t(2491) = -7.315$; $p<.001$). However, physical activity did not affect oxytocin levels in this sample. On a between-person level, the results indicate that the higher age of individuals was again associated with lower average oxytocin levels ($b = -.012$; $t(233) = -3.842$; $p=.002$).

Figure 2. Fluctuations of diurnal oxytocin concentrations.



Note: The graphs depict diurnal oxytocin rhythm throughout individuals who participated in our Ecological Momentary Assessment (EMA) studies during a) the first (April-July 2020) and b) the second (April-July 2021) lockdown of the COVID-19 pandemic. The lines represent oxytocin (pg/ml) concentrations across the two assessment days and all participants in each year, respectively. Error bars represent 95% confidence intervals. Source of the graph a):(Schneider et al., 2023b). Graph b) is based on unpublished preliminary analyses (data in preparation).

Furthermore, after inspecting the illustrations of diurnal oxytocin (Figure 2), it became apparent that the fluctuation pattern is comparable between the years. In addition, it indicated that oxytocin decreases within the first 45 minutes after waking up and seems to increase over the day. To statistically test whether both the decrease and the subsequent increase of oxytocin were significant, I repeated the analyses by separating the measurement time point variable into two variables (one variable for the decrease from time-point 1 to 3 and one variable for the increase from time-point 3 to 6) based on the circadian pattern (Ning & Luo, 2017). The results of these analyses showed that the morning decrease of oxytocin remained significant in the year 2020 ($b = -.141$; $t(2253) = -8.748$; $p<.001$) as well as one year later ($b = -.190$; $t(2490) = -11.869$; $p<.001$). However, the increase of oxytocin levels from time points three to six was only significant in the second year ($b = .047$; $t(2490) = 2.601$; $p=.009$).

To test the correlation of individuals' average oxytocin concentrations between the two years, I conducted an additional analysis, including individuals' mean oxytocin values

from 2020 as an outcome variable and the mean values of 2021 as a predictor. After controlling for age, sex, BMI, and relationship status, the results indicate a significantly positive correlation of individuals' mean oxytocin levels between the years ($b = .882$; $t(166) = 16.691$; $p<.001$).

Although these results are preliminary, they align with previous reports that individual baseline oxytocin levels seem to be stable over time (Feldman et al., 2013; Schneiderman et al., 2012), as indicated by the high correlation of mean oxytocin levels between the years in our data. However, our data also shows significant intraindividual changes in salivary oxytocin levels across the day that need to be accounted for in future studies. This finding supports the previously reported debate that a single measurement of oxytocin is problematic as it most probably does not represent the "real" baseline level (Tabak et al., 2022). Thus, correlating the mean values of several oxytocin measures collected throughout the day may better represent an individual's baseline oxytocin levels than a single measure. Furthermore, our preliminary data indicate that other behavioral factors, such as diet, physical activity, and caffeine, might influence salivary oxytocin levels. However, to date, only a limited number of studies account for these factors when measuring endogenous oxytocin levels (e.g., see de Jong et al., 2015; Rassovsky et al., 2019 for reports on salivary oxytocin and physical activity).

4.1 Future Directions and Recommendations

The results of the present dissertation indicate that further investigation is needed to gain a more profound understanding of the factors that affect endogenous oxytocin levels and their impact on research findings.

To follow up on the results I presented above, future research could expand the assessments of circadian and diurnal fluctuations of salivary oxytocin to analyze further the stability of individual hormonal fluctuations over the years (whether the fluctuation pattern will remain stable within individuals after several years or decades) and whether there are biologically critical time phases for change (e.g., puberty or menopause in women). Since our EMA study only analyzed data from the general population, future studies should address whether circadian and diurnal fluctuations are changed in different populations (e.g., individuals suffering from major depressive disorder or social anxiety disorder). Additionally, data on systematic analyses on lifestyle and behavioral factors regarding oxytocin measures is needed. Thus, to identify various behavioral factors and lifestyle choices (such as physical activity, diet, smoking, sleep schedule, alcohol, caffeine

consumption, etc.) and investigate the time dynamics of their association with oxytocin, further observational studies, as well as experimental studies, should be conducted.

As presented in the introduction and the limitation sections of my dissertation, another critical issue extensively discussed in the literature are the methodological differences in studies questioning the reliability and validity of endogenous oxytocin assessments. Future studies should systematically investigate different approaches to collect, store, and analyze oxytocin measures from CSF, blood, and saliva. More specifically, samples should be examined in parallel with the currently used methods: ELISA, RIA, and Liquid-Chromatography-Mass-spectrometry/Mass-spectrometry (LC-MS/MS). Moreover, different assays from various manufacturers, as well as various extraction methods, including different protocols for solid-phase and liquid-based extraction methods, should be systematically validated based on specificity, accuracy, and recovery, and dilution linearity of measurements (European Medicines Agency, 2022). Additionally, future studies should test the stability of peripheral oxytocin concentrations by manipulating collection devices and storage conditions.

Pursuing these research questions will contribute to current oxytocin research in humans and methodologically improve oxytocin measurements in peripheral bodily fluids. With the obtained information, oxytocin-specific guidelines, and a standard operating procedure (SOP) for sample collection, handling, and assay analyses should be developed to ensure standardization across future studies. This research is highly relevant and may contribute to our understanding of underlying oxytocinergic mechanisms in social cognition and social interaction.

5 Conclusion

Currently, there is a tremendous amount of research on the involvement of oxytocin in regulating social behavior and its potential therapeutic applications. However, the underlying mechanisms and the full extent of endogenous oxytocin's involvement in social processes remain unclear. Although research examining the associations between endogenous oxytocin and social-related processes is increasing, there is skepticism and controversy in the scientific community regarding the significance and accuracy of measuring oxytocin levels in bodily fluids. The present dissertation provides novel empirical data that supports the link between endogenous oxytocin levels and social anxiety (Paper I), affectionate touch (Paper II), and pain perception (Paper III). Additionally, it sheds light on contextual factors that can influence these associations, both on a psychological level (such as basal levels of stress and anxiety) as well as on a biological level (such as

interaction with other hormonal systems like sex hormones). Furthermore, this dissertation demonstrates evidence for salivary diurnal oxytocin variations throughout the day and suggests that individual baseline oxytocin levels remain stable over time. The present dissertation contributes to our understanding of endogenous oxytocin in human (social) everyday life while also offering directions and recommendations for future studies on endogenous oxytocin.

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Appendix I: Paper I

Schneider, E., Müller, L. E., Ditzen, B., Herpertz & Bertsch, K. (2021). Oxytocin and social anxiety: Interactions with sex hormones. *Psychoneuroendocrinology*, 128, 1-7.

Ekaterina Schneider's contribution according to the contributor roles taxonomy (CRediT) author statement (Allen et al., 2019):

Conceptualization, methodology, software, validation, formal analysis, writing – original draft, writing – review and editing, visualization, project co-administration.

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Oxytocin and social anxiety: Interactions with sex hormones

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ABSTRACT

Oxytocin has been associated with anxiolytic and stress reducing effects in a number of studies. Less is known about the associations of endogenous oxytocin concentrations and their interaction with other hormones such as sex hormones in relation to self-reported anxiety levels. In this study, endogenous oxytocin and sex hormone levels were analyzed in 99 high (51 women) and 100 low (50 women) socially anxious individuals. Regression analyses showed that women with high oxytocin and estradiol levels reported a lower total Liebowitz Social Anxiety Score (LSAS) as well as a lower score on the subscale LSAS Fear. This association of hormonal interaction with social anxiety scores was significant in the subsample of high socially anxious women. In men there were no significant associations for endogenous hormones with LSAS scores. These findings suggest that in women the link between oxytocin and anxiety might be dependent on basal anxiety levels as well as on individual sex hormone levels.

1. Introduction

Social anxiety disorder is characterized by pronounced fear and avoidance of evaluation by others in various social situations. It is associated with individual impairment (e.g. social isolation, reduced quality of life) and high economical costs (Dams et al., 2017; Stein et al., 2017). Epidemiological studies have reported gender differences regarding life-time prevalence rates showing that social anxiety disorder affects women more frequently than men (Asher et al., 2017; Hofmann et al., 2010).

A large body of research has focused on the neurocircuitry and neuroendocrine factors modulating fear, one of the main components of anxiety disorder (Shin and Liberzon, 2010). The neuropeptide oxytocin, which is synthesized in the hypothalamus and released into different areas of the brain (e.g., amygdala, hippocampus, etc.) as well as into the blood system, has been suggested to act as a modulator of anxiety-related behavior (Lu et al., 2019). Numerous animal studies have indicated anxiolytic and stress-reducing effects of oxytocin administration (Jones et al., 2017). For example, in rodent studies it has been shown that acquisition and extinction of social fear can alter brain oxytocin system (including oxytocin receptor binding and oxytocin release), whereas administration of oxytocin can reverse social fear (Zoicas et al., 2014) as well as stress-induced social avoidance (Lukas et al., 2011). Even though findings in humans have been less consistent (for a review on previous animal and human research, see (Neumann and Slattery, 2016)), there are suggestions that intranasal oxytocin application affects fear processing and acts as a modulator of fear-related neurocircuitry. For example, amygdala hyperactivity that is common in patients with social anxiety disorder (Shin and Liberzon, 2010) can be reduced by exogenously administered oxytocin (Labuschagne et al., 2010). In healthy individuals, intranasal oxytocin administration reduces amygdala reactivity during fear processing (Kirsch et al., 2005) and extinction (Eckstein et al., 2015). Furthermore, stressful and fearful situations activate the oxytocin system (Landgraf and Neumann, 2004; Wotjak et al., 1998). Measuring central oxytocin in humans is challenging as it involves highly invasive methods e.g. obtaining cerebrospinal fluid for oxytocin analyses (Lefevre et al., 2017). Therefore, a number of studies in the recent past investigated the relationship between peripheral and central oxytocin levels with somewhat inconclusive results (Carson et al., 2015; Kagerbauer et al., 2013; Lefevre et al., 2017), however, evidence from animal research suggests a coordinated increase in central and peripheral oxytocin as

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indicator of an adaptive response to challenge (however, less so in non-stressful situations) (Neumann and Slattery, 2016). In humans for example, peripheral oxytocin levels increase in response to physical challenge and psychosocial stress (de Jong et al., 2015) and have been associated with fewer depressive symptoms in postpartum women with high psychosocial stress levels (Zelkowitz et al., 2014). There are only few studies on peripheral oxytocin associations with anxiety levels and results are mainly inconclusive. Hoge and colleagues reported that after controlling for sex and age higher oxytocin levels were associated with higher anxiety symptoms severity in patients suffering from social anxiety disorder (Hoge et al., 2008). Similarly, in another study with only male subjects higher oxytocin levels were related to higher social anxiety symptoms (higher on total LSAS scores as well as on LSAS Fear subscale) in socially anxious individuals but not in controls (Oh et al., 2018). However, there are studies reporting opposite results. For example Carson and colleagues reported a negative association between oxytocin obtained in both, plasma and cerebrospinal fluid and trait anxiety in children (Carson et al., 2015). Similarly, Scantamburlo and colleagues found a negative relationship between peripheral oxytocin levels and anxiety scores in depressive patients (Scantamburlo et al., 2007). Interestingly, in comparison to the previously mentioned this study included mostly female participants (only four men were included in the sample) indicating that the association between plasma oxytocin and anxiety might be conditional and sex dependent.

A number of studies indicate that oxytocin tends to interact with other neurotransmitter and hormonal systems including gonadal hormones rather than acting independently. Non-human studies have demonstrated that estrogens are not only directly involved in oxytocin production, oxytocin receptor gene expression (Dellovade et al., 1999) and oxytocin receptor binding in different parts of the brain including the amygdala (Young et al., 1998), but also modulate the effects of oxytocin on social behavior, such as, e.g., social recognition (Choleris et al., 2004). With regard to anxiety it has been found that estrogens enhance the anxiolytic effects of oxytocin in female mice (McCarthy et al., 1996). In males, it has been reported that testosterone can increase oxytocin receptor binding, however, authors suggested that this activation was mediated by its metabolites (estradiol and dihydrotestosterone (DHT)) (Johnson et al., 1991). Interestingly, testosterone promotes also vasopressin receptor binding (Delville et al., 1996), a hormone that has been associated with opponent effects of oxytocin (for a review, see (Bos et al., 2012)). Human research investigating the modulatory role of sex hormones on peripheral oxytocin levels is scarce at this point. However, there is evidence for an interaction between reproductive hormones and oxytocin. For example, endogenous oxytocin levels in women vary along the menstrual cycle, with higher levels around the time of ovulation (Engel et al., 2019), the time when estrogen levels are the highest. A few studies have reported sexually dimorphic effects of exogenous oxytocin on social cognition (Ditzen et al., 2013; Hoge et al., 2014; Gao et al., 2016), suggesting that naturally occurring higher levels of estrogens in females and higher levels of testosterone in males could be responsible for the opposite effects.

Thus, in this study we aimed to investigate plasma concentrations of oxytocin and estradiol as well as saliva concentrations of testosterone, along with their interaction in men and women in relation to their self-reported fear and avoidance levels in social situations. Based on previous research, we hypothesized that sex hormones might moderate the association of oxytocin and social anxiety. More specifically, we expected that high sex hormone (estradiol in women, testosterone in men) as well as high oxytocin concentrations would be associated with lower social anxiety levels.

2. Methods and materials

2.1. Participants

A total number of N = 2686 volunteers were screened for social anxiety and avoidance as well as in- and exclusion criteria with online questionnaires. N = 1823 completely filled out online the Liebowitz Social Anxiety Scale (LSAS). 103 female and 99 male participants could be reached via telephone communication and met all inclusion criteria. Exactly 50% of participants (49 men and 52 women) were categorized as high anxiety scorers based on the LSAS scores. Current diagnosis of social phobia (SAD) and avoidant personality disorder (AVPD) were allowed. The sample was recruited for the purpose of a larger research project including functional neuroimaging (data reported elsewhere). Exclusion criteria were any other current psychiatric disorder and psychiatric or psychotherapeutic treatment, lifetime diagnosis of schizophrenia, schizoaffective or bipolar disorder, any organic or neurological disorders, any regular medication except for oral contraceptives. In the female subsample, 35 (out of 51) and 34 (out of 52) participants reported hormonal contraceptive use in the high and low anxiety group respectively. As part of a larger research project this study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg. All participants provided written informed consent and received monetary compensation for their participation.

Due to very high sex hormone levels, one high socially anxious man as well as one high and one low socially anxious woman were excluded from the sample, resulting in 199 participants for the final analyses.

2.2. Procedure

After a screening of interested persons via telephone for eligibility to participate, the included participants were invited to two laboratory appointments (scheduled in the afternoon to avoid circadian rhythm effects). During the first laboratory appointment, participants were asked to fill out questionnaires and were assigned either to the high or low socially anxious group based on their LSAS scores (see Section 2.3). Cut-off scores for the highly anxious group were a total score of 60 or higher and a score of 30 or higher on the subscale “LSAS Fear”(Mennin et al., 2002), whereas for the low anxious group it was a score of 13 or less on the subscale “LSAS Fear”. During the second laboratory appointment, saliva and blood samples were collected (see Section 2.4) after a resting period of at least 30 min. Participants were instructed to refrain from smoking and caffeine intake on the day of the blood and saliva collection and from food and drinks except for water two hours prior to their laboratory appointment. Female participants were scheduled during the early follicular phase of their menstrual cycle (day 2–7 after onset of the menses). Menstrual cycle phase was confirmed via self-report and subsequent plasma estrogen and progesterone analyses.

2.3. Psychometric measures

2.3.1. Liebowitz Social Anxiety Scale (LSAS)

The levels of participants’ individual social anxiety and avoidance were assessed using the LSAS (Heimberg et al., 1999). The LSAS measures fear and avoidance in 13 social and 11 performance situations. Participants rated their fear and their avoidance behavior in each situation on a Likert scale from 0 (none) to 4 (very much/often). The internal consistency for the LSAS in our sample was excellent ($\alpha = 0.96$).

2.4. Neuroendocrine measures

For the analyses of endogenous oxytocin levels blood samples were collected from antecubital veins of all participants into 5 ml vacutainer blood monovettes containing EDTA and were immediately cooled in ice-chilled water at 4 °C. Monovettes were centrifuged at 4 °C at 1.500 g for 5 min. Plasma was removed and stored at − 80 °C until completion of

the study. Plasma samples were first extracted in order to exclude plasma proteins and subsequently analyzed at the RIAgnosis laboratory in Munich, Germany, using a sensitive radioimmunoassay with a detection limit of 0.1 pg/ml. The coefficient variation for intra-assay precision was below 10%.

Another blood sample was collected in women only for basal progesterone and estradiol analyses into 7.5 ml heparin-plasma vacutainer tubes and stored at − 80 °C until analyzed at the Central Laboratory of the University Hospital in Heidelberg, Germany, using commercially available chemiluminescence immunoassays (CLIA). Both assays show high sensitivity and low cross reactivity with other hormones tested suggesting that synthetic steroids were not detected. The detection limit of the assays was 0.2 ng/ml for progesterone and 10.0 pg/ml for estradiol. The levels of both hormones were used to confirm the menstrual cycle phase of female participants. Two women were excluded from further analyses because their progesterone and estradiol values were too high (z > 3.29) indicating that the early follicular phase has been missed.

For testosterone analyses, saliva samples were collected from all participants using salivette devices (Sarstedt, Nümbrecht, Germany) and stored at − 20 °C. Testosterone concentration was measured at the laboratory of the Technical University Dresden, Germany, also using CLIA with a high analytical sensitivity of 1.8 pg/ml (IBL). Due to very high testosterone concentration (z = 6.02; cut-off: z > 3.29) of one male participant we could not rule out the possibility that his value might have been influenced by one or several factors we could not control for in our statistical analyses (use of synthetic steroids, hyperandrogenism etc.) and thus, was excluded from further analyses.

2.5. Statistical analyses

Data managing and statistical analyses were conducted using IBM SPSS version 25. For both questionnaires used in this sample the internal consistency was calculated using Cronbach's alpha. Outliers were identified via z-transformation and multivariate outliers via Mahalonobis distance measure. First, we analyzed whether the women's hormonal levels (including estradiol, progesterone, oxytocin and testosterone) significantly varied depending on their use of oral contraception using Mann-Whitney *U* tests. Additionally we analyzed whether hormonal concentrations differed between highly and low socially anxious group also using Mann-Whitney *U* test (these analyses were conducted for men and women separately). In order to test for the relationship of self-reported LSAS scores and basal hormonal levels, multiple regression analyses were conducted. Prior to running the regression models general assumptions for multiple regression analyses, such as linearity, homoscedasticity, normality and independence of error terms were tested (Field, 2013). Normality of error terms was tested using the Kolmogorov-Smirnov test, which was not significant. The independence of residuals was tested using the Durbin-Watson test. No violations of the assumptions were detected. The total scores of the LSAS as well as the LSAS subscales Fear and Avoidance were included as dependent variables. As independent variables the group variable, hormone variables as well as oxytocin by estradiol (for women)/-testosterone (for men) interaction were entered into the model. Since estradiol and progesterone concentrations were analyzed only in the female sub-sample, regression models for men and women were conducted separately. Specifically, we analyzed whether the interaction of oxytocin and estradiol in women as well as the interaction of oxytocin and testosterone in men (variable was created by multiplying variables of oxytocin and the particular sex hormone) were associated with self-reported LSAS scores. To avoid multicollinearity, hormone variables of men and women were centered around their group mean. Bonferroni correction was used in order to adjust the p value for multiple comparisons. Significance threshold was adjusted to p < .006 in this sample.

3. Results

The analyses of hormonal differences in women showed that the use of oral hormonal contraceptives was associated with significantly higher oxytocin ($z = -5.46$; $p < .001$) but lower estradiol ($z = -4.00$; $p < .001$) and progesterone ($z = -2.87$; $p = .004$) plasma levels compared to naturally cycling women. However, due to a counterbalanced distribution of women using hormonal contraceptives in the high and low socially anxious group, women in these two groups did not differ in relation to their hormone levels (see Table 1). Thus, in highly anxious women levels of plasma estradiol, progesterone, and oxytocin as well as saliva testosterone were statistically not different compared to low anxious women.

The regression analyses showed that in the female subsample there were no significant main effects of oxytocin and estradiol on the outcome variable total LSAS score but a significant association with the group variable ($R = 0.94$, $R^2 = .90$; $F(4,90) = 192,48$, $p < .001$; $\beta = 0.932$; $T = 26.589$, $p < .001$) as well as with the oxytocin x estradiol interaction variable ($\beta = -0.103$; $T = -2.946$, $p = .004$). We further analyzed the association of oxytocin x estradiol interaction with the subscales of the LSAS by following the same analytical approach. Here, we found that the outcome variable LSAS Fear was also significantly associated with the group variable ($R = 0.96$, $R^2 = .93$; $F(4,90) = 287,70$, $p < .001$; $\beta = 0.936$; $T = 32.412$, $p < .001$) as well as

Table 1
Sample characteristics for the female subsample (N = 101).

Sample characteristics (*N* = 101)	Mean (SD); *N* (%)		
	low anxious	highly anxious	χ^2 (p)
N	50	51	
Age (years)	23.66 (4.29)	23.06 (5.46)	
Years of education	16.20 (2.94)	15.37 (2.76)	
Current SAD and/or AVPD	0 (0)	19 (37.25%)	
Use of hormonal contraceptives	35 (70%)	34 (66.67%)	0.130 (0.719)
Questionnaire data			
Liebowitz Social Anxiety Scale (LSAS total)	19.62 (7.02)	73.71 (12.67)	
LSAS Fear	8.34 (2.97)	38.16 (6.27)	
LSAS Fear of social interaction	2.86 (1.73)	18.94 (4.25)	
LSAS Fear of performance	5.48 (2.05)	19.22 (3.82)	
LSAS Avoidance	11.28 (5.68)	35.55 (7.69)	
LSAS Avoidance of social interaction	4.66 (3.07)	17.55 (4.29)	
LSAS Avoidance of performance	6.62 (3.48)	18.00 (4.62)	
Hormonal data			
Comparison of anxiety groups	**low anxious**	**highly anxious**	**Z (p)**[a]
Peripheral blood plasma oxytocin (pg/ml)	5.68 (3.90)	5.54 (3.87)	-0.235 (0.814)
Progesterone level (ng/ml)	0.49 (0.25)	0.52 (0.23)	-1.021 (0.307)
Estradiol level (pg/ml)	31.39 (19.36)	30.80 (19.29)	-0.175 (0.861)
Testosterone level (pg/ml)	18.63 (18.37)	17.98 (11.97)	-0.696 (0.486)
Comparison of contraception groups	**OC**	**NOC**	**Z (p)**[a]
Peripheral blood plasma oxytocin (pg/ml)	6.97 (3.76)	2.57 (1.87)	-5.460 (0.000)
Progesterone level (ng/ml)	0.46 (0.23)	0.61 (0.24)	-2.872 (0.004)
Estradiol level (pg/ml)	26.44 (16.78)	41.57 (20.52)	-4.007 (0.000)
Testosterone level (pg/ml)	18.20 (16.54)	18.57 (12.92)	-0.821 (0.412)

Note: OC: oral contraceptive use; NOC: no oral contraceptive use.
[a] Mann-Whitney *U* Test.

with the oxytocin x estradiol interaction variable ($\beta = -0.115$; $T = -3.956$, $p < .001$) (see Fig. 1). However, the subscale LSAS Avoidance was significantly associated only with the group variable ($R = 0.88$, $R^2 = .77$; $F(4,90) = 73,98$, $p < .001$; $\beta = 0.860$; $T = 16.542$, $p < .001$) but not with oxytocin x estradiol interaction ($p > .100$).

Furthermore, detailed analyses indicated that the significant association of oxytocin x estradiol interaction with LSAS scores is mainly driven by the group of high socially anxious women rather than by the group of low anxious women. Within the group of high socially anxious women the association of total LSAS scores with oxytocin x estradiol interaction did not reach Bonferroni-corrected significance probably due to loss of power ($R = 0.39$, $R^2 = .15$; $F(3,44) = 2.38$, $p = .083$; $\beta = -0.403$; $T = -2.201$, $p = .033$). However, the subscale LSAS Fear showed significant association with oxytocin x estradiol interaction ($\beta = -0.621$; $T = -3.78$, $p = .001$). In contrast, the regression analysis in the low socially anxious group showed a non-significant trend of oxytocin x estradiol interaction and total LSAS scores ($R = 0.29$, $R^2 = .09$; $F(3,45) = 1.31$, $p = .285$; $\beta = -0.281$; $T = -1.749$, $p = .088$) and the LSAS Fear subscale ($R = 0.26$, $R^2 = .07$; $F(3,45) = 1.00$, $p = .402$; $\beta = -0.274$; $T = -1.692$, $p = .098$) (see Fig. 2).

Additionally, we conducted separate regression analyses for women using hormonal contraception and naturally cycling women, respectively. The results indicated that in the group of contraceptive users the oxytocin x estradiol interaction remained significant in relation to the LSAS total score ($R = 0.95$, $R^2 = .90$; $F(4,62) = 138.25$, $p < .001$; $\beta = -0.148$; $T = -2.945$, $p = .005$) and the LSAS Fear subscale ($R = 0.97$, $R^2 = .94$; $F(4,62) = 218.08$, $p > .001$; $\beta = -0.118$; $T = -2.90$, $p = .005$). This hormonal interaction in the subgroup of normally cycling women was not significant. However, the statistical power in this model might be too low, as only 28 normally cycling women were included.

Similarly, for men, we first analyzed potential group differences based on their hormonal levels. We found no significant variation of hormones between high and low socially anxiety scorers (see Table 2).

Findings of the regression analyses revealed no significant associations for oxytocin, testosterone, and oxytocin x testosterone interaction variables with neither total LSAS scores nor with the LSAS Fear and LSAS Avoidance subscales.

In order to follow the same analytical approach as we did in female subsample, we then split the group of high and low anxious men and conducted separate regression models. In high socially anxious (but not low anxious) men the oxytocin x testosterone interaction showed a non-significant trend with total LSAS scores ($R = 0.35$, $R^2 = .12$; $F(3, 47) = 2.00$, $p = .128$; $\beta = 0.281$; $T = 1.966$, $p = .056$) as well as with LSAS Fear ($R = 0.40$, $R^2 = .16$; $F(3, 47) = 2.82$, $p = .050$; $\beta = 0.280$; $T = 2.006$, $p = .051$) but not with LSAS Avoidance These trend associations did not reach significance possibly due to low statistical power, and thus cannot be sufficiently interpreted.

4. Discussion

Oxytocinergic mechanisms have been related to anxiety symptoms in a number of studies indicating somewhat inconclusive and sex-specific results (for a review, see (Neumann and Slattery, 2016)). However, to the best of our knowledge, to date there is no human study investigating the interactional effects of endogenous oxytocin and sex hormones in relation to individual levels of social anxiety. Thus, in the current study we examined hormonal concentrations and their interactions in relation to self-reported anxiety levels in high and low socially anxious scorers.

The results of this study show that in women total LSAS scores as well as the LSAS Fear subscale were significantly negatively associated with oxytocin x estradiol interaction and this association was mostly driven by the group of high socially anxious women. These findings indicate that women with high basal oxytocin and estradiol concentrations reported less anxiety as compared to those with low oxytocin and estradiol concentrations. Separate analyses for high and low socially anxious women showed that this relationship remained significant in highly anxious women. Numerous previous oxytocin administration studies have already demonstrated that oxytocin can act as a modulator in a number of aspects of social behavior (for an overview, see (Shamay-Tsoory and Abu-Akel, 2016)). Moreover, oxytocin's anxiolytic and stress reducing effects have been reported in social anxiety patients (Labuschagne et al., 2010) as well as in healthy controls (Ditzen et al., 2009; Eckstein et al., 2015; Heinrichs et al., 2003; Kirsch et al., 2005). However, less attention has been paid to the associations of basal hormonal concentrations and self-reported anxiety levels. The results of our data support previous work by Scantamburlo and colleagues, who reported a negative association of oxytocin with anxiety and depressive symptom severity in a clinical population of mostly women (Scantamburlo et al., 2007). However, it is noteworthy that in contrast other studies have found that high basal oxytocin concentrations were positively associated with severity of anxiety levels in men (Oh et al., 2018) as well as in mixed male and female study samples (Hoge et al., 2008). This suggests that other sex-specific factors and hormones interact with the oxytocin system and might be responsible for the divergent results. Indeed, it has been repeatedly suggested to investigate the interactive role of estradiol and oxytocin, because estradiol directly modulates the oxytocin system including oxytocin production and receptor binding (Dellovade et al., 1999; Young et al., 1998) as well as oxytocin effects on social behavior (Choleris et al., 2004). Thus, our findings that in women

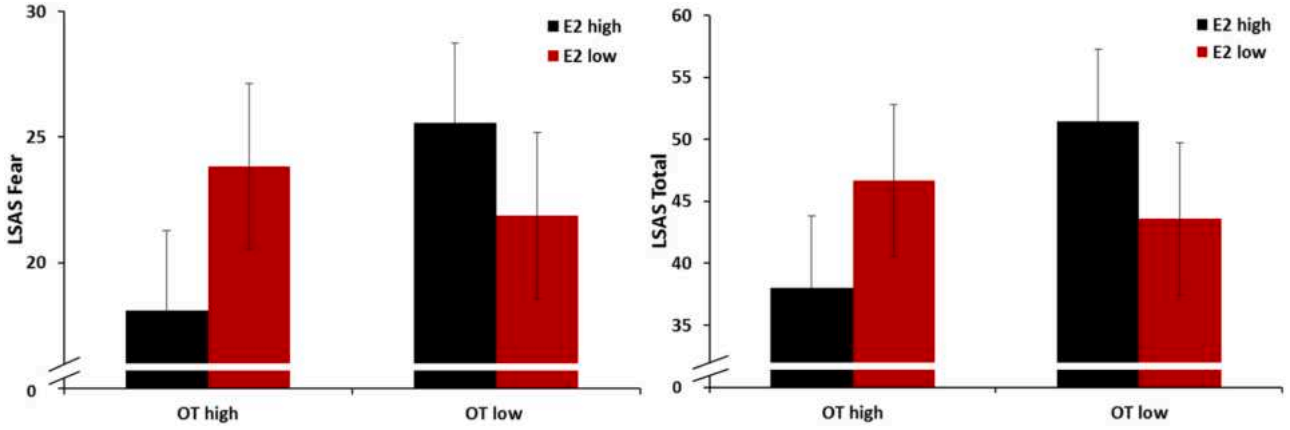

Fig. 1. Median hormone concentrations and self-reported anxiety in all women. LSAS total score and LSAS Fear in women in relation to endogenous oxytocin and estradiol levels. LSAS: Liebowitz Social Anxiety Scale; LSAS Fear: Subscale "Fear" of the Liebowitz Social Anxiety Scale; OT: Oxytocin; E2: Estradiol. The assignment of the hormones in high and low was made based on median split.

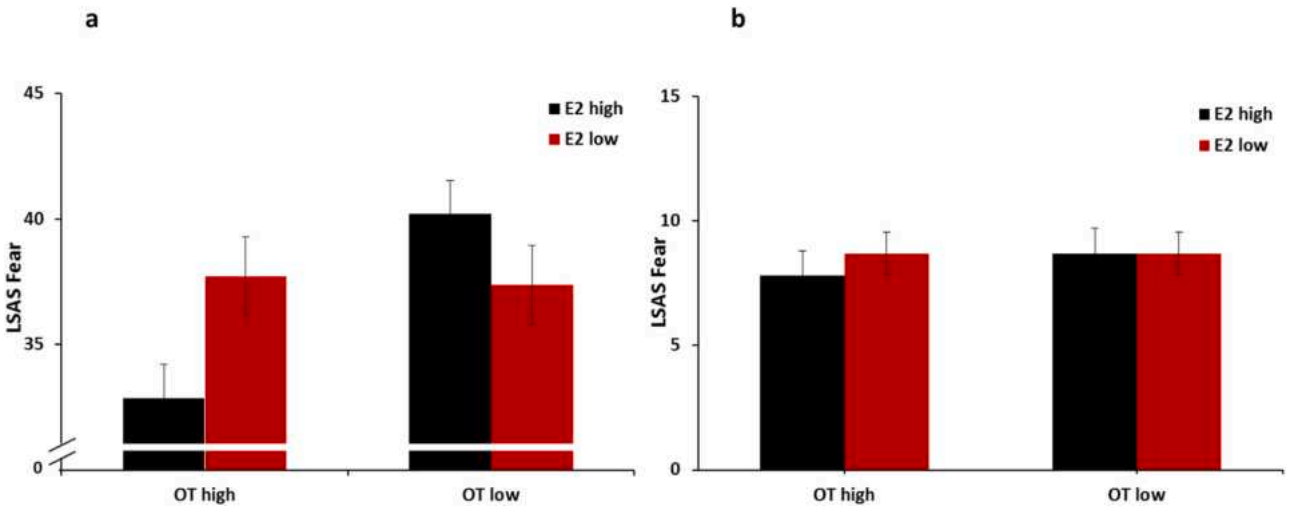

Fig. 2. Median hormone concentrations and self-reported fear in high and low anxious women. LSAS Fear in high (a) and low (b) anxious women in relation to endogenous oxytocin and estradiol levels. LSAS Fear: Subscale "Fear" of the Liebowitz Social Anxiety Scale; OT: Oxytocin; E2: Estradiol. The assignment of the hormones in high and low was made based on median split.

Table 2
Sample characteristics for the male subsample (N = 98).

Sample characteristics ($N = 98$)	Mean (SD); *N* (%)		
	low anxious	highly anxious	Z (p)[a]
N	50	48	
Age (years)	24.60 (3.61)	24.65 (5.31)	
Years of education	17.11 (2.55)	16.70 (2.92)	
Current SAD and/or AVPD	0 (0)	25 (52%)	
Questionnaire data			
Liebowitz Social Anxiety Scale (LSAS total)	18.88 (6.88)	75.33 (14.11)	
LSAS Fear	7.70 (3.183)	39.50 (7.29)	
LSAS Fear of social interaction	2.96 (1.90)	19.08 (4.25)	
LSAS Fear of performance	4.74 (2.15)	20.42 (3.75)	
LSAS Avoidance	11.18 (5.22)	35.83 (7.75)	
LSAS Avoidance of social interaction	5.26 (2.98)	17.73 (4.12)	
LSAS Avoidance of performance	5.92 (2.98)	18.10 (4.35)	
Hormonal data			
Peripheral blood plasma oxytocin (pg/ml)	3.81 (2.82)	3.00 (2.59)	-1.477 (0.140)
Testosterone level (pg/ml)	86.59 (51.03)	78.55 (39.10)	-0.231 (0.817)

[a] Mann-Whitney *U* Test.

anxiety levels are lower when both oxytocin and estradiol levels are high may be considered as a preliminary human-data based support for the data previously reported by animal studies (McCarthy et al., 1997). Also, in mice the same authors could show that estradiol enhanced the anxiolytic effects of oxytocin (McCarthy et al., 1996). While our human data are consistent with these results, caution is required, since our work provides only correlational data; thus, no causality can be concluded.

In men, we did not find a significant association of oxytocin x testosterone interaction with LSAS anxiety levels in either group. The role of testosterone in anxiety-related behavior is somewhat inconclusive. Rodent studies report both anxiolytic as well as anxiogenic effects of testosterone (for a review, see (McHenry et al., 2014)). Interestingly, it has also been demonstrated that in gonadectomized male rats testosterone decreases depressive-like symptoms and that this effect was mediated by estradiol (Carrier and Kabbaj, 2012). Moreover, testosterone can increase vasopressin receptor binding (Delville et al., 1996) as well as oxytocin receptor binding after being metabolized into estradiol and DHT (Johnson et al., 1991). These findings suggest that the role of testosterone in regard to anxiety-related behavior is more complex due to its conversion into metabolites and its promoting effects on receptor binding of other hormones, such as oxytocin and vasopressin. Both hormones have been associated with opposite effects and specifically with regard to anxiety and depression-related behavior (Neumann and Landgraf, 2012).

Interestingly, similar to our findings, other studies found a significant association of oxytocin and self-reported anxiety levels only in the clinical population but not in healthy controls (Hoge et al., 2008; Oh et al., 2018). Thereby, these results support previous findings in rodent (Slattery and Neumann, 2010) as well as human (Labuschagne et al., 2010) studies indicating that oxytocin administration can attenuate anxiety-related behavior and amygdala reactivity in highly but not low anxious (healthy control) subjects.

Additionally, we found significantly different hormonal concentrations in women depending on their use of hormonal contraceptives. Specifically, we found that women using hormonal contraceptives showed significantly higher oxytocin levels but lower estradiol and progesterone levels in plasma, as compared to naturally cycling women. This finding is in contrast to the study of de Jong and colleagues reporting lower baseline salivary oxytocin levels in contraceptive users compared to naturally cycling women (de Jong et al., 2015). However, as the authors pointed out the sample of naturally cycling women was very small (n = 4) and it was not controlled for menstrual cycle phase making it difficult to draw conclusions from these results. Our results are consistent with a previous study, which found elevated oxytocin concentrations due to contraceptive use in a within-subject study design (Silber et al., 1987). Similar results have been reported in another study (Amico et al., 1981). Additionally, in the latter study the authors were able to mimic the elevation of plasma oxytocin by administering estradiol suggesting that estrogen component of the contraceptives might be responsible for the oxytocin elevation in plasma. This conclusion seems reasonable as fluctuations of oxytocin levels throughout the menstrual cycle in naturally cycling women have been observed (for a meta-analysis on this topic, see (Engel et al., 2019)).

A number of limitations need to be considered at this point. First, we want to point out that in men only testosterone but no estradiol concentrations were analyzed, which makes it impossible to conclude whether the significant association of oxytocin x estradiol interaction with anxiety levels in women could also be found in men. Moreover, no

manipulation of the hormonal levels was provoked via administration, instead only endogenous and peripheral levels of hormones were assessed and linked to self-reported anxiety levels. In this line, it should be noted that peripheral hormonal levels may only partially reflect central hormonal release. Additionally, the reliability of measurement of peripheral oxytocin in plasma and saliva has been debated (MacLean et al., 2019).

However, this study can provide a first step towards an understanding of hormonal interactions – oxytocin and sex hormones specifically - in human anxiety disorders, which can help to understand some divergent and sex dependent findings in oxytocin related research. While further research is needed in order to support these results, our data suggests that investigation of hormonal interactions is crucial not only in order to understand the underlying mechanisms of human social behavior, but also to improve treatment of psychopathological disorders such as social anxiety disorder.

CRediT authorship contribution statement

All authors have substantially contributed to the manuscript. K.B., L.E.M. and S.C.H. have designed the study, L.E.M. has collected the data, E.S. and L.E.M. have prepared and analyzed data which were interpreted by K.B., E.S., L.E.M and B.D. E.S. has prepared a first draft of the manuscript which was revised and approved by all authors.

Conflict of interest

Authors declare to have no conflicts of interest.

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Appendix II: Paper II

Schneider, E., Hopf, D., Aguilar-Raab, C., Scheele, D., Neubauer, A. B., Sailer, U., Hurlemann, R., Eckstein, M. & Ditzen, B. (2023b). Affectionate touch and diurnal oxytocin levels: An ecological momentary assessment study. *Elife*, 12, 1-18.

Ekaterina Schneider's contribution according to the CRediT author statement (Allen et al., 2019):

Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project co-administration.

RESEARCH ARTICLE

Affectionate touch and diurnal oxytocin levels: An ecological momentary assessment study

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Abstract

Background: Affectionate touch, which is vital for mental and physical health, was restricted during the Covid-19 pandemic. This study investigated the association between momentary affectionate touch and subjective well-being, as well as salivary oxytocin and cortisol in everyday life during the pandemic.

Methods: In the first step, we measured anxiety and depression symptoms, loneliness and attitudes toward social touch in a large cross-sectional online survey (N = 1050). From this sample, N = 247 participants completed ecological momentary assessments over 2 days with six daily assessments by answering smartphone-based questions on affectionate touch and momentary mental state, and providing concomitant saliva samples for cortisol and oxytocin assessment.

Results: Multilevel models showed that on a within-person level, affectionate touch was associated with decreased self-reported anxiety, general burden, stress, and increased oxytocin levels. On a between-person level, affectionate touch was associated with decreased cortisol levels and higher happiness. Moreover, individuals with a positive attitude toward social touch experiencing loneliness reported more mental health problems.

Conclusions: Our results suggest that affectionate touch is linked to higher endogenous oxytocin in times of pandemic and lockdown and might buffer stress on a subjective and hormonal level. These findings might have implications for preventing mental burden during social contact restrictions.

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Editor's evaluation

This important study combines a large cross-sectional survey with detailed ecological momentary assessment to examine the relationship between affectionate touch and well-being during the first wave of the COVID-19 pandemic in Germany. The manuscript reports valuable and solid findings

extending previous research in this domain. Specifically, the combination of ecologically momentary assessment data with repeated measurements of salivary cortisol and oxytocin adds to the current understanding of how affectionate touch relates to psychological burden and affect. Due to its correlational nature, the causality of effects remains speculative and needs to be addressed by future work.

Introduction

Social integration and close social contact have been shown to improve mental and physical health as well as increase longevity (***Holt-Lunstad, 2018***). This effect has been suggested to be mediated through physical proximity and affectionate touch, with touch serving as a social safety signal (***Eckstein et al., 2020***). Affectionate touch has been associated with beneficial effects on human development and psychological well-being throughout the lifespan (***Atzil et al., 2018***; ***Cascio et al., 2019***). Touch activates reward-related brain regions (***Kreuder et al., 2017***) and reduces stress-induced cortisol (***Ditzen et al., 2007***) and pain (***Kreuder et al., 2019***). On a neuroendocrine level, the stress-buffering effects of affectionate touch on subjective measures and activity of the hypothalamic–pituitary–adrenal (HPA) axis have been hypothesized to be mediated by the neuropeptide hormone oxytocin (***Eckstein et al., 2020***).

The outbreak of the Covid-19 pandemic was a continuous stressor with major health and societal consequences (***Fancourt et al., 2021***; ***Mata et al., 2021***; ***Pierce et al., 2020***). Immediate restrictions and physical distancing were necessary measures to control the spread of the virus. The resulting physical isolation has been linked to higher self-reported loneliness, especially as a result of the first lockdown (***Fancourt et al., 2021***; ***Mata et al., 2021***; ***Pierce et al., 2020***) and in individuals with previous higher loneliness (***Bu et al., 2020***). In general, loneliness and social isolation have been associated with poorer mental and physical health as well as increased mortality (***Lee et al., 2021***; ***Leigh-Hunt et al., 2017***). Thus, it is not surprising that several recent studies emphasize the potential impact of loneliness during the Covid-19 pandemic on mental health (***Brooks et al., 2020***; ***Campion et al., 2020***; ***Rozenkrantz et al., 2020***). Large population-based studies suggest that levels of mental distress with clinical significance increased from 18.9% in 2018–19 to 27.3% during the pandemic (***Pierce et al., 2020***), and the increase was most prominent in the initial phase of the lockdown (***Chandola et al., 2022***). On the other hand, perceived social support as well as frequent social contact during the pandemic were associated with lower depression scores (***Sommerlad et al., 2021***).

The request to minimize social contact and increase physical distance during lockdown consequently led to less physical contact and lower frequency of interpersonal touch, potentially increasing the feeling of longing for touch. Moreover, higher longing for touch was associated with prolonged and more severe Covid-19 restrictions (***Meijer et al., 2022***). Literature on touch deprivation suggests that a lack of touch is associated with lower levels of general well-being and an increased risk of mental health problems (***Banerjee et al., 2021***). A recent study by von Mohr and colleagues showed that self-reported deprivation of intimate touch (but not other types such as friendly or professional touch) during the Covid-19 lockdown was associated with higher loneliness scores. In addition, they found that intimate touch deprivation was associated with higher anxiety levels; however, this association was no longer significant when accounting for loneliness (***von Mohr et al., 2021***). The authors suggested that the lack of intimate touch may increase anxiety in individuals with higher loneliness. Burleson and colleagues reported that reduced affectionate touch was associated with more psychological distress, especially for those participants, who typically use touch for affect regulation (***Burleson et al., 2022***).

Initial laboratory research has demonstrated that receiving touch such as a massage can have beneficial effects evident in reduced self-reported anxiety and stress levels (***Kirschner and Kirschner, 2019***), as well as decreased cortisol (***Maratos et al., 2017***) and increased oxytocin (***Morhenn et al., 2012***) concentrations. Similarly, a more recent study found a significant increase in plasma oxytocin and corresponding neural responses after a foot massage. Interestingly, basal oxytocin concentrations, as well as oxytocin increase after the massage were associated with more positive attitudes toward social touch (***Li et al., 2019***). Moreover, touching a dog as compared to merely observing it was associated with not only decreased self-reported stress, but also increased self-reported happiness (***Sokal et al., 2021***). Based on these findings, we hypothesized that a positive attitude toward touch and increased loneliness would be associated with higher anxiety and depression symptoms during

the lockdown. On a momentary level, we expected that affectionate touch would be associated with decreased subjective anxiety, distress, and decreased HPA axis activity (cortisol levels), as well as with higher endogenous oxytocin levels. Furthermore, we expected that the link between subjective anxiety and distress with affectionate touch would be mediated by elevated oxytocin levels. To the best of our knowledge, there has not yet been a study investigating the associations of affectionate touch with mental health and neuroendocrine variables during the Covid-19 lockdown. We addressed this gap using ecological momentary assessment (EMA) of both repeated psychological and endocrine measures in a large sample with frequent repeated everyday life measuring.

Methods

For this study, ethical approval was granted from the ethics committee of the Heidelberg University Medical Faculty (approval no. S-214/2020), and the study was registered online at https://drks.de/search/en/trial/DRKS00021671. All participants provided written informed consent. We used the disclosure of interest form of the International Committee of Medical Journal Editors (ICMJE) to report no conflicts of interest. STROBE protocol was used to standardize reporting.

Study design and population

In a large online survey launched in April 2020, structural social factors, such as housing situation, anxiety, and depressive symptoms, as well as subjective psychosocial burden, loneliness, and the perception of touch during the physical distancing measures, were assessed (***Hopf et al., 2022***). Study participants were recruited via local newspapers, radio programs, and social media. In an attempt to more actively involve the study participants in the research (collecting data, carrying out measurements in open formats, reporting unexpected results, i.e. citizen science approach), all participants (N = 1050) who had completed the online survey were invited to take part in a 2-day psychobiological EMA. Participants were given standardized instructions via phone on how to use their smartphones to collect momentary subjective data, as well as saliva samples via a passive drool method at six time points per day over the course of two consecutive days (i.e. in total, each individual provided 12 saliva samples). They received the collecting devices via mail along with the informed consent documents to sign. Sampling times on each day were adapted to the individual wake-up time and were taken directly after awakening, 30 min after, 45 min after, 2½ hr after, 8 hr after, and directly before going to sleep. To reduce potential missing values, minimize irregularities, and increase adherence, the data sampling was monitored by study members.

Measures

Hospital Anxiety and Depression Scale (HADS)

General psychological distress was assessed using the total score of the Hospital Anxiety and Depression Scale (HADS) (***Hinz and Brähler, 2011***). Sum scores were calculated for anxiety and depression subscales as well as for the total score. The internal consistency of the global HADS score in our data was high (HADS total score: Cronbach's α = 0.89; HADS Anxiety subscale: Cronbach's α = 0.82; HADS Depression subscale: Cronbach's α = 0.82).

UCLA Loneliness Scale

Loneliness was measured using the 20-item UCLA Loneliness Scale (***Döring and Bortz, 1993***). Participants rated how often they felt in a certain way during the past 2 wk, with higher scores indicating higher levels of loneliness. The sum scores were used for statistical analyses. In our sample, the scale showed a high internal consistency (Cronbach's α = 0.91).

Social Touch Questionnaire (STQ)

To measure attitudes toward social touch, we used the Social Touch Questionnaire (STQ) (***Wilhelm et al., 2001***), assessing different aspects of social touch such as touch involving family and friends vs. touch involving strangers, touch occurring in different settings, as well as touch with sexual vs. without sexual connotation. Internal consistency in our data was high with Cronbach's α = 0.84. Low values of STQ indicate a high liking of social touch, whereas high values indicate a high aversion to social

touch. To interpret the results more intuitively, individual scores of the STQ were inverted (i.e. high STQ values indicate a more positive attitude towards touch).

Ecological momentary assessment

Momentary levels of well-being (anxiety, stress, general and Covid-19 related burden, as well as happiness levels) were assessed through single items ('Please indicate how you feel at the moment …') using visual analog scales from 0 (not at all) to 100 (very much). Affectionate touch was assessed with the question 'Since the last time point, did you experience touch, hugs, kisses, cuddles, etc.?' and an additional visual analog scale for the intensity rating of the experienced touch from 0 (low intensity) to 100 (high intensity).

Neuroendocrine measures

On seeing the prompt on their smartphones, participants self-sampled their saliva into Salicaps (small plastic tubes) via passive drool and stored each sample immediately after collection in their home freezers. At the end of data collection, the study team personally visited to collect the samples on dry ice. The saliva samples were stored at –80°C until analyses at the Institute of Medical Psychology's biochemical lab at Heidelberg University Hospital.

For the analyses of endogenous oxytocin concentrations, saliva samples were thawed and centrifuged at 4°C at 1.500 × *g* for 15 min and subsequently analyzed without extraction (50% of the samples in duplicates) following the protocol of oxytocin enzyme-linked immunosorbent assay from Enzo Life Sciences (ELISA; ENZO Life Sciences, Switzerland). The detection limit was 15 pg/ml, and the variation coefficient for intra- and inter-assay precision was 6.12 and 11.13%, respectively. For cortisol analyses, 20% of the samples were analyzed in duplicates and an ELISA from Demeditec Diagnostics (Demeditec Diagnostics, Germany) was used with a reported detection limit of 0.019 ng/ml. Intra- and inter-assay variations in our sample were 2.95 and 7.51%, respectively.

Statistical analyses

For data processing, IBM SPSS version 27 was used. Statistical analyses were conducted using R studio (R version 4.1.1) and Mplus (version 8.6). We analyzed the relationship of attitudes toward social touch (STQ) and loneliness (UCLA Loneliness) with anxiety and depression symptoms (HADS total) controlling for age, sex, and presence of mental disorder using multiple regression analyses. No violations of general assumptions for multiple regression (linearity, homoscedasticity, normality, and independence of errors) were detected. The total score of HADS, as well as HADS Anxiety and HADS Depression subscales, were included as dependent variables, whereas STQ, UCLA Loneliness, as well as the interaction variable (STQ × UCLA Loneliness) were entered as independent variables into the model. STQ and UCLA Loneliness scores were centered around their respective means. Missing data were deleted listwise.

To test whether affectionate touch was associated with well-being and neuroendocrine markers in everyday life, we conducted multiple hierarchical linear models. To separate within-person and between-person effects, self-reported touch (yes/no) and the intensity of touch were centered around each person's mean and the person's mean was centered on the grand mean. First, we included momentary affectionate touch (yes/no) controlling for age, sex, and day as independent variables to predict individual momentary self-reported anxiety, stress, general and Covid-19 related burden, as well as happiness levels in separate models. Subsequently, we analyzed whether the intensity of experienced touch was associated with these momentary psychological states following the same analytical approach. For models including cortisol and oxytocin measures as dependent variables, we additionally controlled for body mass index (BMI) and several potential confounders: momentary food and drink intake, alcohol, caffeine, and cigarette consumption, as well as physical activity, sleep duration, and quality, problems falling asleep, intake of sleeping pills, forced awakening, and brushing teeth. Furthermore, we controlled for assessment time points by including time (coded from 0 to 3 for the assessment time points 3–6) to control for linear diurnal changes after the awakening response (***Ning and Luo, 2017***). Additionally, for these models, we conducted random slope models and compared the fit of these models to models without random slope for the focal predictor (touch; touch intensity) using likelihood ratio tests. For the models on affectionate touch as a binary variable (yes/no), we report random intercept and random slopes models in the 'Results' section since these

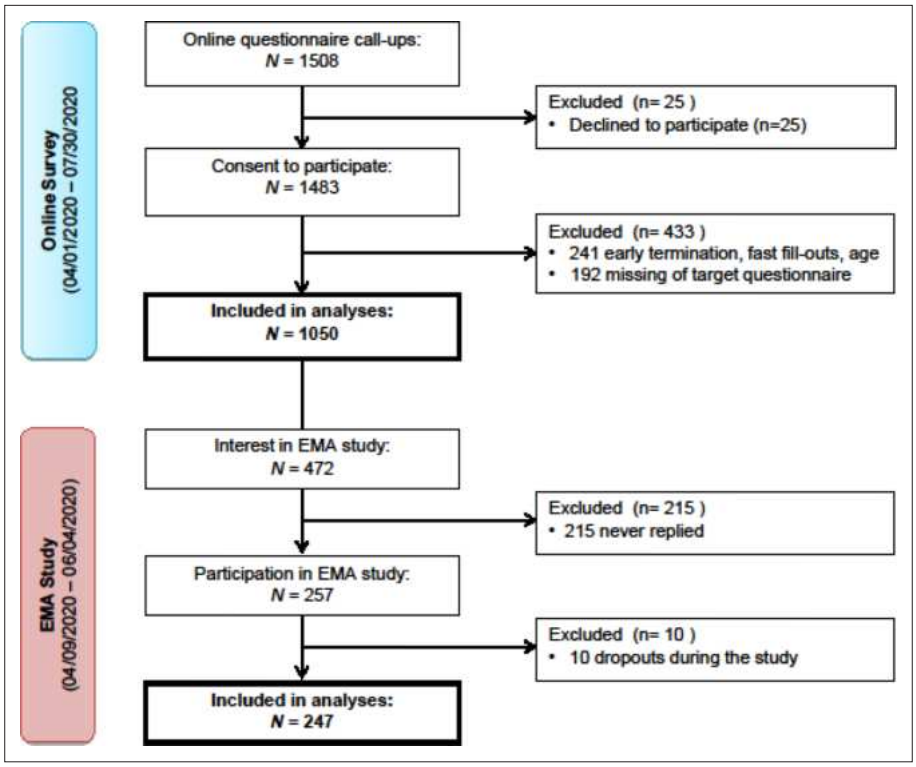

Figure 1. Flowchart of the recruitment process. ***Figure 1*** depicts the recruitment stages of both the online and the ecologically momentary assessments (EMA) study. Participants were recruited between April 1 and July 30, 2020, via online media and local newspapers. Inclusion criteria: fluency in German, minimum age of 18 y, and willingness to participate voluntarily. In total, 1483 individuals agreed to participate, of whom 1050 participants filled out the online questionnaires of interest. Out of the 472 participants who were interested in the EMA study, 247 finished the assessments.

showed a statistically better fit compared to random intercept and fixed slopes models. However, for the dimensional intensity of affectionate touch, we report random intercept and fixed slopes models since the random slope models did not yield a better model fit. Before analyses, cortisol and oxytocin levels were log-transformed (natural logarithm) to normalize the distribution. Cortisol and oxytocin awakening response was calculated using the formula for calculating the area under the curve concerning increase (***Pruessner et al., 2003***).

Results

Sample characteristics

From April to August 2020, 1483 participants filled out the online survey, of whom 433 were excluded from data analysis (see ***Figure 1***). A total of 1050 participants (n = 815 women, n = 227 men, n = 4 non-binary, n = 4 no information on gender) were included in the analyses. Participants' age ranged from 18 to 81 y, with a mean age of 36.34 (SD = 14.77). 20.2% (n = 212) indicated that they suffered from a diagnosed mental disorder. The most frequent single diagnosis was depression (35%) followed by anxiety disorders (10%). Of those with at least one diagnosis, 27.5% indicated having multiple diagnoses.

After completion of the online survey, 472 individuals indicated that they were interested in the EMA study, of whom 257 confirmed their participation after receiving detailed information. Ten participants withdrew from the study due to personal reasons, resulting in a total of 247 participants (n = 173 women, n = 74 men) completing the 2 d EMA. The mean age of the sample was 32.02 y (SD = 13.12) ranging from 18 to 78 y (for more details on sample characteristics, please see ***Table 1***).

Table 1. Sample characteristics of online survey and ecological momentary assessment.

Sample characteristics of online survey participants

	Men (n = 227)	Women (n = 815)	Non-binary (n = 4)	Missing (n = 4)
	M (SD)	M (SD)	M (SD)	M (SD)
Age (years)	34.67 (15.18)	36.74 (14.58)	45.50 (24.73)	40.25 (15.39)
General psychological distress*	10.22 (6.75)	13.18 (7.49)	21.00 (5.89)	19.50 (14.66)
Anxiety†	5.15 (3.68)	6.95 (4.12)	10.50 (3.11)	9.25 (7.68)
Depression ‡	5.07 (3.55)	6.22 (4.10)	10.50 (3.32)	10.25 (8.18)
Loneliness §	37.18 (10.15)	39.33 (10.95)	53.00 (13.24)	47.50 (19.50)
Attitude toward social touch ¶	33.58 (10.18)	34.76 (12.27)	38.50 (23.39)	40.75 (16.92)

Sample characteristics of ecological momentary assessment participants

	Men (n = 74)		Women (n = 173)	
	M	SD	M	SD
Age (years)	30.99	13.62	33.05	12.41
Cortisol (ng/ml) **	8.40	2.02	8.68	2.31
Oxytocin (pg/ml)**	176.12	106.18	164.54	96.74
Covid-19-related burden**	36.98	24.61	41.78	23.49
General burden	39.96	23.89	47.20	21.78
Stress levels††	29.49	15.88	35.62	17.00
Anxiety levels††	18.39	15.98	24.14	20.08
Happiness levels††	71.13	17.09	67.87	18.42
Intensity of affectionate touch††	65.21	20.00	56.57	23.13

Table depicts means (M) and standard deviations (SD). Number of participants indicated as (n).
*Hospital Anxiety and Depression Scale (HADS total score).
†HADS Anxiety subscale.
‡HADS Depression subscale.
§University of California, Los Angeles Loneliness Scale (UCLA Loneliness).
¶Social Touch Questionnaire (STQ).
**Out of 2964 possible data points, n = 2724 remained for analysis after excluding outliers, samples that were not stored as instructed or below detection limit, sampling problems.
††Momentary self-reported state.

Attitude toward touch and its association with anxiety, depression, and loneliness

On average, participants' HADS total scores were M = 12.58 (SD = 7.49, range = 0–37). 39.7% of the sample had values above the cut-off score (>13) compared to a reference sample (***Hinz and Brähler, 2011***). The average HADS Anxiety subscale score was M = 6.58 (SD = 4.19, range = 0–20), whereas the HADS Depression subscale score was M = 5.99 (SD = 4.04, range = 0–21) with values exceeding the cut-off scores (>8) in 29.8 and 24.5% of cases, respectively. The results of multiple regression analyses showed significant main effects of sex (β = 0.111; t(1031) = 4.655, p<0.001), presence of diagnosed mental disorder (β = 0.151; t(1031) = 5.882, p<0.001), UCLA Loneliness (β = 0.548; t(1031) = 20.403, p<0.001), STQ (β = -0.052; t(1031) = −2.083, p=0.038), as well as a significant interaction of UCLA Loneliness × STQ (β = 0.052; t(1031) = 2.104, p=0.036) on the outcome variable total HADS score. Thus, anxiety and depression symptoms were higher in women, individuals with a mental disorder and participants with higher loneliness; and lower in participants with a more positive attitude toward touch. In contrast, although the moderation effects were small, they indicate that the association of loneliness with anxiety and depression symptoms was more pronounced for individuals with a more

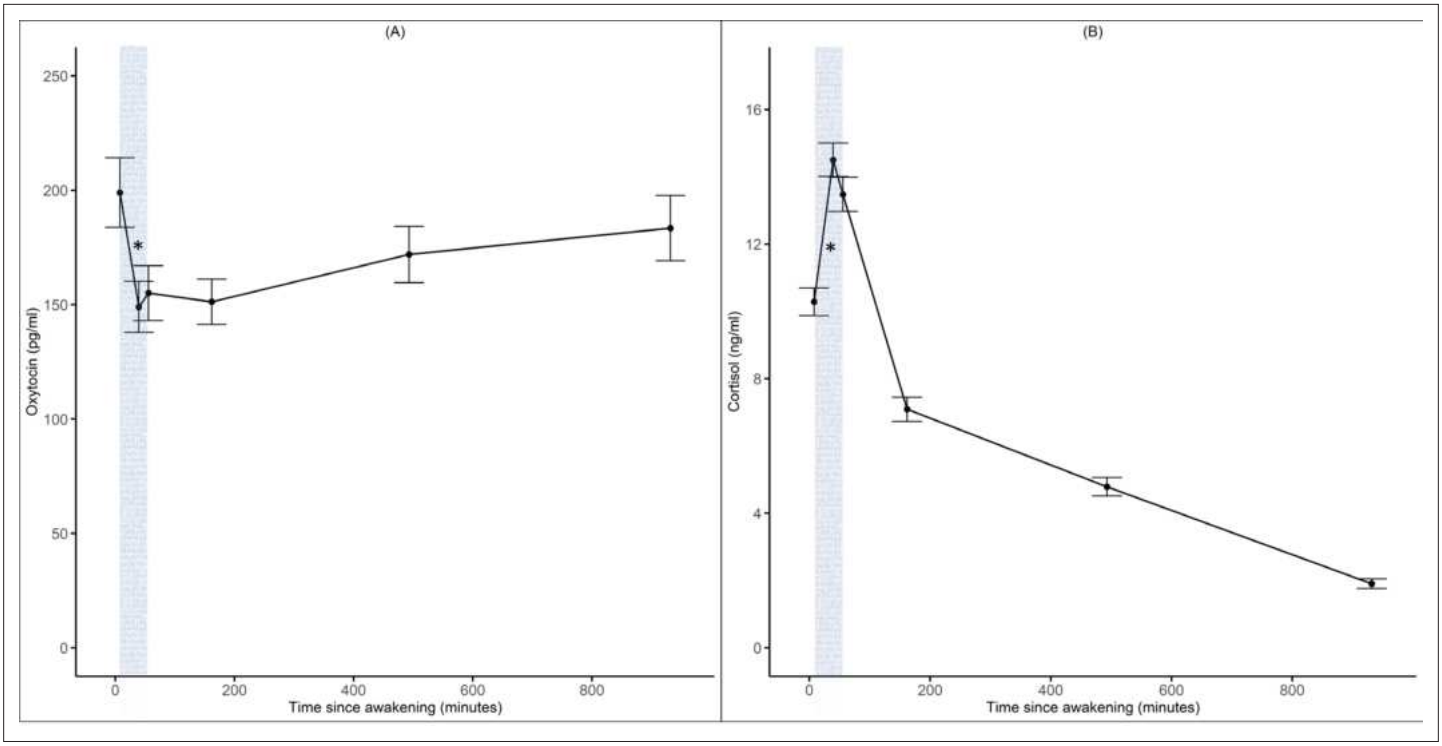

Figure 2. Diurnal oxytocin and cortisol trajectories. Panels (**A**) and (**B**) illustrate the daily oxytocin (pg/ml) and cortisol (ng/ml) trajectories across 2 d and all participants. Gray area indicates cortisol and oxytocin awakening response. Error bars indicate 95% confidence intervals.

The online version of this article includes the following figure supplement(s) for figure 2:

Figure supplement 1. Diurnal oxytocin levels depending on relationship status (**A**) and living arrangements (**B**).

positive attitude toward social touch. The model tested here was significant overall ($F(6,1031) = 125.1$, $p<0.001$) with an R^2 of 0.421.

Next, we analyzed the association of the UCLA Loneliness × STQ interaction with the subscales of the HADS by following the same analytical approach. Here, we found that the outcome variable HADS Anxiety was also significantly and positively associated with female sex ($\beta = 0.142$; $t(1031) = 5.369$, $p<0.001$), presence of mental disorder ($\beta = 0.180$; $t(1031) = 6.3$, $p<0.001$), and UCLA Loneliness ($\beta = 0.404$; $t(1031) = 13.54$, $p<0.001$). Furthermore, we observed a significant interaction of UCLA Loneliness × STQ ($\beta = 0.079$; $t(1031) = 2.907$, $p=0.004$). However, the subscale HADS Depression showed only a significant association with sex ($\beta = 0.060$; $t(1031) = 2.593$, $p=0.010$), presence of mental disorder ($\beta = 0.097$; $t(1031) = 3.838$, $p<0.001$), and UCLA Loneliness ($\beta = 0.603$; $t(1031) = 22.974$, $p<0.001$). The UCLA Loneliness × STQ interaction was not significant ($p=0.539$). Both models with the outcome variable HADS Anxiety as well as with HADS Depression were overall significant ($F(6,1031) = 69.25$, $p<0.001$; with an R^2 of 0.287 and $F(6,1031) = 139.1$, $p<0.001$; with an R^2 of 0.447, respectively).

Affectionate touch, anxiety, oxytocin, and stress-related outcomes on a momentary level

Descriptive statistics of outcomes of interest are displayed in ***Table 1***. In addition, an explorative graphical illustration of daily profiles of oxytocin and cortisol shows their variation throughout the day (***Figure 2***). The patterns of daily profiles did not appear to differ based on participants' relationship status (single vs. in a relationship) or living situation (alone vs. with others) (see ***Figure 2—figure supplement 1***). A positive correlation between the two assessment days was found for individual (ln-transformed) mean values of oxytocin ($r(227) = 0.850$, $p<0.001$), as well as cortisol ($r(243) = 0.571$, $p<0.001$) levels. Additionally, we found a significant negative correlation between mean cortisol and oxytocin awakening response ($r(181) = –0.195$, $p=0.008$).

Results from separate random intercept and random slopes multilevel analyses showed that on a momentary (within-person) level, presence of affectionate touch was significantly and negatively associated with stress (b = −4.187; t(793) = −2.100; p=0.036), but not with general burden, anxiety, happiness, cortisol, or with oxytocin levels (see ***Tables 2 and 3***, respectively). The negative association with Covid-19-related burden did not reach statistical significance (b = −2.660; t(792) = −1.867; p=0.062).

On a between-person level, affectionate touch was significantly associated with lower cortisol (b = −0.121; t(128) = −2.118; p=0.036) (see ***Table 3***), stress (b = −7.534; t(223) = −2.592; p=0.010), as well as with higher happiness (b = 12.420; t(223) = 4.049; p<0.001) levels (see ***Table 2***), but not with general burden or anxiety. The negative association with Covid-19-related burden did not reach statistical significance (b = −7.478; t(223) = −1.838; p=0.067) (see ***Figure 3***).

We also analyzed the intensity of experienced affectionate touch as a predictor for psychological and hormonal outcomes, separating within-person and between-person effects. We found that within a person there were significant and negative associations of the intensity of momentary affectionate touch with anxiety (b = −0.065; t(430) = −2.232; p=0.026), stress (b = −0.148; t(430) = −3.363; p<0.001), general burden (b = −0.077; t(430) = –2.687; p=0.008), and positive associations with momentary happiness (b = 0.085; t(430) = 2.795; p=0.005). The negative association with Covid-19-related burden, however, did not reach statistical significance (b = −0.068; t(428) = −1.900; p=0.058) (see ***Table 2***).

Momentary oxytocin levels were significantly higher with more intensive affectionate touch (b = 0.006; t(149) = 3.058; p=0.002) and cortisol levels were descriptively slightly lower; however, this effect was not statistically significant (see ***Table 3***).

Furthermore, on the between-person level, higher intensity of affectionate touch was significantly associated with less stress (b = −0.223; t(158) = −3.318; p=0.001) and greater happiness (b = 0.314; t(159) = 4.764; p<0.001) (see ***Figure 4***), but not with anxiety, general burden, Covid-19-related burden or hormonal levels. No statistically significant sex effects emerged in any of these analyses.

In a final set of analyses, we conducted multilevel structural equation models to investigate whether there was evidence for oxytocin mediating the effects of affectionate touch on cortisol and/or self-report outcomes. None of the indirect effects of the presence of affectionate touch (p>0.773) or intensity of affectionate touch (p>0.194) on the within-person level were statistically significant. Furthermore, on the within-person level, the correlations of momentary oxytocin with cortisol (r = −0.016, p=0.521), Covid-19-related burden (r = −0.030, p=0.210), stress (r = −0.020, p=0.442), anxiety (r = −0.013, p=0.660), and happiness (r = 0.031, p=0.328) were not statistically significant.

Discussion

This study investigated the associations of affectionate touch with self-reported mental health and mood, as well as with momentary endogenous oxytocin and cortisol levels during the first Covid-19 lockdown in the spring of 2020.

In our online survey data, we found significant main effects of sex, psychopathology, and loneliness on psychological distress (HADS total score) and, more specifically, on anxiety (HADS Anxiety) and depressive (HADS Depression) symptoms. Individuals reported higher levels of depression and anxiety, especially if they were female or burdened by mental illness or loneliness. These data are in line with previous studies (***Fancourt et al., 2021***; ***Mata et al., 2021***; ***Pierce et al., 2020***). Interestingly, analyses showed that the attitude toward touch significantly moderated the association between loneliness and the HADS total score, as well as the HADS Anxiety subscale. Thus, individuals with a positive attitude and affect toward social touch experiencing loneliness showed higher distress and anxiety in times of Covid-19-related lockdown. Whether these moderation effects are apparent outside of pandemic-caused physical restrictions is unknown and should be addressed in future studies. These findings support our hypothesis that touch deprivation and loneliness could be related to anxiety symptoms. However, it is further important to note that about 20% of the participants reported having at least one psychiatric diagnosis. In comparison, the pre-pandemic 12 mo prevalence in the general population in Germany is about 28% (***Jacobi et al., 2014***). Thus, our sample seems to be slightly less burdened compared to the general population, which partly limits the generalizability of the results.

Results of the psychobiological EMA study in a large sample of a broad age range showed that the presence of affectionate touch was negatively associated with stress and cortisol levels and positively linked with happiness. Moreover, the more intensely affectionate touch was experienced, the

Table 2. Results of the associations between affectionate touch and self-reported psychological affective states.

(A) Random intercept and random slopes models

Effects	General burden	Covid-19 burden	Stress	Anxiety	Happiness
Fixed effects Within-person					
Intercept	47.734 (4.668); p<0.001	43.145 (5.009); p<0.001	34.431 (3.596); p<0.001	21.096 (3.958); p<0.001	71.080 (3.778); p<0.001
Touch*	0.462 (1.561); p=0.767	–2.660 (1.424); p=0.062	–4.187 (1.994); p=0.036	–0.217 (1.510); p=0.886	1.557 (1.599); p=0.331
Between-person					
Touch*	–5.560 (3.791); p=0.144	–7.478 (4.068); p=0.067	–7.534 (2.907); p=0.010	–1.483 (3.210); p=0.645	12.420 (3.068); p<0.001
Covariates					
Age	–0.186 (0.122); p=0.128	–0.158 (0.131); p=0.228	–0.136 (0.094); p=0.150	–0.090 (0.103); p=0.388	0.051 (0.099); p=0.605
Sex†	4.709 (3.325); p=0.158	3.761 (3.565); p=0.293	4.986 (2.524); p=0.050	6.568 (2.814); p=0.021	–3.318 (2.681); p=0.217
Day	–2.196 (0.864); p=0.011	–2.602 (0.981); p=0.008	–4.189 (1.228); p<0.001	–3.145 (0.875); p<0.001	1.637 (0.901); p=0.070
Random effects (SD)					
Intercept	21.487	22.794	13.694	17.647	16.597
Touch*	9.141	0.642	8.094	7.906	8.945
Residual	12.821	14.864	18.741	13.076	13.478

(B) Random intercept and fixed slopes models

Effects	General burden	Covid-19 burden	Stress	Anxiety	Happiness
Fixed effects Within-person					
Intercept	44.439 (6.130); p<0.001	39.748 (6.114); p<0.001	32.966 (4.609); p<0.001	24.466 (5.277); p<0.001	71.437 (4.626); p<0.001
Touch intensity	–0.077 (0.028); p=0.008	–0.068 (0.036); p=0.058	–0.148 (0.044); p<0.001	–0.065 (0.029); p=0.026	0.085 (0.030); p=0.005
Between-person					
Touch intensity	–0.121 (0.086); p=0.163	–0.138 (0.087); p=0.115	–0.223 (0.067); p=0.001	–0.102 (0.074); p=0.171	0.314 (0.066); p<0.001
Covariates					
Age	–0.031 (0.161); p=0.847	–0.082 (0.161); p=0.610	–0.090 (0.125); p=0.475	–0.158 (0.143); p=0.270	0.007 (0.122); p=0.952
Sex†	2.647 (4.253); p=0.535	4.143 (4.214); p=0.327	2.650 (3.069); p=389	5.932 (3.645); p=0.106	–0.030 (3.171); p=0.993
Day	–3.788 (1.056); p<0.001	–4.930 (1.302); p<0.001	–4.791 (1.583); p=0.003	–3.718 (1.078); p<0.001	3.695 (1.112); p=0.001
Random effects (SD)					
Intercept	22.467	21.601	13.206	18.622	15.842

Table 2 continued on next page

Table 2 continued

(A) Random intercept and random slopes models

Effects	General burden	Covid-19 burden	Stress	Anxiety	Happiness
Fixed effects					
Within-person					
Residual	11.812	14.626	18.300	12.121	12.591

Table depicts coefficients (standard errors in parentheses) and p-values of associations between (A) the presence and (B) intensity of affectionate touch and psychological variables. Number of observations = 593–1023, Number of participants 162–227.

*0 = no, 1 = yes.

†0 = male, 1 = female.

lower were subsequent subjective anxiety, general burden and stress levels. Higher intensity of affectionate touch was associated with elevated oxytocin and self-reported happiness. Note that in this data assessment in everyday life affectionate touch could not be experimentally manipulated and these correlative results should be interpreted with caution. However, the results could be interpreted that affectionate touch during the Covid-19 pandemic buffers anxiety and stress and downregulates the HPA response, particularly cortisol. At the same time, the intensity of affectionate touch was associated with increased endogenous oxytocin levels and subjective happiness. Interestingly, a recent study demonstrated that foot massage was rated as more pleasurable and rewarding and was associated with a higher increase of oxytocin after the massage administered by hand as compared to machine-administered massage, although the intensity of the massage was rated similarly (***Li et al., 2019***). Furthermore, the oxytocin system and its potential stress-ameliorating effects seem to be triggered by meaningful and intense touch in particular (***Eckstein et al., 2020***).

To our knowledge, the present data provide the first empirical evidence to suggest that affectionate touch is related to reduced anxiety, stress, and general burden, as well as stress-responsive cortisol levels and at the same time is linked to higher endogenous oxytocin levels and well-being in an ecologically valid everyday life setting.

Up to now, no systematic data on daily oxytocin profiles and momentary oxytocin levels in a large sample of men and women from varying age groups have been available. Based on single peripheral oxytocin measures from relatively small samples so far (see ***Valstad et al., 2017***, for an overview), it was the object of debate whether peripheral oxytocin levels might be interpretive of emotional functioning or correspond with stressful experiences (***Engel et al., 2019***). Moreover, in the last decade, there has been an extensive discussion about the reliability and validity of peripheral oxytocin measures (***Martins et al., 2020***; ***Szeto et al., 2011***; ***Tabak et al., 2023***). There are several methodological issues and challenges associated with measuring oxytocin in blood plasma and saliva samples (***Tabak et al., 2023***). For example, studies have shown that oxytocin concentrations after sample extraction are much lower compared to unextracted oxytocin measurements (***Szeto et al., 2011***). Additionally, the correlations between extracted and unextracted oxytocin levels as well as between saliva and plasma oxytocin concentrations have been inconsistent across studies (e.g. ***Hoffman et al., 2012***; ***Martins et al., 2020***; ***Nagahashi-Araki et al., 2022***; ***Szeto et al., 2011***). These inconsistencies might be due to numerous reasons including differences in the study populations, methods of sample processing, and analyses. In particular, using different assay types (e.g. radioimmunoassay vs. enzyme immunoassay), as well as sample preparation (extraction vs. non-extraction), may contribute to these inconsistencies and make it difficult to compare results between studies (***Tabak et al., 2023***). Since unextracted samples were used in this study, the concentrations probably represent both free and bound oxytocin (***MacLean et al., 2019***), thereby potentially limiting the comparability with studies using extracted samples.

Another important issue is the intraindividual stability of oxytocin over time (***Feldman et al., 2013***; ***Martins et al., 2020***; ***Schneiderman et al., 2012***). A recent study reports no correlation of single oxytocin measures between several assessments, indicating that single measures of oxytocin might not be reliable to represent oxytocin baseline levels (***Martins et al., 2020***). In our sample, we found a significant positive correlation of mean oxytocin values between the two assessment days. As the fluctuations of oxytocin throughout the day were apparent in our study, correlating mean values of six

Table 3. Results of the associations between affectionate touch and hormonal levels.

(A) Random intercept and random slopes models			(B) Random intercept and fixed slopes models		
Effects	**Cortisol**	**Oxytocin**	**Effects**	**Cortisol**	**Oxytocin**
Fixed effects Within-person			**Fixed effects Within-person**		
Intercept	2.941 (0.165); p<0.001	4.973 (0.405); p<0.001	Intercept	2.744 (0.370); p<0.001	4.657 (0.798); p<0.001
Touch*	–0.019 (0.060); p=0.756	–0.030 (0.076); p=0.688	Touch intensity	–0.001 (0.001); p=0.367	0.006 (0.002); p=0.003
Between-person			**Between-person**		
Touch*	–0.121 (0.057); p=0.036	–0.145 (0.147); p=0.329	Touch intensity	–0.001 (0.002); p=0.504	0.002 (0.003); p=0.489
Covariates			**Covariates**		
Age	–0.001 (0.002); p=0.633	–0.013 (0.005); p=0.011	Age	–0.001 (0.003); p=0.776	–0.019 (0.007); p=0.007
Sex†	–0.020 (0.044); p=0.647	–0.129 (0.123); p=0.293	Sex†	–0.001 (0.063); p=0.989	–0.167 (0.151); p=0.272
Day	–0.050 (0.035); p=0.155	–0.010 (0.059); p=0.863	Day	–0.032 (0.051); p=0.528	0.077 (0.086); p=0.370
Time-fall ‡	–0.476 (0.023); p<0.001	0.051 (0.035); p=0.145	Time-fall ‡	–0.471 (0.032); p<0.001	0.025 (0.049); p=0.611
Body mass index	–0.014 (0.005); p=0.009	0.012 (0.015); p=0.422	Body mass index	0.005 (0.009); p=0.585	0.014 (0.022); p=0.528
Eating*	–0.077 (0.075); p=0.302	0.032 (0.117); p=0.788	Eating*	–0.125 (0.099); p=0.210	0.099 (0.149); p=0.506
Drinking*	–0.007 (0.081); p=0.933	0.045 (0.126); p=0.722	Drinking*	–0.011 (0.108); p=0.919	–0.039 (0.161); p=0.810
Caffeine*	0.127 (0.043); p=0.004	–0.099 (0.070); p=0.158	Caffeine*	0.131 (0.064); p=0.043	0.157 (0.102); p=0.125
Alcohol*	–0.030 (0.063); p=0.628	–0.174 (0.096); p=0.070	Alcohol*	–0.012 (0.072); p=0.865	–0.154 (0.112); p=0.171
Cigarettes*	0.104 (0.072); p=0.153	0.053 (0.139); p=0.705	Cigarettes*	0.089 (0.098); p=0.368	–0.054 (0.179); p=0.766
Physical activity*	0.039 (0.040); p=0.332	0.108 (0.065); p=0.098	Physical activity*	–0.072 (0.056); p=0.199	0.014 (0.086); p=0.873
Sleep duration §	–0.003 (0.004); p=0.371	–0.009 (0.008); p=0.272	Sleep duration §	–0.029 (0.029); p=0.321	0.046 (0.060); p=0.438
Sleep quality ¶	–0.001 (0.001); p=0.289	–0.000 (0.002); p=0.912	Sleep quality ¶	–0.001 (0.001); p=0.477	–0.000 (0.002); p=0.960
Problem falling asleep*	0.031 (0.053); p=0.552	–0.245 (0.101); p=0.015	Problem falling asleep*	0.030 (0.081); p=0.709	–0.133 (0.148); p=0.370
Sleeping pills*	0.032 (0.109); p=0.772	0.031 (0.254); p=0.904	Sleeping pills*	–0.203 (0.290); p=0.487	0.771 (0.453); p=0.093
Forced awake*	–0.008 (0.041); p=0.838	0.012 (0.088); p=0.894	Forced awake*	0.016 (0.058); p=0.788	0.075 (0.117); p=0.520
Brushing teeth*	0.045 (0.036); p=0.217	0.096 (0.056); p=0.090	Brushing teeth*	0.029 (0.051); p=0.568	0.016 (0.077); p=0.839
Random effects (SD)			**Random effects (SD)**		
Intercept	0.149	0.542	Intercept	0.179	0.546
Touch*	0.273	0.166	Touch intensity	–	–
Residual	0.334	0.477	Residual	0.339	0.461

Table depicts unstandardized coefficients (standard errors in parentheses) and p-values of hormonal associations with (A) the presence and (B) intensity of affectionate touch. Number of observations = 251–545. Number of participants = 88–152.

*0 = no, 1 = yes.

†0 = male, 1 = female.

‡0 = time point 1–3, 1 = time point 4, 2 = time point 5, 3 = time point 6.

§In hours.

¶1 = very bad, 101 = very good.

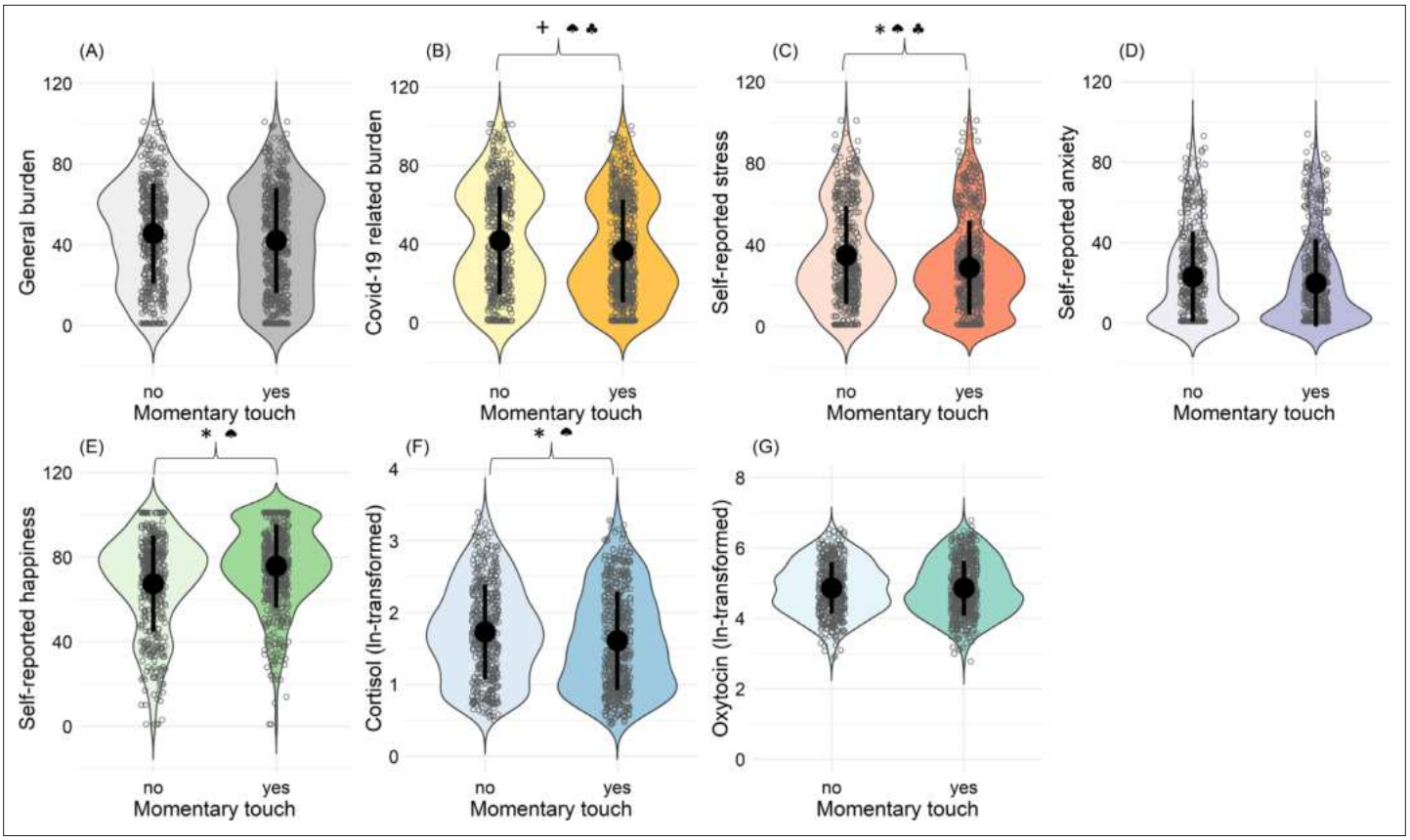

Figure 3. Associations between occurrence of affectionate touch and psychological and hormonal state. Panels (**A**) to (**G**) illustrate violin plots with density distributions of subjective ratings of general and Covid-19-related burden, stress, anxiety, happiness, cortisol, and oxytocin, depending on whether touch occurred or not. Each dot represents one assessment. Central dots (black) represent each mean. Black lines represent the standard deviations. * indicates statistically significant results ($p<0.05$). +indicates a statistical trend ($p<0.1$). ♠ indicates statistically significant between-person effect. ♣ indicates statistically significant within-person effect.

oxytocin measures over the day might represent the individual baseline oxytocin levels better than single measures (this issue has been also discussed in ***Tabak et al., 2023***). However, the difference between our data and the findings reported by ***Martins et al., 2020*** might also reflect methodological differences as previous studies with unextracted samples also reported positive correlations over time (***Feldman et al., 2013***; ***Schneiderman et al., 2012***).

In our study, we found a positive association between momentary oxytocin levels and the intensity of affectionate touch on a within-person level. Our findings are in line with previous studies showing an increase in salivary oxytocin after self-touch (***de Jong et al., 2015***), standardized touch (***Portnova et al., 2020***), and massage (***Li et al., 2019***; ***Moussa et al., 2021***). Notably, in recent animal research, it has been shown that the density of oxytocin neurons in the brains of male mice increased after social isolation and that oxytocin neurons are involved in regulating social craving (***Musardo et al., 2022***). Moreover, social touch has been associated with central nervous system oxytocin activation and secretion into the periphery (***Tang et al., 2020***). Our data are in line with this research; however, we did not find support for the hypothesis that peripheral oxytocin directly mediated the effects of affective touch on momentary subjective distress and well-being on a statistical level. Central nervous oxytocin dynamics (receptor sensitivity, real-time levels, local gene expression, or methylation) and their interaction with the HPA axis cannot be measured in the living human brain presently (***Quintana et al., 2019***). Thus, although speculative, our results might suggest that central nervous system oxytocin mechanisms as triggered by touch can modulate endocrine outcomes (peripheral oxytocin, cortisol) and subjective distress, as well as well-being. Furthermore, affectionate touch might influence different outcome levels in parallel (not mediated) processes, or the effects of oxytocin on perceived distress, well-being, and cortisol could unfold across a longer time span, instead of on a moment-to-moment

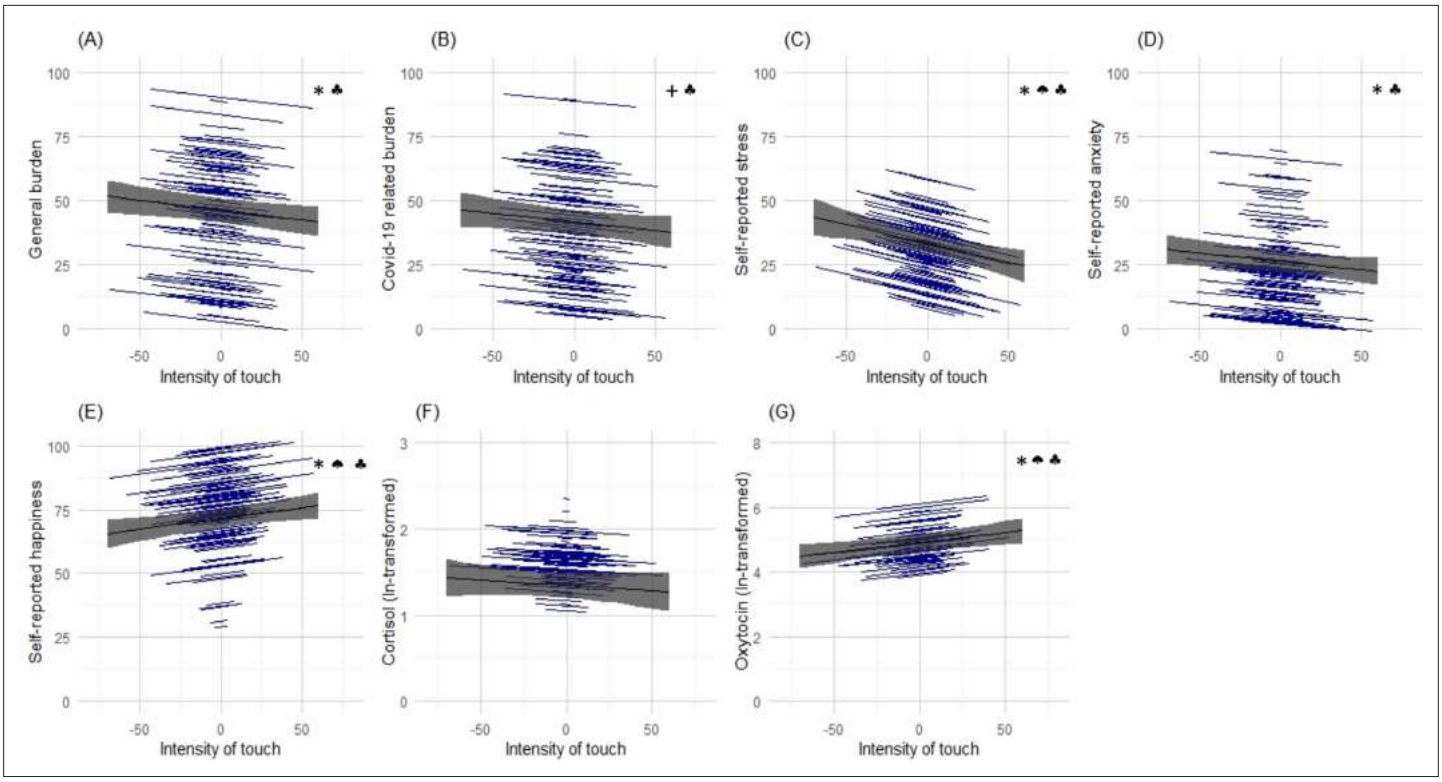

Figure 4. Associations between momentary affectionate touch intensity and psychological and hormonal state. Panels (**A**) to (**G**) illustrate the results of random intercept and fixed slopes models depicting associations of momentary intensity of touch with self-reported general burden and Covid-19 related burden, stress, anxiety, happiness, cortisol, and oxytocin. Gray lines indicate the overall predicted slope, whereas the blue lines indicate the individual's predicted slopes with their minimum and maximum predicted values as endpoints. Gray areas depict the 95% confidence band. * indicates statistically significant results ($p<0.05$).+indicates a statistical trend ($p<0.1$). ♠ indicates statistically significant between-person effect. ♣ indicates statistically significant within-person effect.

basis. Alternatively, it is also possible that initially higher levels of participants' well-being (including lower stress, anxiety, or burden) might have increased feelings of closeness and therefore promoted affectionate touch. Also, other aspects that accompany physical contact such as eye contact, compliments, or affective closeness with loved ones could have contributed to the beneficial effects.

The study has some limitations that need to be addressed. The assessment of individual depression and anxiety levels was based on the self-reports using the HADS. Although this instrument has been validated and repeatedly used in clinical practice (***Hinz and Brähler, 2011***), it does not replace clinical interviews and might be influenced by self-report bias. Furthermore, in the EMA measures we used single items to minimize the drop-out rate during the study, but these items might not comprehensively reflect the individual's experience of burden, stress, anxiety, or happiness. In addition, touch from strangers was restricted during the pandemic; thus, affectionate touch experiences were probably mostly from family contacts. Therefore, we cannot draw differential conclusions about varying contexts of touch. While in general, being touched by strangers may be rated as less pleasant, during times of a pandemic it is also associated with a higher risk for infection. In contrast, during the lockdown, touch at home may be experienced either as harmless and pleasant (***Sorokowska et al., 2021***) or as too close to feel comfortable during times of limited distraction and constant and close physical contact with family members. The latter is particularly relevant when it comes to the association of touch (yes/no) with cortisol and oxytocin. While touch, per se, seemed to be associated with reduced cortisol levels in the present sample, oxytocin secretion appears to be related to the intensity of touch. However, since this is a cross-sectional study, we here interpret associations rather than causal effects. The Covid-19-related lockdown provided us with a social situation to study the effects of touch between family/household members (romantic couples, parent–child dyads, etc.) in a relatively controlled setting. While the situation was quite specific and limited the generalizability of the results to everyday life in pre- or post-pandemic conditions, the risk of viral infection was not the only

concern among the population. Participants reported significant concerns about being isolated from others and how long it might take for them to get back to normal (***Hopf et al., 2021***). These concerns related, at least in part, to the fear of loneliness, defined as a perceived lack of social connection and the distress this causes (***Bekhet et al., 2008***). Thus, these results obtained in the general population during pandemic-related restrictions can be partially generalized to other situations, such as a lack of social contacts due to migration, physical illnesses/disabilities, or other reasons.

Conclusion

Taken together, the present findings provide support for the links of affectionate touch with more positive mental health outcomes during times of prolonged stress. Notably, the above associations with lower anxiety, better mood, and reduced cortisol levels in everyday life during the Covid-19 lockdown showed that more intense affectionate touch is related to higher salivary oxytocin levels on a moment-to-moment basis. This suggests that endogenous oxytocin might be stimulated through targeted behavior (e.g. social touch), which could have implications for prevention and interventions for individuals who are particularly vulnerable during times of stress and social isolation.

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Ekaterina Schneider, Data curation, Formal analysis, Investigation, Visualization, Methodology, Writing – original draft; Dora Hopf, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – review and editing; Corina Aguilar-Raab, Conceptualization, Methodology, Writing – review and editing; Dirk Scheele, Conceptualization, Resources, Investigation, Methodology, Writing – review and editing; Andreas B Neubauer, Uta Sailer, Formal analysis, Methodology, Writing – review and editing; René Hurlemann, Methodology, Writing – review and editing; Monika Eckstein, Conceptualization, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Project administration, Writing – review and editing; Beate Ditzen, Conceptualization, Resources, Software, Formal analysis, Supervision, Funding acquisition, Validation, Investigation, Methodology, Project administration, Writing – review and editing

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Ethics

For this study ethical approval was granted from the ethics committee of the Heidelberg University Medical Faculty (approval no. S-214/2020) and the study was registered online at https://drks.de/search/en/trial/DRKS00021671. All participants provided written informed consent.

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Additional files

Supplementary files

- MDAR checklist

Data availability

The datasets analyzed and presented in this manuscript are openly available online (https://doi.org/10.11588/data/WFNWJT).

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database and Identifier
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Appendix III: Paper III

Schneider, E., Hopf, D., Eckstein, M., Scheele, D., Aguilar-Raab, C., Herpertz, S. C., Grinevich, V. & Ditzen, B. (2023a). Stress during the COVID-19 Pandemic Moderates Pain Perception and Momentary Oxytocin Levels. *Journal of Clinical Medicine*, 12(6), 1-12.

Ekaterina Schneider's contribution according to the CRediT author statement (Allen et al., 2019):

Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project co-administration.

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Article

Stress during the COVID-19 Pandemic Moderates Pain Perception and Momentary Oxytocin Levels

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Abstract: Self-reported pain levels have been associated with increased stress levels during the COVID-19 pandemic. Less is known about the long-term effects of stress on individuals' physical and emotional pain levels and their associations with the neuropeptide hormone oxytocin. We aimed to predict momentary pain through individual stress levels and momentary oxytocin levels at genuinely high-stress phases, namely during COVID-related lockdowns. In a cross-sectional (n = 254) and a longitudinal (n = 196) assessment during lockdowns in Germany, participants completed a 2-day ecological momentary assessment (EMA) protocol (collecting six saliva samples on two consecutive days each and simultaneously reporting on stress, physical, and emotional pain levels) in 2020, as well as one year later, in 2021. Hierarchical linear modeling revealed significant positive associations between individuals' stress levels and physical pain, both cross-sectionally (b = 0.017; t(103) = 3.345; p = 0.001) and longitudinally (b = 0.009; t(110) = 2.025; p = 0.045). Similarly, subjective stress ratings showed significant positive associations with emotional pain on a within-person (b = 0.014; t(63) = 3.594; $p < 0.001$) as well as on a between-person (b = 0.026; t(122) = 5.191; $p < 0.001$) level. Participants further displayed significantly lower salivary oxytocin when experiencing higher levels of emotional pain (b = −0.120; t(163) = −2.493; p = 0.014). In addition, high-stress levels significantly moderated the association between physical pain and salivary oxytocin (b = −0.012; t(32) = −2.150; p = 0.039). Based on mechanistic and experimental research, oxytocinergic mechanisms have long been suggested to modulate pain experiences, however, this has not yet been investigated in everyday life. Our data, which was collected from a large sample experiencing continued stress, in this case, during the COVID-19 pandemic, suggests that individuals experience more intense physical pain and elevated stress levels, as shown by particularly low salivary oxytocin concentrations.

Keywords: stress; physical pain; emotional pain; oxytocin; ecological momentary assessment; COVID-19

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1. Introduction

The outbreak of the COVID-19 pandemic made it necessary to implement immediate restrictions and physical distancing to control the spread of the virus. The threat of the virus in combination with restrictions and quarantines has been associated with increased rates of anxiety, depression, stress, and decreased well-being [1–4]. In parallel, self-

reported pain increased [5–7], especially when patients suffering from chronic pain did not have access to treatment [8], suggesting that adequate clinical care can prevent pain augmentation during a pandemic [9,10]. Further, self-reported pain during the pandemic was associated with decreased physical activity and increases in psychological stress [6].

Initial research has demonstrated that stress per se modulates pain perception and vice versa, that is, the association between stress and pain seems to be bidirectional [11,12]. However, while acute stress can reduce pain perception, chronic stress, in contrast, has been reported to increase pain (for review see [13,14]; for reports on sex differences see [15,16]). Moreover, negative emotions, as well as their intensity, seem to modulate this relationship [17]. Research regarding emotional pain, however, is very scarce [18], despite the knowledge that the assessment and understanding of emotional pain can have important implications in clinical work [19]. One previous study found that previously experienced emotional pain (e.g., childhood abuse, parent's psychopathology) has detrimental effects on medical pain conditions and increases depression and anxiety later in life [20].

Data on neuroendocrine correlates of pain suggest a critical role of oxytocin, as the exogenously administrated neuropeptide elicits analgesic effects and increases pain tolerance [21]. More specifically, in men with chronic back pain, oxytocin administration attenuated pain intensity when acute thermal pain was applied, but not when back pain was occurring spontaneously [22], indicating that the analgesic effects of oxytocin might depend on the type of pain. Likewise, in a study using heat pulses generated by an infrared laser that selectively activates Ad- and C-fiber nerve endings [23], analgesic effects of oxytocin have been found, but not for noxious thermode heat stimuli [24]. Interestingly, the anti-nociceptive effects of oxytocin appear to be sex-specific and have been mostly observed for men (e.g., [25], but also see [26]).

The literature on the associations between peripheral oxytocin and pain is scarce. For example, elevated oxytocin concentration was reported in migraineurs [27], whereas, in children with abdominal pain and inflammatory bowel disease [28] and patients with low back pain [29], oxytocin levels were decreased. In line with this, another study suggests that, in women, higher oxytocin was linked to higher pain tolerance [30]. Interestingly, recent studies demonstrated that pain, as well as oxytocin, can be modulated simultaneously by social factors. Storytelling [31] or the mother's voice [32] can reduce pain and increase oxytocin concentrations in children, possibly due to the neuropeptide's stress-reducing effects. However, to the best of our knowledge, there is currently no study investigating momentary and long-term effects of stress on pain and their associations with oxytocin concentrations in daily life during the pandemic. Furthermore, studies on emotional pain are underrepresented, in general, and even less is known about the role of emotional pain in everyday life and its associations with stress and salivary oxytocin. To fill this gap, we conducted an ecological momentary assessment (EMA) in everyday life during the first lockdown in Germany and repeated the assessment one year later during the second lockdown to probe long-term effects. We specifically investigate distinct associations with physical pain, as compared to emotional pain. Before this study, we hypothesized that self-reported stress would be associated with increased individual pain levels on a momentary level within one assessment phase (1) and one year later (2). Furthermore, we expected a negative association between oxytocin and pain on a momentary level (3), and that this link would be moderated by individuals' subjective stress levels (4).

2. Materials and Methods

2.1. Study Design and Population

During the first lockdown in Germany, which began in March 2020, we launched the initial part of our study to investigate the impact of physical contact restrictions on individuals' well-being (data published elsewhere [33,34]). During the first lockdown between April and August 2020, a total sample of 247 participants completed a 2-day EMA (t1);

254 participants (n = 196, plus n = 58 newly recruited) completed a second 2-day EMA (t2) in 2021.

Participants were recruited via social media, local newspapers, and radio and were included for participation if they were over 18 years of age and German-speaking. In addition to participating in an online questionnaire assessment, interested individuals were invited to take part in a 2-day psychobiological ecological momentary assessment (EMA). At six time points per day, participants collected saliva samples via the passive drool method and simultaneously assessed their momentary subjective data. Sampling time points were adapted based on individual wake-up times and were taken directly after awakening, 30 min after, 45 min after, 2 ½ hours after, 8 h after, and directly before going to sleep. All participants received the informed consent documents as well as the collecting devices via mail. Participants received standardized instructions on the saliva collection devices, the use of their smartphone to collect momentary ratings and proper storage of the samples. The experimenters provided individual instructions via e-mail and by phone, constantly monitored the daily assessments and were available to be contacted for questions. One year after the completion of the first EMA study (t1), participants were asked to participate in the second EMA study (t2) with an identical study design. Of the 472 individuals who originally expressed interest in the study, 257 were enrolled. Ten individuals did not finish data collection due to personal reasons (e.g., psychological burden, loss of a family member, etc.) resulting in 247 individuals with a complete dataset. In 2021, of the 288 interested individuals, 259 were enrolled in the study. Further, 254 individuals completed the two-day data collection, whereas 5 individuals withdrew for personal reasons.

The ethics committee of Heidelberg University Medical Faculty approved the study (approval no. S-214/2020) and all participants provided written informed consent. The study was registered online athttps://drks.de/search/en/trial/DRKS00021671 (accessed on 5 December 2022).

2.2. Measurements

2.2.1. Subjective Ratings of Ecological Momentary Assessment in t1 and t2

In both years, t1 and t2, participants received links via short messages on their phones to collect subjective ratings. Momentary levels of stress were assessed through single items ("Please indicate how you feel at the moment…") using visual analog scales from 0 (relaxed) to 100 (stressed). Pain levels were assessed in the second year of EMA (t2) only, with the questions "Do you feel any physical or emotional pain at this moment?" and "If yes, please indicate the intensity of your experienced physical/emotional pain" ranging from 1 (little pain) to 6 (extreme pain). Additionally, participants were asked where in the body they felt the pain. Momentary stress levels were assessed at four of six time points: 45 min, 2 ½ hours, 8 h after waking up; as well as directly before going to bed, whereas pain levels were assessed at the first and last measurement time points per day (directly after awakening and directly before going to bed) resulting in 1016 possible pain measures (2 times per day, from 254 individuals in 2 days) and 3600 stress measures (4 times per day, from 196 and 254 individuals on 2 days in 2020 and 2 days in 2021, respectively).

2.2.2. Neuroendocrine Measures

After receiving the corresponding link, participants self-sampled their saliva using Salicaps® (IBL International, Hamburg, Germany) (small plastic tubes). Immediately after the collection of each sample, participants stored them in their home freezers until the study team collected the samples on dry ice. The saliva samples were stored at −80 °C until the biochemical analyses were performed at the Institute of Medical Psychology's biochemical lab at Heidelberg University Hospital.

For the analyses of endogenous oxytocin concentrations, saliva samples were thawed and centrifuged at 4 °C at 1500× g for 15 min and subsequently analyzed following the protocol of oxytocin enzyme-linked immunosorbent assay from Enzo Life Sciences

(ELISA; ENZO Life Sciences, Lörrach, Germany) with a detection limit of 15 pg/mL. The analyses were performed without extraction and variation coefficients for intra- and inter-assay precision were 6.12% and 11.13%, respectively, for the analyses of the first year (t1), whereas in the second year (t2) the coefficients were 5.9% and 13.63%, respectively.

2.3. Statistical Analyses

For data processing and statistical analyses, we used IBM SPSS version 27 and R studio (R version 4.1.1). We conducted multiple hierarchical linear models to test whether momentary stress levels were associated with the intensity of experienced physical and emotional pain in everyday life. To separate within-person and between-person effects, self-reported stress was centered around each person's mean and the person's mean was centered on the grand mean for both years (t1 and t2, respectively).

First, we included momentary stress levels during the second lockdown (t2) controlling for age, sex, being in a relationship (yes/no), and measurement time points as independent variables to predict momentary self-reported pain levels during the second lockdown (t2). Subsequently, we examined whether higher stress levels during the first lockdown would predict the intensity of experienced pain one year later. Therefore, in this model, we included the grand mean centered stress ratings of the first lockdown (t1) instead of the stress ratings of the second lockdown (t2).

Next, we tested the hypothesis that momentary oxytocin would be associated with the intensity of experienced emotional and physical pain. To normalize the distribution, oxytocin levels were log-transformed (natural logarithm). We included the person mean, as well as the grand mean centered intensity of experienced pain, as predictors and oxytocin levels as the dependent variable. For models including oxytocin measures as dependent variables, we additionally controlled for body mass index (BMI), momentary food and drink intake, alcohol, caffeine, and cigarette consumption, medication intake, as well as physical activity and teeth brushing.

Finally, we tested whether individual stress levels moderated the link between momentary pain and oxytocin. Oxytocin levels were included as the dependent variable, whereas stress levels (t2), the intensity of pain, the product term (stress (t2) x intensity of pain), and the aforementioned control variables were included as predictors in the model.

For all these models, we also conducted random intercept and random slope models and compared the fit of these models to models without random slope using Likelihood Ratio tests. Since none of the random slope models showed a better model fit ($p > 0.414$), we report only the random intercept models.

3. Results

To test our first, third, and fourth hypotheses we used the whole t2 sample (t2all), whereas for the analysis of the second hypothesis we only used the subgroup of individuals, who participated in our study twice. The mean age of the sample was 34.07 (SD = 13.06) ranging between 19 and 79 years (for more details of sample characteristics please see Table 1). Of the whole sample 9.6% indicated that they have been diagnosed with depression, 3.1% with multiple diagnoses, and 1.9% with posttraumatic stress disorder.

Table 1. General characteristics of the sample.

Ecological Momentary Assessment in 2021						
	t2 Men (*n* = 76)		**t2 Women (*n* = 176)**		**t2 all (*n* = 254 *)**	
	M	**SD**	**M**	**SD**	**M**	**SD**
Age (years)	33.89	13.91	34.23	12.67	34.07	13.06
Oxytocin (pg/mL) [a]	133.66	113.61	135.27	117.31	135.437	116.95
Stress levels [b]	32.84	18.04	39.26	19.13	37.46	18.93
Physical pain intensity [b]	1.42	0.49	1.73	0.82	1.64	0.76
Emotional pain intensity [b]	1.71	0.66	1.88	1	1.85	0.92
Repeated Measures of Ecological Momentary Assessment						
	t1 (*n* = 196)		**t2 (*n* = 196)**			
	M	**SD**	**M**	**SD**		
Age (years)	32.72	12.94	33.86	13.05		
Oxytocin (pg/mL) [a]	160.02	117.56	130.05	114.24		
Stress levels [b]	33.00	17.13	36.78	19.46		
Physical pain intensity [b]			1.66	0.79		
Emotional pain intensity [b]			1.87	0.92		

Note. Table depicts means (M) and standard deviations (SD). Number of participants indicated as (*n*). [a] Oxytocin raw values, before ln-transformation and [b] momentary self-reports (using a visual analogue scale from 0 (relaxed) to 100 (stressed) and a Likert scale ranging from 1 (little pain) to 6 (extreme pain). * Including individuals (*n* = 2) without indication of their sex.

Of the whole sample, 154 participants (60.6%) with a mean age of 33.34 (SD = 12.99) reported physical pain, whereas 147 participants (57.9%) with a mean age of 34.08 (SD = 13.03) reported emotional pain at any time point of the 2-day EMA. The most frequent forms of physical pain were 1) head, neck, or shoulder at 31.3%, followed by a combination of different types of pain at 30.7% and back pain at 12.9%. The overall mean for the intensity of physical pain was 1.64 (SD = 0.76) and 1.85 (SD = 0.92) for emotional pain indicating that most individuals experienced low levels of pain. Mean stress levels were M = 37.46 and showed a broad range (SD = 18.89).

3.1. Self-Reported Stress Levels as Predictors for Physical and Emotional Pain Intensity

Results from separate random intercept multilevel analyses showed that on a between-person level, self-reported stress (t2) was significantly and positively associated with the intensity of physical pain (b = 0.017; t(103) = 3.345; $p = 0.001$), but not on a within-person level (b = 0.004; t(48) = 1.007; $p = 0.319$). Similarly, individual stress levels during the first lockdown in 2020 (t1) predicted subsequent physical pain levels during the second lockdown in 2021 (t2) on a between-person level (b = 0.009; t(110) = 2.025; $p = 0.045$) (for more details please see Table 2A).

For emotional pain, we found a significant positive association with subjective stress ratings on within-person (b = 0.014; t(63) = 3.594; $p < 0.001$), as well as between-person (b = 0.026; t(122) = 5.191; $p < 0.001$) levels. Individual stress levels during the first year of the lockdown (t1), however, showed only marginal association with emotional pain in the subsequent year (t2) (b = 0.009; t(101) = 1.858; $p = 0.066$) (for more details please see Table 2B).

Table 2. Hierarchical linear models of the associations between stress, pain, and oxytocin.

(A) Physical Pain Intensity				
Effects	**Stress(t2) → Physical Pain (t2)**	**Stress(t1) → Physical Pain (t2)**	**Physical Pain (t2) → Oxytocin (t2)**	**Physical Pain (t2) × Stress(t2) → Oxytocin (t2)**
Fixed Effects				
Intercept	**2.057 (0.427); $p < 0.001$**	**1.207 (0.310); $p < 0.001$**	**4.986 (0.374); $p < 0.001$**	**4.777 (0.788); $p < 0.001$**
Stress (t2) [a]	0.004 (0.004); $p = 0.319$	---	---	**−0.010 (0.003); $p = 0.003$**
Physical pain [b]	---	---	−0.105 (0.056); $p = 0.064$	−0.049 (0.090); $p = 0.593$
Stress (t2) x Physical pain (t2)	---	---	---	**−0.011 (0.004); $p = 0.040$**
Between-person				
Stress (t1) [a]	---	**0.009 (0.004); $p = 0.045$**	---	---
Stress (t2) [a]	**0.017 (0.005); $p = 0.001$**	---	---	−0.002 (0.004); $p = 0.703$
Physical pain (t2) [b]	---	---	−0.056 (0.087); $p = 0.524$	−0.030 (0.122); $p = 0.804$
Stress (t2) x Physical pain (t2)	---	---	---	−0.000 (0.004); $p = 0.982$
Covariates				
Age (t2)	0.009 (0.007); $p = 0.242$	**0.013 (0.006); $p = 0.043$**	−0.008 (0.005); $p = 0.124$	**−0.019 (0.007); $p = 0.004$**
Sex [c]	0.190 (0.231); $p = 0.413$	0.334 (0.182); $p = 0.069$	0.067 (0.153); $p = 0.662$	0.052 (0.201); $p = 0.798$
Partner (t2) [d]	−0.315 (0.239); $p = 0.191$	−0.268 (0.191); $p = 0.164$	0.143 (0.165); $p = 0.387$	**0.481 (0.217); $p = 0.029$**
Time point (t2) [e]	**−0.053 (0.021); $p = 0.014$**	−0.002 (0.014); $p = 0.895$	−0.013 (0.012); $p = 0.267$	−0.003 (0.017); $p = 0.837$
Body Mass Index (t2)	---	---	−0.003 (0.012); $p = 0.801$	−0.010 (0.016); $p = 0.532$
Eating (t2) [f]	---	---	0.197 (0.206); $p = 0.341$	0.197 (0.301); $p = 0.519$
Drinking (t2) [f]	---	---	−0.167 (0.221); $p = 0.452$	0.254 (0.614); $p = 0.682$
Coffein (t2) [f]	---	---	**−0.345 (0.154); $p = 0.027$**	−0.319 (0.178); $p = 0.082$
Alcohol (t2) [f]	---	---	−0.144 (0.157); $p = 0.362$	−0.032 (0.169); $p = 0.848$
Cigarettes (t2) [f]	---	---	−0.166 (0.190); $p = 0.385$	0.167 (0.262); $p = 0.526$
Physical activity (t2) [f]	---	---	0.075 (0.117); $p = 0.522$	0.025 (0.137); $p = 0.857$
Brushing teeth (t2) [f]	---	---	0.144 (0.119); $p = 0.230$	0.042 (0.131); $p = 0.751$
Medication (t2) [f]	---	---	−0.103 (0.153); $p = 0.502$	0.116 (0.197); $p = 0.560$
Random effects (SD)				
Intercept	0.76	0.585	0.636	0.691
Residual	0.706	0.774	0.545	0.439
(B) Emotional Pain Intensity				
Effects	**Stress(t2) → Emotional Pain (t2)**	**Stress(t1) → Emotional Pain (t2)**	**Emotional Pain (t2) → Oxytocin (t2)**	**Emotional Pain (t2) × Stress (t2) → Oxytocin (t2)**
Fixed Effects				
Intercept	**1.625 (0.360); $p < 0.001$**	**1.378 (0.308); $p < 0.001$**	**5.235 (0.318); $p < 0.001$**	**5.244 (0.583); $p < 0.001$**

Stress (t2) [a]	**0.014(0.004); $p < 0.001$**	---	---	−0.002 (0.003); $p = 0.474$
Emotional pain [b]	---	---	**−0.120 (0.048); $p = 0.014$**	−0.151 (0.077); $p = 0.058$
Stress (t2) x Emotional pain (t2)	---	---	---	−0.004 (0.003); $p = 0.164$
Between-person				
Stress (t1) [a]	---	0.009 (0.005); $p = 0.066$	---	---
Stress (t2) [a]	**0.026 (0.005); $p < 0.001$**	---	---	0.001 (0.004); $p = 0.834$
Emotional pain (t2) [b]	---	---	−0.022 (0.060); $p = 0.715$	−0.065 (0.094); $p = 0.491$
Stress (t2) x Emotional pain (t2)	---	---	---	0.002 (0.003); $p = 0.579$
Covariates				
Age (t2)	**0.014 (0.007); $p = 0.042$**	0.005 (0.007); $p = 0.426$	−0.009 (0.004); $p = 0.050$	**−0.017 (0.006); $p = 0.002$**
Sex [c]	0.092 (0.197); $p = 0.413$	0.186 (0.198); $p = 0.352$	0.018 (0.123); $p = 0.882$	−0.029 (0.150); $p = 0.849$
Partner (t2) [d]	−0.315 (0.239); $p = 0.641$	0.034 (0.196); $p = 0.864$	0.210 (0.123); $p = 0.091$	**0.342 (0.155); $p = 0.033$**
Time point (t2) [e]	−0.034 (0.022); $p = 0.129$	0.016 (0.016); $p = 0.303$	−0.017 (0.011); $p = 0.120$	−0.004 (0.015); $p = 0.780$
Body Mass Index (t2)	---	---	−0.014 (0.011); $p = 0.214$	−0.021 (0.014); $p = 0.140$
Eating (t2) [f]	---	---	**0.462 (0.167); $p = 0.006$**	0.250 (0.205); $p = 0.228$
Drinking (t2) [f]	---	---	**−0.467 (0.189); $p = 0.015$**	−0.053(0.417); $p = 0.900$
Coffein (t2) [f]	---	---	−0.208 (0.127); $p = 0.105$	−0.179 (0.137); $p = 0.200$
Alcohol (t2) [f]	---	---	−0.158 (0.124); $p = 0.204$	−0.131 (0.142); $p = 0.362$
Cigarettes (t2) [f]	---	---	−0.019 (0.146); $p = 0.898$	0.266 (0.189); $p = 0.166$
Physical activity (t2) [f]	---	---	0.059 (0.103); $p = 0.566$	0.054 (0.118); $p = 0.651$
Brushing teeth (t2) [f]	---	---	0.173 (0.100); $p = 0.085$	0.127 (0.113); $p = 0.268$
Medication (t2) [f]	---	---	−0.2093 (0.138); $p = 0.132$	**0.412 (0.161); $p = 0.014$**
Random effects (SD)				
Intercept	0.683	0.658	0.504	0.6
Residual	0.859	0.919	0.522	0.454

Note. Table depicts unstandardized coefficients (standard errors in parentheses) and p values of random intercept models on associations with (A) physical pain and (B) emotional pain. Number of observations = 143–304. Number of participants = 100–139. [a] momentary self-reports (using a visual analogue scale from 0 (relaxed) to 100 (stressed) and [b] a Likert scale ranging from 1 (little pain) to 6 (extreme pain); [c] 0 = male, 1 = female; [d] 0 = single, 1= in a relationship; [e] time-point = 1–12; [f] 0 = no, 1 = yes. Significant results are highlighted in bold.

3.2. Associations of Oxytocin Levels with the Intensity of Pain, and Its Interaction with Self-Reported Stress (t2)

The analysis of our third hypothesis revealed a significant negative main effect of emotional pain and oxytocin concentrations (b = −0.120; t(163) = −2.493; $p = 0.014$), as well as a negative statistical trend association of physical pain intensity and oxytocin concentrations on a within-person level (b = −0.105; t(141) = −1.867; $p = 0.064$), but not on a between-person level. In addition, to analyze whether stress levels moderated the association between pain intensity and oxytocin concentrations, we included self-reported stress levels (t2), as well as the interaction variable of pain x stress (t2) as additional predictors. We found a significant association between momentary stress levels (b = −0.010; t(32) = −3.254; $p = 0.003$) and a significant interaction of physical pain x stress (b = −0.012; t(32) = −2.150; $p = 0.039$), with oxytocin concentrations showing a negative association of physical pain and oxytocin in individuals with high levels of stress (see Figure 1). No significant associations on a between-person level were found. In none of the analyses did we detect any effect of sex.

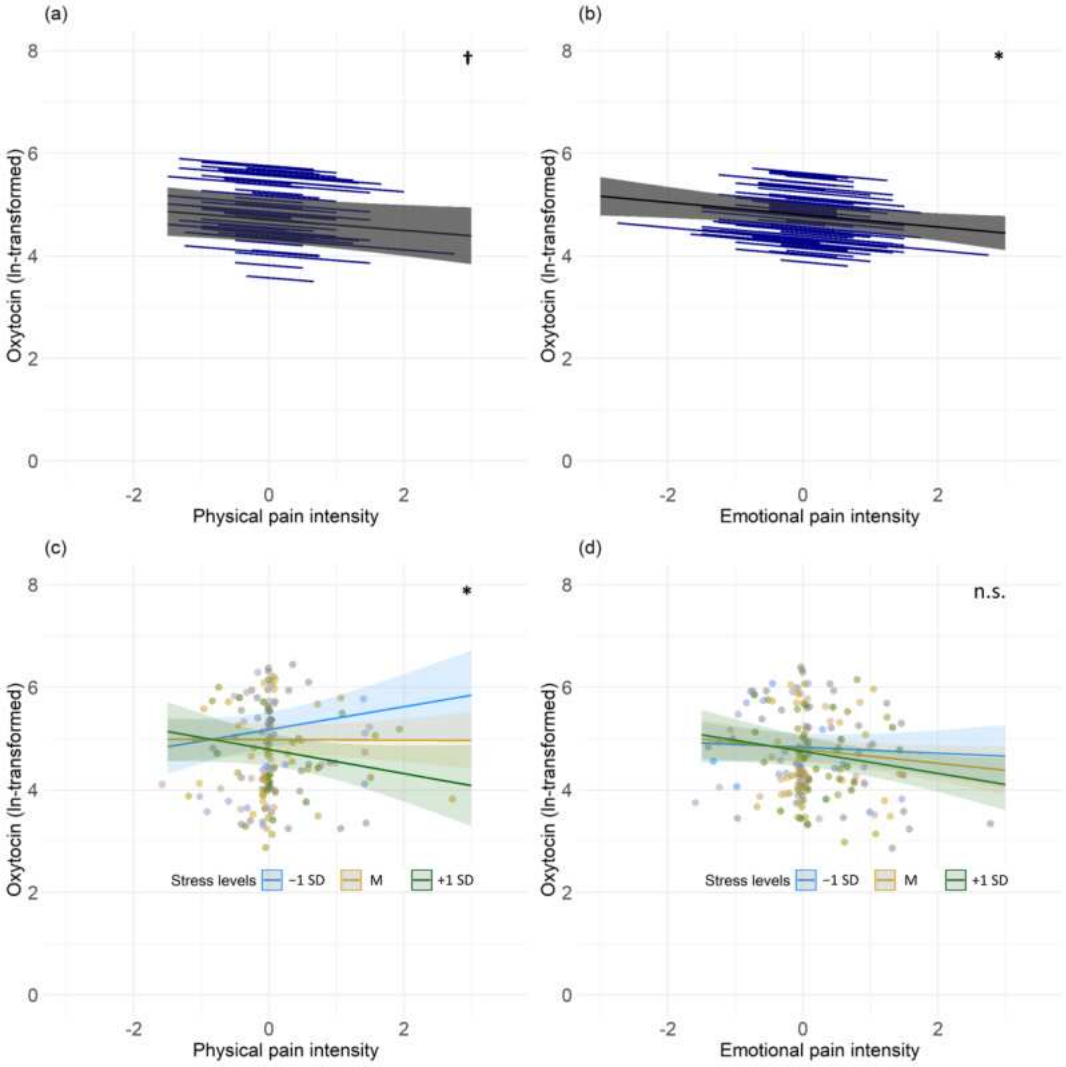

Figure 1. Associations of oxytocin levels with pain intensity and its interaction with stress. Panel (**a**) illustrates the associations of person's mean-centered physical pain and (**b**) emotional pain intensity with oxytocin concentrations (pg/mL) on a within-person level. The grey lines indicate the overall predicted slope, whereas the blue lines indicate the individual's predicted slopes with their minimum and maximum predicted values as endpoints. The grey area depicts 95% confidence band .

Panels (**c**) and (**d**) illustrate individual stress levels as a moderator of the associations between pain intensities and oxytocin (pg/mL) concentrations. Colored lines represent the effect of stress on the association between pain and oxytocin using mean (M) and 1 standard deviation below (−1 SD) and above (+1 SD). Each dot represents individual values. * represents significant results, whereas † represents a statistical trend and n.s. a non-significant moderation.

4. Discussion

In the present study, we investigated short and long-term associations between self-reported stress levels and the intensity of physical and emotional pain in the general population during the COVID-19 pandemic. Additionally, we analyzed whether salivary oxytocin levels were associated with the intensity of pain and whether this association was moderated by stress levels.

Results of our longitudinal psychobiological EMA study during the COVID-19 lockdowns revealed that individuals with higher stress levels are more likely to report higher intensity of physical and emotional pain in daily life, and that higher stress levels during the first lockdown predicted higher pain intensity during the second lockdown. These findings are in line with recent studies reporting that increased psychological stress was associated with pain augmentation in times of pandemic [6] and elevated levels of stress predicted higher rates of low back pain one year later [35]. On a within-person level, we found that higher momentary stress levels predicted emotional pain levels. However, in contrast to our hypothesis and previous findings, which showed that psychosocial stress induction can increase physical pain sensitivity [36,37], we did not find a within-person association between subjective stress and physical pain levels.

As for psychobiological mechanisms underlying the association of stress and pain, we found a significant association between momentary emotional pain levels and oxytocin concentrations, indicating that oxytocin concentrations were lower when emotional pain was higher. With regard to physical pain, there was a marginally significant negative association between the momentary intensity of physical pain and salivary oxytocin. Interestingly, analyses showed that momentary self-reported stress levels significantly interacted with the intensity of pain to predict oxytocin levels. Thus, individuals experiencing more intense physical pain and elevated stress levels show particularly low salivary oxytocin concentrations during the COVID-19 pandemic. These results support previous findings reporting a negative association between oxytocin, pain and stress in human subjects [28,29]. For example, Anderberg and Uvnäs-Moberg found that patients suffering from fibromyalgia syndrome had lower plasma oxytocin concentrations when their stress and pain ratings were high [38]. In line with previous studies [26], we did not find any role of sex in the association of oxytocin and pain. Although in animal models, oxytocin has been shown to directly decrease pain signaling to the spinal cord [39,40], it has also been suggested to elicit stress-induced hyperalgesia [13]. Furthermore, our observation of stress as a moderator of endogenous oxytocin effects is consistent with a previous study showing that higher levels of plasma oxytocin were associated with fewer depressive symptoms and more sensitive maternal behavior among women who reported high levels of psychosocial stress [41]. As such, stress seems to moderate oxytocin effects in various domains.

Our study has several strengths and limitations that need to be addressed. To minimize the drop-out rate during the study, we assessed individual stress and physical pain using single items, which led to a relatively large sample size in an ecologically valid setting. However, these items might not comprehensively reflect the individual's experience of stress and pain during times of pandemic, as standard questionnaires would. Moreover, these data might be influenced by self-report bias, since standard or experimental induction of stress or pain was not used in this study. To investigate the long-term effects of the pandemic on the psychobiological state, we conducted a 1-year follow-up assessment (t2) of our initial assessment (t1). Thus, we were able to analyze whether initial stress levels during the first lockdown predicted pain intensity in the following year. Unfortunately, we did not assess pain ratings during the first lockdown, which makes it impossible for us to study the

bidirectional link between stress and pain in the long-term and to analyze whether high pain levels in the first year would also predict higher self-reported stress in the following year. On the one hand, the restrictions and lockdowns, resulting from the pandemic, affected most of the population creating a unique social situation to investigate the relationship between stress, pain, and oxytocin in a relatively controlled setting. Thus, including the general population with a broad variance of momentary stress in our study made the interpretation of the results more generalizable. On the other hand, the relatively low pain ratings in our sample make it difficult to extrapolate our findings to patients with high pain intensity. To the best of our knowledge, this is the first study investigating emotional pain and its associations with subjective stress as well as oxytocin levels. Emotional pain might overlap with general psychological burden, and thus its association with stress does not seem surprising, but the data collected might contribute to a more fine-grained analysis of neuroendocrine factors in emotional and physical pain. As for the limitations of the mode of collection of the neuroendocrine factor, it is possible that peripheral oxytocin measured in saliva or blood may not necessarily reflect oxytocin release in the brain [42]. However, in stressful situations such positive correlations have been reported in animal studies [43], thus supporting the notion that peripheral oxytocin may signal corresponding responses with central stress and pain-related mechanisms.

5. Conclusions

Taken together, our findings indicate that individuals experiencing more stress might be at higher risk of experiencing more pain in times of pandemic. Moreover, individual stress levels were predictive for future pain ratings showing that individuals with higher stress during the initial phase of the pandemic reported more intensive pain one year later. Additionally, we found that momentary oxytocin levels were decreased in individuals experiencing more stress and higher pain intensity. This suggests that subjectively experienced stress during a pandemic can impair the analgesic effects of oxytocin. Thus, reducing stress and promoting healthy coping strategies in stressful situations might reduce pain perception.

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Institutional Review Board Statement: The study was conducted according to the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study was approved by the ethics committee of the Heidelberg University Medical Faculty (approval no. S-214/2020).

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2022 **Travel Grant for Early Career Researcher**
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2022 **Poster Award by University Hospital Heidelberg**
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2017 **Research Grant by Medical Faculty of Heidelberg University**
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6.000, - €, Co-applicant: Monika Eckstein, Ph.D.

PEER-REVIEWED JOURNAL ARTICLES

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