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Does Oxytocin influence early mate preference in female ring doves ‘Streptopelia risoria’?

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ABSTRACT

Oxytocin, often called the 'Love Hormone,' plays a key role in regulating prosocial behaviors, especially in partner preference and social bonding. Many studies have shown its involvement in pair bonding in mammals; however, its function is less understood in bird species. This study examined female ring doves' (*Streptopelia risoria*) partner preferences during early courtship by testing the effects of intranasal oxytocin administration on this process. To do this, females were placed in a habituation cage with a randomly assigned male to form a bond without physical contact, preventing the fulfillment of a complete courtship, as the goal was to test oxytocin's effect during early courtship. After two days in the habituation cage, the females underwent a classical partner preference test following administration of either intranasal oxytocin or saline solution, to observe potential partner preference cues. During this test, females were placed in a large cage between two smaller cages—one housing the familiar male and the other an unfamiliar male. The entire session was recorded for behavioral analysis—including proximity, beak and body orientation, preening, and feeding—to assess oxytocin's influence on mate preference at the early stage of courtship before sexual contact. I predicted that female ring doves would 1. show a preference toward the familiar male and 2. that oxytocin would enhance this preference. The results showed no significant effect of treatment on female preference for the familiar male. However, there were trends toward an increase in beak and body orientation toward the familiar male, suggesting a possible influence of oxytocin. Furthermore, a significant difference was observed in the females' preening proportion between the two phases of our experiment, namely the baseline phase and the experimental phase, with the only difference being that during the experimental phase, the individuals could see each other. Females preened more when they could see the males, supporting preening's role as a social behavior, even though the treatment did not affect this behavior. Overall, this study provides initial evidence that intranasal oxytocin does not significantly influence female mate preferences during the early stages of courtship in ring doves. It suggests that oxytocin's role might become apparent later in the courtship process or could depend on specific contexts rather than early mate preference alone. The absence of clear effects may be due to the timing of testing, the relatively low dose used, and the focus on spatial measures instead of a wider range of behaviors reflecting mate preference. Future research should use higher and systematically varied oxytocin doses, longer habituation periods, and extend the partner preference test to observe more consistent behavioral patterns.

CONTENT TABLE

1. INTRODUCTION

1.1. Background

Oxytocin (OT), also known as the 'Love Hormone' or 'Feel-Good Hormone,' plays an important role in various social behaviors. This peptide was first identified and synthesized by du Vigneaud and his colleagues in 1953 (du Vigneaud et al., 1953). OT is a nonapeptide as it is composed of nine amino acids in its sequence. At the position one and six of the amino acid sequence are cysteine residues, which form a disulfide bridge, resulting in a cyclic structure of OT. The gene structure of OT was first elucidated in rats (*Rattus norvegicus domestica*) (Ivell and Richter, 1984), followed by the structure of OT receptors (OTR) in humans (*Homo sapiens sapiens*) (Kimura et al., 1992).

It should be highlighted that OT is a highly conserved molecule that is over 600 million years old (Knobloch and Grinevich, 2014). OT evolved through a local duplication of the ancestral gene Vasotocin (VT) via DNA transposable elements. Additionally, OT receptors (OTR) evolved millions of years before the OT ligand, suggesting that the ancestral VT may have originally acted through the OTR before OT even existed, as proposed by Theofanopoulou and colleagues (Theofanopoulou et al., 2021). They also demonstrated that OT has affinity for five different VT receptors—VTR1A, VTR1B, VTR2A, VTR2B, and VTR2C—highlighting the strong interaction with the vasotocinergic system (Theofanopoulou et al., 2021). It is not surprising that OT is found across many different taxa but historically has been referred to various other names; for example, the mammalian homologue of OT in birds has been called mesotocin, in fish isotocin, in nematodes nematocin, and in insects inotocin (Theofanopoulou et al., 2021). Unfortunately, this was likely also the reason why research has remained isolated for each species. Hence, the presence of this peptide in so many taxa underscores the importance of this molecule. Recently, Theofanopoulou and colleagues (2021) showed that the sequences of these nonapeptides are identical across taxa and proposed simplifying the nomenclature by referring to all homologues as 'Oxytocin' (see Figure 1).

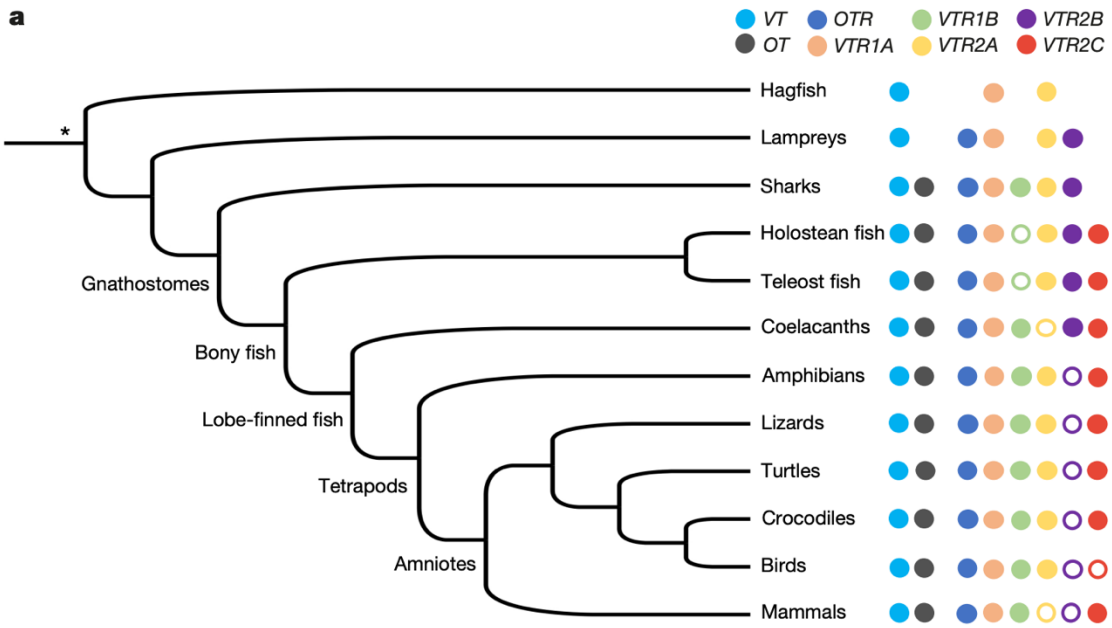

Figure 1: "Phylogenetic distribution of OT, VT , OTR and VTR genes among vertebrates". Reproduced from Theofanopoulou et al. (2021), licensed under CC BY 4.0. OTR and VTR genes codes for the receptors. Birds don't code for two receptors; however, the remaining receptors show high affinity towards both VT and OT

Several studies have demonstrated the role of OT in various behaviors, especially social behaviors. Social behaviors are actions performed by an individual that influence other members of the social group (Goodson, 2013). There is a wide range of social behaviors an individual can display, often depending on the context. Some social behaviors are prosocial, meaning they are affiliative and benefit the well-being of others (Goodson, 2013). However, not all social behaviors are prosocial, as some can have the opposite effect, such as aggression following a threat or social avoidance (Goodson, 2013). Moreover, the different social behaviors exhibited by an individual are species-specific and highly individual (Carter, 1998). Some social behaviors involve same-sex interactions, such as cooperation or group hunting, for example. Conversely, others benefit offspring, like the mother-infant bond (Kendrick et al., 1987; van Leengoed et al., 1987; Bowlby, 1958). Additionally, social behaviors can occur between opposite sexes, including mating, bond formation, or biparental care of the young. Nonetheless, all social behaviors have evolved in response to environmental and social challenges to benefit the social group. It is not surprising that this hormone gained a lot of attention. In the next section, I will discuss the influence of OT, emphasizing the two different regulatory mechanisms (central and peripheral) of the OT system and their impacts.

1.2. Neuro-endocrine mechanism of OT-system:

The nonapeptide is produced within the major nuclei of the hypothalamus, such as the paraventricular nucleus (PVN) and the supraoptic nucleus (SON), by magnocellular neurons. It is stored in vesicles within the Herring bodies until released through the posterior pituitary into

the bloodstream (Jurek and Neumann, 2018). It should be noted that it has 2 distinct regulatory mechanisms:
First, OT acts locally on key brain nuclei and thus regulates centrally, primarily affecting social behaviors (see 1.2.2. Behaviors Centrally Regulated by Oxytocin). Since hormones must cross the blood-brain barrier (BBB) to act centrally, it is important to use a method that facilitates this, like intranasal administration through a nebulizer, in order to study central effects and subsequent behavior modulation. However, OT administration can also happen peripherally by delivering the hormone intravenous or intramuscular via peripheral routes. For the hormone, delivered through peripheral administration, to affect behavior, it must pass the BBB to reach the brain. In opposition, central administration refers to administration methods where the substance is directly delivered to the brain and acts on the central nervous system by bypassing the BBB.
Second, it also plays an important role in the periphery by acting on specific target tissues after being released into the bloodstream (see 1.2.1. Peripheral Effects of Oxytocin).

1.2.1. Peripheral effects of OT:

1.2.1.1. Uterine contraction:

In mammals OT plays an important role in labor and pregnancy. In fact, there is a constant fluctuation in basal OT level and OT receptors during pregnancy which are modulated by Estrogens (Uvnäs-Moberg, 2024). At the end of the pregnancy, circulating OT levels rise to facilitate stimulating uterine contractions, by acting on myometrial OTR on the smooth muscle cells of the uterus (Uvnäs-Moberg, 2024). The foetus also induces a positive feedback loop, called Ferguson reflex, by pressing with his head on the cervix. The cervix will then send signals via positive feedback system to the higher brain center of the mother, such as the hypothalamus. This will result in more OT release in response (Ferguson, 1941). Due to the Ferguson reflex, the uterine contractions will get stronger and more frequent until delivery of the child due to the opening of the cervix. Even after the child's birth, the uterus contraction is still responsive to OT, to minimize bleeding by compressing the blood vessels and to expel the placenta (Uvnäs-Moberg, 2024).

1.2.1.2. Milk let down reflex:

OT is also responsible for the milk let down reflex. While milk production is regulated by prolactin hormone (Crowley, 2015), OT modulates the release of the stored milk to feed the offspring. In fact, also here it undergoes a positive feedback system, as stimulation of the breast (sucking) by the child will trigger sensory nerves that will send signals to the hypothalamus (Walter et al., 2021). The triggers are not only about sucking the nipple, but it can also be due to other triggers like hearing the baby cry, seeing and smelling the baby or by physical stimulation of the nipples- e.g. breast pump. Once the signals arrived at the higher brain centre, the posterior pituitary gland will release instantly OT into the bloodstream. As a result of this mechanism, OT will bind to its receptors in the breast and cause the contraction of myopithalial cells around the alveoli. Those contractions will lead to the release of the milk from the alveoli into ducts towards the nipple (Newton and Newton, 1948; Deif et al., 2021).

1.2.2. Behaviors Centrally Regulated by Oxytocin:

As mentioned earlier, hormones can have both peripheral and central effects depending on where they bind to their receptors. A central effect happens when the hormone acts directly on the central nervous system, either by being produced in the brain or by crossing the BBB if it is produced elsewhere. Binding to its receptor in different brain areas involved in behavior regulation—such as the hypothalamus—will influence behavior by affecting various neurological processes, like neuronal gene expression or neurotransmitter modulation.

1.2.2.1. Social bonding:

OT is believed to be involved in social bonding, as it may help inhibit defensive behaviors and promote positive social interactions, ultimately leading to the formation and development of social bonds (Jurek and Neumann, 2018; Carter, 1998). Social bonding refers to the formation and maintenance of selective and lasting affiliative relationships between individuals within a social group, mainly to enhance reproductive success (Goodson, 2013). It encompasses different behaviors such as the mother-infant bond, mating, and subsequent pair bonding. However, the expression of social bonds varies by species and individual (Carter Sue, 1998; Goodson, 2013). In chimpanzees (*Pan troglodytes*), OT appears to reinforce existing positive relationships by encouraging future cooperation, regardless of blood relation (Crockford et al., 2013). A study on bonobos (*Pan paniscus*) by Brooks and colleagues found that self-directed behaviors decreased when they received OT, and allogrooming—an important social behavior for strengthening bonds—also increased with OT administration, highlighting its role in bonding in bonobos (Brooks et al., 2022). In meerkats (*Suricata suricatta*), peripheral OT administration led to increased cooperative behaviors such as guarding and pup-feeding, along with decreased aggression, demonstrating that peripheral OT administration can have an impact on behaviour too (Madden and Clutton-Brock, 2011). In dogs (*Canis lupus familiaris*), OT, intranasally administred, enhances social motivation to approach and connect with familiar partners, forming the basis for social bonds and supporting their long-term maintenance (Romero et al., 2014). However, this effect is context-dependent, as Hernádi and colleagues (2015) suggested. Dogs given OT through intranasal administration exhibit more affiliative behaviors toward familiar individuals and increased aggression toward unfamiliar ones (Hernádi et al., 2015). Similarly, in pigs (*Sus scrofa domesticus*), OT's effects depend on the context. Camerlink and colleagues (2016) demonstrated that intranasal OT administration in familiar groups reduces social contact in neutral and positive situations but increases it in negative scenarios (Camerlink et al., 2016). This result highlights the importance of social context for the influence of OT.

1.2.2.2. Social recognition:

Social recognition, which is the ability to recognize and remember conspecifics, is an important factor for social behaviors such as mating and bonding. OT is known to promote social recognition, especially in rodents (Popik et al., 1992; Ferguson et al., 2000; Bielsky and Young., 2004). To recognize a conspecific, an individual must be able to perform social discrimination, a specific type of memory (Goodson, 2013; Ferguson et al., 2000). Mice (*Mus musculus*) lacking the OT gene, because they are OT knockout mice (OTKO mice), cannot

recognize familiar individuals but perform well in other recognition tasks such as spatial memory tasks compared to mice with the OT gene, suggesting that OT plays a key role in social memory (Ferguson et al., 2000). However, central administration of OT in the OTKO mice can reverse the impaired social recognition (Ferguson et al., 2000). OT is released during social interactions in mice and acts as a social reward mechanism, with the help of the dopamine system, to reinforce prosocial behaviors (Oettl et al., 2016). In another study, subcutaneous OT administration in rats led to a decrease in social investigation behavior when encountering a familiar individual, compared to an unfamiliar one, suggesting that OT facilitates social recognition in this species as well (Popik et al., 1992). Engelmann and colleagues (1998) found similar results, demonstrating that central administration of OT enhances social memory, whereas OT antagonist has opposite effects. Additionally, central OT administration into the olfactory bulb, a key brain area for recognition in rodents, facilitates rat maternal behavior due to an improved ability to recognize offspring (Yu et al., 1996). However, the dose of OT administration appears to play a significant role in its effectiveness on social recognition. In fact, too low or too high a dose inhibits social recognition, as shown in several studies (Popik et al., 1992; Engelmann et al., 1998; Dluzen et al., 1998; Benelli et al., 1995).

1.2.2.3. Mother-infant bond:

Oxytocin (OT) is essential for maternal bonding across mammalian species (Bowlby, 1958), although its role varies between species and taxa. In sheep, pregnancy-related hormonal states such as low estrogen and high progesterone stimulate OT production (Broad et al., 1994), while hormonal changes around birth increase OT receptor expression (Insel, 1990). Maternal care is very specific, with ewes rejecting non-filial young (Poindron et al., 1979). OT activity in the olfactory bulb helps lamb recognition (Kendrick et al., 1992), and vaginocervical stimulation triggers OT release in the brain, inducing maternal behavior even in non-pregnant ewes as OT bind to its receptors in the brain leading to behavior modulation (Keverne et al., 1983). Epidural anesthesia blocks maternal interest, but intracerebral OT administration can restore it (Levy et al., 1992). Central OT also encourages mothers to stay close to their lambs, even in the presence of competing stimuli like food (Kendrick et al., 1987). In rats, virgin females given OT show maternal behaviors (Pedersen and Prange, 1979; Pedersen et al., 1982; Fahrbach et al., 1985), and increases in OT receptor expression after birth are key for maternal care (Pedersen, 1995; Young et al., 1997). OT production is also regulated by steroid hormones (Bale et al., 1995). Blocking OT with antagonists delays maternal behavior (van Leengoed et al., 1987). However, OT is mainly crucial for initiating maternal behavior, not maintaining it over time (Francis et al., 2000). Overall, these findings suggest that OT acts as a conserved neuroendocrine mechanism for triggering maternal care across mammals, although the specific pathways and behaviors vary by species and life stages.

1.3. Partner preference and Pair bonding in monogamous species:

A non-classical study model, the prairie vole (*Microtus ochrogaster*), which is a socially monogamous species, has been proven to be an excellent study model to investigate the neurobiology of pair bonding (Beery et al., 2018; Cho et al., 1999; DeVries et al., 1997; Williams et al., 1994; Keebaugh and Young, 2011). Several studies show that administering OT centrally,

affects pair bond formation: For instance, injecting OT into the cerebral ventricles of female prairie voles accelerates pair bonding (Williams et al., 1994), while blocking OTR entirely prevents pair bond formation (Insel and Hulihan, 1995). Moreover, infusing an OTR antagonist into the prefrontal cortex of female prairie voles disrupts mating-related partner preferences (Young et al., 2001). OT exerts a stronger influence on females than on males, indicating a possible sex-specific effect. However, during development OT appears to have an influence also in males, as early-life OT exposure increases the likelihood of developing partner preferences, meaning that the individuals exposed to OT during early life are more likely to create a bond with a particular conspecific (Bales and Carter, 2003). Furthermore, a comparative study of different vole species with varying mating systems has revealed the importance of OTR expression and distribution. Socially monogamous prairie voles have higher densities of OTR in regions involved in pair-bond formation, such as the caudate putamen and nucleus accumbens than the promiscuous montane vole (*Microtus montanus*) (Young et al. 1996). Both brain regions are also involved in the dopamine reward system, supporting the hypothesis that the interaction between OT and the dopaminergic system is necessary for pair bond formation, as bonding can be seen as rewarding (Lim et al., 2004). Additionally, increasing OTR expression in the nucleus accumbens before sexual maturity in female prairie voles enhances the formation of partner preferences as adults (Keebaugh and Young, 2011). Overall, it can be concluded that OTR expression plays an essential role in the expression of social monogamous behavior in voles and modulating other neuroendocrine mechanisms important for establishing a social monogamous mating system. In another study, Lim and colleagues (2004), have shown enhanced partner preference in a promiscuous species, namely the meadow vole, by manipulating the expression of a single gene. This could explain the importance of receptor expression patterns, which affect the shape of neural circuitry, leading to different social behaviors and, consequently, different mating strategies across species.

1.4. Oxytocin effects in avian species:

Most studies investigating the role of OT on social behavior have been conducted on classical model species, especially mammals such as humans, apes, voles, or rodents. However, as mentioned above, OT is evolutionarily quite conserved and the question arises about the role of OT in other taxa, such as birds. Interestingly, more than 70% of bird species are socially monogamous and live in complex social societies. This raises the question of whether avian OT, formerly named 'Mesotocin,' might be involved in the regulation of social behavior, comparable to mammals. Birds also exhibit a wide range of mating and parenting systems, which offers opportunities to select appropriate models for studying the behavioral endocrinology of OT, including analyzing its variation and evolution across different environmental conditions. So far, only a few studies, detailed below, have investigated the role of OT in birds, suggesting that its effects on behavior and physiology are comparable to its mammalian homolog.

From the limited evidence so far, it seems that OT plays a crucial role in social behavior among birds. In zebra finches (*Taeniopygia guttata*), which have a monogamous mating system, central infusion of OT encourages spending more time near familiar conspecifics (Goodson et al., 2009). Conversely, intramuscular administration of an OT antagonist in zebra finches results in more time spent near novel conspecifics (Pederesen and Tomaszycki,

2012). The effect is more pronounced in females, highlighting the sex-specific nature of OT in birds similar to voles. Furthermore, to demonstrate OT's influence on social affiliation in zebra finches, an experiment was conducted where females chose between 2 groups consisting of either 10 or 2 familiar conspecifics (Goodson et al., 2009). The results show that an intramuscular administration of OT antagonist leads to a decrease in time spent close to the larger group, while centrally infusing OT has the opposite effect. Additionally, OTR expression plays a significant role in social behavior across bird species. In fact, closely related finch species with different social structures exhibit varying OTR expression in the lateral septum, underscoring the importance of receptor expression (Goodson et al., 2009), similar to the different results in mammals mentioned previously. OT influences the formation of pair relationships in zebra finches, as intramuscular OT antagonist-treated individuals failed to form a pair, which they normally should, since this species has a socially monogamous mating system (Pedersen and Tomaszycki, 2012). Furthermore, OT regulates parental behaviors in birds. Peripheral OT antagonist administration leads to less parental care, in zebra finches, and changes in the chicks' outcomes by higher mortality rates (Kelly and Adkins-Regan, 2020). Another study done on turkeys (*Meleagris gallopavo*) suggests that OT is involved in maternal and brooding behaviors. In fact, increasing OT concentration by intracerebroventricular administration enhances brooding and chick care, while blocking OT receptors reduces parental care toward the chicks (Thayananuphat et al., 2011). Those findings are similar to those in mammals, highlighting the comparable effects of avian OT and its mammalian homolog. In another study on pinyon jays (*Gymnorhinus cyanocephalus*), researchers hypothesized that OT impacts cooperative behaviours, which is important for bonding. In fact, intranasal OT administered individuals increased cooperation by preferring to deliver food to their partner rather than to themselves (Duque et al., 2018). Therefore, in this thesis we would like to investigate the role of intranasal administred OT in pair-preference in a bird species, the ring dove (*Streptopelia risoria*). By using the intranasal administration method, we were confident that OT would be delivered to the brain and bind to its receptors to influence behavior, which we could observe and quantify using different behavioral indices, mostly spatial ones.

1.5. Why ring doves?

As mentioned before, OT is not well studied in birds yet, however there are some indications that this hormone has effects on behavior and physiology similar to its mammalian homolog. Ring doves appear to be a suitable species to study the involvement of OT in pair formation, as they are socially monogamous and form strong pair bonds. Additionally, the behavioral and endocrine aspects of the ring dove courtship cycle are well known and documented, making it an ideal species for investigating the effects of OT on pair bonding and all the processes involved in pair bond formation and maintenance (Lehrman, 1961; Fusani et al., 2001). It is important to study the early courtship, as it lays the foundation for the later stages of courtship leading finally to pair bonds. Further, studying the effect of OT in ring doves, a non-mammalian species, is important to fill the gaps in comparative neuroendocrinology between different taxa, for a better overall understanding of the effect of OT. Also, it is important to

study it in birds since we know that the brain structure of avian species differs from that of mammals.

1.6. Why using intranasal OT administration instead of other techniques?

Intranasal administration of substances is a technique that allows scientists to bypass the blood-brain barrier and deliver substances effectively into the brain. This method is considered non-invasive because it causes no harm to the animals, contrary to the most central administration techniques, where surgery is necessary. This technique presents numerous opportunities for the field, as it now enables the targeting of brain regions involved in social interaction. The intranasal delivery of OT utilizes pathways provided by the olfactory and trigeminal nerves to transport it from the nasal cavity into the brain (Winterton et al., 2021). Several studies have demonstrated the effectiveness of this technique in various species, mainly mammals, for delivering OT to the CNS (Lee et al., 2020; Temesi et al., 2017). But this technique has also been used in avian species, as intranasal administration of OT has been shown to be effective in other bird species, i.e. pinyon jays (Duque et al., 2020, Duque et al., 2018).

1.7. Hypothesis and Predictions:

Generally, it is known that OT plays an important role in partner preferences and in the recognition of familiar individuals, not only in mammals but also in birds (Goodson et al., 2009; Goodson et al., 2012; Oettl et al., 2016; Ferguson et al., 2000), as well as in pair bond formation and maintenance (Pedersen and Tomaszycki, 2012). The aim of this study was first to test whether a female prefers a familiar versus an unknown male during early courtship in ring doves. Second, to test whether intranasal administration of oxytocin modulates female mate preference. Third, it sought to analyze the behavior of both females and males, since we know that one influences the other. Ultimately, this study will contribute to a broader understanding of the neuroendocrinological role of OT in mate preference in ring doves. In the present thesis I have the following two Hypothesis and Predictions:

Hypothesis 1: I hypothesize that in a preference test, the female ring doves favour a familiar male over an unfamiliar one.
Prediction 1: Female preference will be measured through mostly spatial indices as the location next to partner index, body postured toward partner index and beak directed toward partner index. I predict that those indices will have high values.
Hypothesis 2: Next, I hypothesize that intranasal administration of OT strengthens the preference for the familiar male compared to the non-familiar one.
Prediction 2: I predict that OT treatment will lead to an increase in the indices used to quantify female partner preference, i.e. higher values in the experimental group than the control group.

2. METHODS

2.1. Experimental animals:

The animals used in this study originate from the Fusani Lab colony and are housed at the University of Vienna in the Biology Building (UBB) or at the Konrad Lorenz Institute of Ethology (KLIVV) in Vienna. Those kept at KLIVV were transported to the UBB for the experiment and were given time to adapt to their new environment, which is essential for them not to be stressed for the future experiment. In total we used 32 animals for this experiment, 16 females and 16 males. However, there was a problem with the video files of one group explaining why we have 8 females in the OT group and only 7 in the control group. The age range of the individuals used for this experiment varies between 2 and 6 years. The subjects were randomly assigned to the control group (SL) or the experimental group (OT).

2.2. Experimental design:

First, doves were captured from single-sex aviaries and transported to the laboratory. Next, they were placed in habituation cages with an assigned individual of the opposite sex for two consecutive days to let them habituate to each other. After the **habituation phase** (see 2.3.), each "female" underwent the "**preference test**" (see 2.4.), after being intranasally administered with either an OT solution or a saline solution (see Figure 2). The whole study design was thought by Virginie Canoine & Saverio Lubrano & Cliodhna Quigley as pilot study for their research.

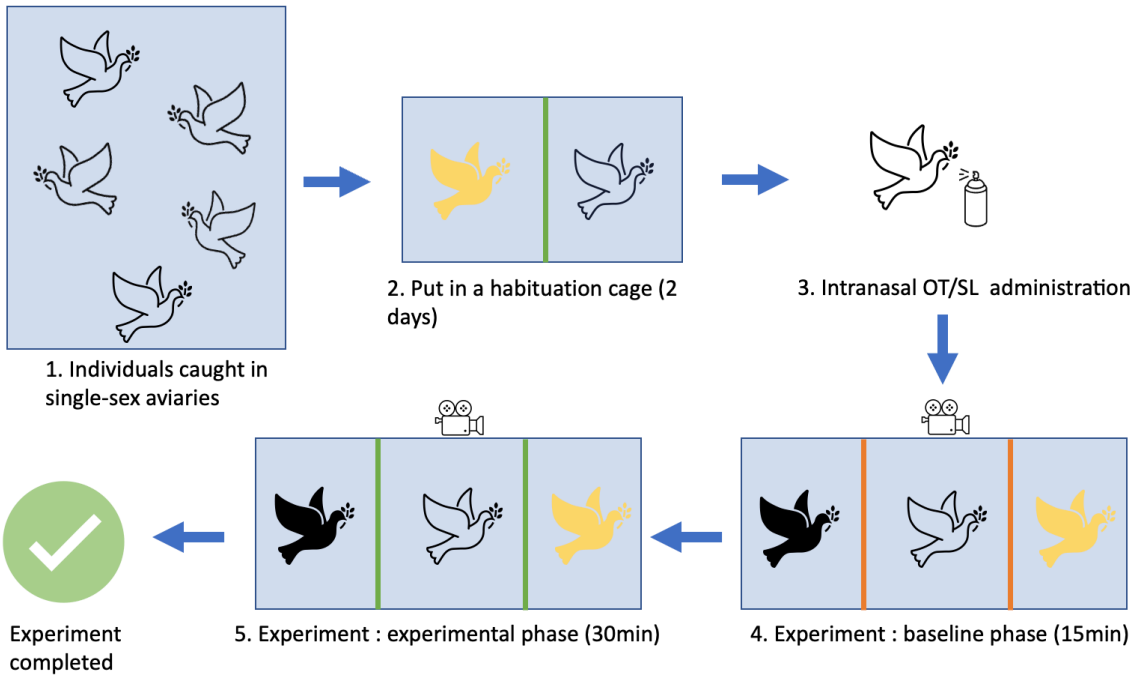

Figure 2: Representation of the experimental design. The green vertical lines indicate grids through which the individuals could see each other, whereas the orange lines represent panels through they could not see each other. The small camera symbolizes the act of recording. The white doves represent the females and the yellow ones the males that shared the habituation cage with the females. The black doves represent an unfamiliar male, which is a novel social stimulus.

2.3. Habituation phase prior to experiment:

Per session, we captured four female and four male doves in the single-sex aviaries. We placed them individually in cardboard boxes for transport. Then, we randomly paired them in cages separated by gridded panels, so the pairs could not physically interact with each other. Essentially, they could only see, hear, and smell each other to get used to one another, preventing them from copulating. While all the assigned male-female "pairs" could see their partner, the different pairs were visually separated from each other with cloth covers over the cages. They could only hear each other to reduce stress, because knowing they are not alone helps decrease anxiety. All individuals were provided with food and water. Further, no nesting material was added to avoid nesting behavior, which might initiate pair formation, since we want to test them at the early courtship phase, before having sexual contact. Additionally, the birds were fed, and the cages cleaned daily at the same time. The pairs were kept in those cages for two consecutive days to give them enough time to habituate to one another and create a bond.

2.4. Intranasal administration of OT

The OT solution, with a concentration of 10,000 pg/mL, and the saline solution, with a concentration of 0.9%, were prepared in advance, along with the aliquots used for the experiment involving these solutions. The aliquots for the control group contained 1 mL of saline solution, while those for the experimental group contained 1 mL of OT solution. Before being placed in the preference test setup, females were either administered OT or saline solution using a nebulizer spray.

2.5. Partner preference test setup:

To setup the preference test, the large female cage was located between two smaller cages housing the males (see Figures 3 and 4). Each cage was provided with water and food allowing the animals to feed if needed, for their well-being. In the female's cage, there were three perches: one in the middle, one on the left side, and one on the right side. The perches were positioned to facilitate easy observation of the female's voluntary movements toward one of the males. The males however were kept in smaller cages than the females and had only one perch in their cages. Following each test, the cages were cleaned and tidied.

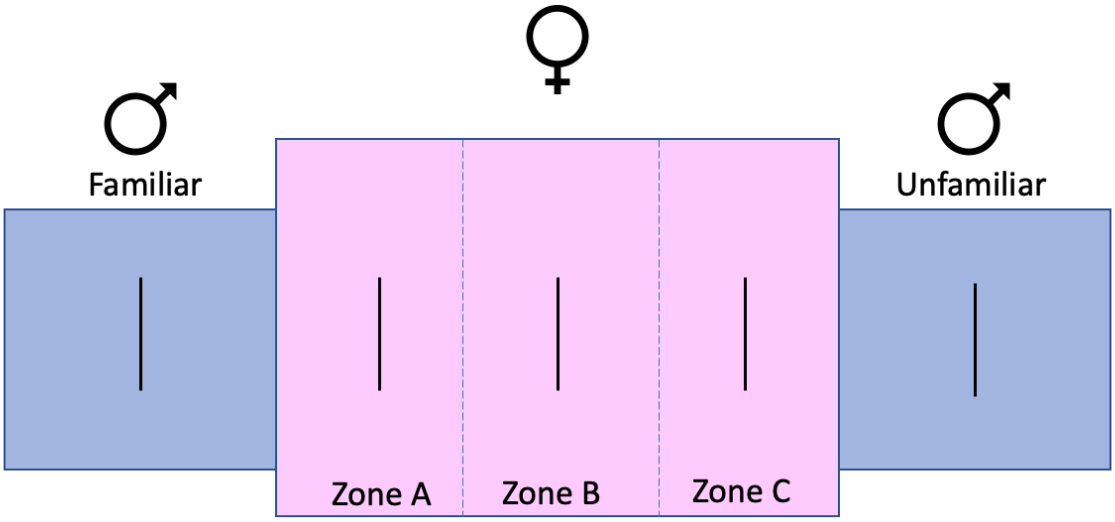

Figure 3: Representation of the partner preference test setup, viewed from above. The pink rectangle represents the female cage with her three perches (vertical black lines), and the blue ones represents the cages of the males with single perches. Note that the location of the familiar and unfamiliar male was randomized across pairs. The dotted blue lines in the females' cage represent the boundaries of the different hypothetical zones (Zone A on the left, Zone B in the middle, and Zone C on the right) used to score female location since the female was able to move freely around the cage.

2.6. Partner preference test:

The cage position of the familiar male and the unfamiliar one was changed across pairs, as this could create bias in the experiment. After having placed every individual in their assigned cages, we turned on the camera and left the experimental room to go to the observer room. We made sure to remain quiet and avoid abrupt movements during this process. We waited fifteen minutes in the observer room to give the animals time to habituate to their new cages (baseline phase). Once the time was up, we returned to the experimental room and removed the separators between the cages so the animals could see each other. After removing the separators, we went back to the observer room and waited an additional thirty minutes (experimental phase). When this was done, we returned to the experimental room and replaced the separators. We turned off the camera to stop recording, then placed the animals in separate carton boxes to return them to their habituation cages. Next, we cleaned and tidied the experimental cages until they looked as they did at the start of the experiment. Finally, we downloaded the data, renamed the files, and saved them. We repeated this method for each pair during 4 different sessions and per session we tested 4 pairs/morning. Additionally, we made sure that the different sessions happened at the same time of day, in the morning around 9 am -12 am in order to place them back in their aviary in the afternoon. The first session occurred in February 2023 and the other ones in June 2023. All the tests were performed by Virginie Canoine and Saverio Lubrano.

Figure 4: Representation of the partner preference test and the hypothetical zone borders (blue vertical lines) of the female cage.

2.7. Video and Data analysis:

2.7.1. Renaming of the files:

Videos were recorded with a GoPro camera in full HD resolution (1920 x 1080 px) at 30 fps. Once the data was collected from the recording setup, I had to rename the videos. To do this, I made sure that each filename included the experiment code (in our case: mT_Behav_exp), the ID of each involved animal (with the order of their IDs indicating their spatial arrangement in the cages: male_female_male), the session date (YYYY/MM/DD), and the file part number (1, 2, 3, 4, or 5) as the camera saves long videos into smaller segments. This was necessary to improve workflow and keep the main information in the filenames.

2.7.2. Scoring in Loopy:

To process the data from video recordings, I used *Loopy-video tracking and coding* software from Loopbio (http://loopb.io, Loopbio GmbH, Vienna, AUSTRIA). I uploaded the various files obtained from our experiments into Loopy. Afterward, I created my behavioral coding, within the software, to score behaviors using Loopy's coding tool. For this, I defined different behaviors related to actions of interest relevant to our study. The ethogram in Table 1 shows all analysed scored behaviors (see Appendix for full ethogram). Finally, I started the behavioral scoring of the videos. Each video was viewed twice, once focusing on the female and once on the calls (note that calls are not included in data analysis). The idea of the experiment was also to score the behavior of the males, but the video quality did not allow me to do so- the males were difficult, sometimes even impossible to see in their smaller cages, so I de-

cided to leave them out (see Figure 4). Once this was done with all my video data, I downloaded the output data as a CSV format. Note that scoring was done while blinded to the treatment group each female was in and the familiar/unfamiliar status of each male.

2.7.3. Ethogram:

Table 1: Ethogram used for the scoring of the females in Loopy, full ethogram in the appendix

Subject	Behavior	Definition
Female	Preening (Duration)	If the beak is clearly visible, the start of the behavior is when the beak touches the 'preening zone' for the first time. The end of a preening behavior is when the dove lifts her head and stays heads-up for a minimum of 3 sec; then search for the frame where her beak touches her feathers for the last time (=stop frame). Nevertheless, in some cases the beak is not clearly visible. Use the head and the feathers movement as a cue. Watch how the head moves down, while the feathers start shaking. Then search for an appropriate frame where you are certain that the dove is preening and use this as start frame for the behavior.
Female	Visibility (Duration)	A dove is coded as *visible* if you can clearly see her head. This variable serves as an indicator for the head direction - if the dove is not *visible*, you will not be able to code the *head/beak direction* behavior.
Female	Beak_direction_R (Duration)	This behavior can only be coded if the head is clearly visible. When the dove is directly looking into the camera, the head direction is neutral, and you must imagine a hypothetical neutral line. During the video analysis you can search for the frame where the dove is directly looking at the camera; search on the following frames for the one where you can see the first deviation from the neutral line to start the coding of the behavior. Any deviation to the right of this ' hypothetical neutral line' will be coded as beak_direction_R.
Female	Beak_direction_L (Duration)	This behavior can only be coded if the head is clearly visible. When the dove is directly looking into the camera, the head direction is neutral, and you must imagine a hypothetical neutral line. During the video analysis you can search for the frame where the dove is directly looking at the camera; search on the following frames for the frame where you can see the first deviation from the neutral line to start the coding of the behavior. Any deviation to the left of this 'hypothetical neutral line' will be coded as beak_direction_L.
Female	Location_A (Duration)	Indicates that the female is located in zone A (see Figure 1). The behavior starts on a frame when more than half of the dove's body is in zone A and ends on a frame when less than half of her

		body is in zone A.
Female	Location_B (Duration)	Indicates that the female is located in zone B (see Figure 1). The behavior starts on a frame when more than half of the dove's body is in zone B and ends on a frame when less than half of her body is in zone B.
Female	Location_C (Duration)	Indicates that the female is located in zone C (seeFigure 1). The behavior starts on a frame when more than half of the dove's body is in zone C and ends on a frame when less than half of her body is in zone C.
Female	Body_posture_R (Duration)	Use the feet of the doves as a cue. The behavior *body_posture_R i*s coded whenever the dove's 3 forward toes are pointing towards the right. Search for the frame where you can clearly see the forward toes pointing towards the right and start coding. In case doves switch their body posture, the end of the body_posture behavior will happen when the forward toes are clearly pointing in the other direction and at this moment start the opposite body_posture behavior. If you cannot see the feet, use the longitudinal body gradient as a cue.
Female	Body_posture_L (Duration)	Same methodology as Body_posture_L except the direction is changing.
Female	Feeding (Duration)	In the female cage, there are two feeding areas - one for water and one for food. To code this behavior, wait until you are certain that the dove is feeding. Then go back with the frames and search for the first moment when you see the dove's head going down towards the feeding areas. Once you have found the appropriate frame, start coding. The end of this behavior is the first time the dove lifts her head up and stays head up for a minimum of 3sec after a feeding interval.

2.7.4. Data wrangling:

Data analysis programs were written with assistance of Dr. Cliodhna Quigley. To process the output data from the scoring, I needed to convert it into a suitable format for data analysis. Data wrangling was performed in R Studio version 2025.05.1+513 (R Core Team, 2025) using the *dplyr* (Wickham et al., 2023) and *tidyr* (Wickham et al., 2024) packages. The experiment was split into two phases: baseline and experiment (see Figure 5). The baseline phase was defined as the 15 minutes before the removal of the first visual separator (screen). The experimental phase was defined as 30 minutes from the time the experimenter left the room. This ensured consistent phase durations across all scored videos.

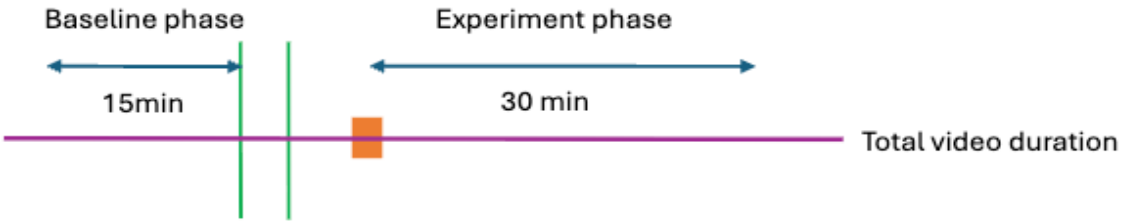

Figure 5: Schematic representation of the different phases of the experiment. The total video duration is divided into two distinct phases: a baseline phase (15 minutes) and an experiment phase (30 minutes). Green vertical lines indicate the moment of screen removals, while the orange marker represents the moment when the experimenter left the room. Scored behaviors occurring outside of these phases are not included in data analysis.

Additionally, I corrected the time alignment across different video segments obtained from the experiment data by adjusting the start_s and stop_s times, so multiple video chunks aligned into a continuous timeline. This was necessary because the original videos were cut into five smaller pieces by the camera. Then, I added information about the status of each male (familiar / unfamiliar) and treatment group of the females (OT / saline). This must be done, since I did the behavioral scoring blindly, to avoid experimenter bias. Female behaviors were summarized by experiment phase, yielding individual measures for preening (time and bouts), feeding (time and bouts), head direction (left or right), locations (zones A, B, and C), body posture (left or right), and visibility (time visible). Furthermore, I calculated behavior proportions relative to phase duration and preference indices based on partners' positions, meaning the data was no longer split into left and right but into familiar and unfamiliar. I also computed indices for time spent in the neutral zone (B), which was located in the center of the female cage, away from either male, and movement rate (the number of transitions between zones). See Table 2 for details.

Table 2: Table representing the main measures used for the statistical analysis. The left column represents the name of the different indicators, and the right column represents the formulas used to calculate the different indicators.

Name of the indicators	**Formules**
Index_location_partner	= Time in zone next to partner / (Time in zone next to partner + Time in zone next to unfamiliar)
Index_body_posture_partner	= Time body postured toward partner / (Time body postured toward partner + Time body postured toward unfamiliar)
Index_beak_direction_partner	= Time beak directed toward partner / (Time beak directed toward partner + Time beak directed toward unfamiliar)

Index_neutral_location	=Time in zone B / (Time in zone A + Time in zone B + Time in zone C)
Deplacement_rate	= Zone A counts + Zone B counts + Zone C counts / phase duration in sec
Proportion_preening	= Preening time in sec / phase duration in sec
Proportion_feeding	= Feeding time in sec / phase duration in sec

2.8. Statistical analysis:

For the statistical analysis, I used different statistical tests. First, I employed two-way mixed ANOVAs , using the *car* (Fox and Weisberg, 2019*)* and *ez* (Lawrence, 2016) packages in R studio version 2025.05.1+513 (R Core Team, 2025) because the data in this study come from a mixed design—each bird has repeated measures (the within-subject factors) but also belongs to different treatment groups (the between-subject factor). This test reveals the effects of treatment (OT / saline), the effect of phase (baseline / experiment), and the interaction between these effects. However, there are assumptions for this test, including the normality of the dependent variable, the homogeneity of variances, sphericity, and the independence of observations. For the last two, no checks were needed since the sphericity assumption is met, as our within-subject factors only had two levels, and independence is ensured because subjects were randomly assigned to different treatment groups. Normality was tested using the Shapiro-Wilk test, and homogeneity was assessed with Levene's test provided by *car* package. If these assumptions were violated, I used a nonparametric longitudinal factorial test using the *nparLD* (Noguchi et al., 2012) package instead of the two-way mixed ANOVA. Additionally, a Welch two-sample t-test, using *rstatix* package (Kassambara, 2023) was performed when sample sizes are unequal (or Wilcoxon rank-sum tests, provided by *rstatix*, if normality is not met), compared treatment groups on key indices. All the plots, illustrating the results, were created in R Studio by using the packages ggplot2 (Wickham, 2016).

2.9. Ethical approval:

The present study was approved (approval ID: No. 2023-008), by the Animal Ethics and Experimentation Board at the Faculty of Sciences, University of Vienna, on 03/15/2023 in accordance with the Austrian national legislation on animal experimentation and the University of Vienna's institutional guidelines. Every effort was made to reduce animal stress and ensure welfare.

3.RESULTS:

Note that there are 7 females in the control saline group and 8 in the OT group.

3.1. Index location next to partner:

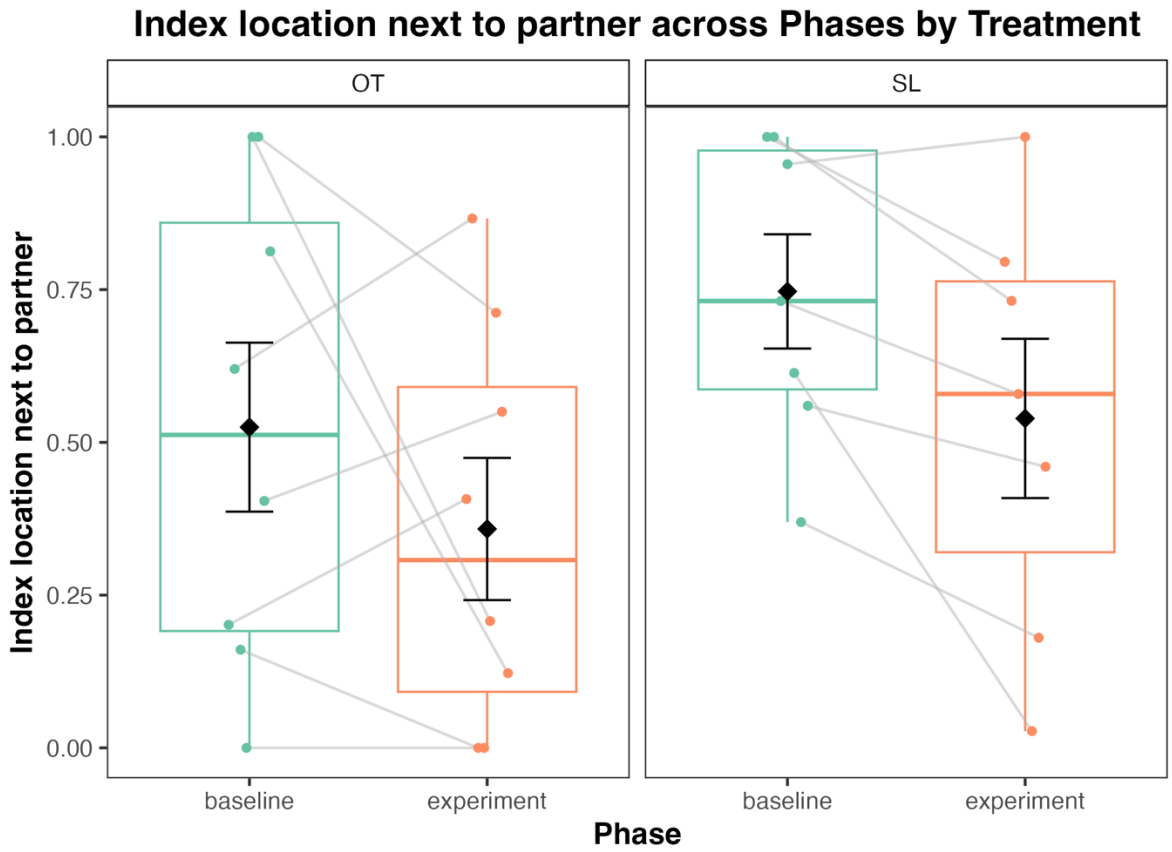

Figure 6: Illustration of the location next to partner index across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The index location next to the partner shows the proportion of time the female spends in the area next to her partner compared to the total time spent near either her partner or unfamiliar male. Values above 0.5 indicate more time near the familiar male, while values below 0.5 indicate more time near unfamiliar male.
A two-way mixed ANOVA was performed to assess the effects of treatment and phase on the time females spent near the familiar male. There was no significant effect of treatment, $F(1,12) = 0.90$, $p = 0.362$. However, there was a significant effect of phase, $F(1,12) = 5.09$, p

= 0.044, demonstrating that the females spend less time near the familiar partner in experiment than baseline. Lastly, there was no significant interaction between treatment and phase, F (1, 12) = 0.01, p = 0.923. A complementary analyse confirmed no significant effect of the treatment: a welch two sample t-test showed no treatment effect in the experimental phase (p=0.320)

3.2. Index neutral location:

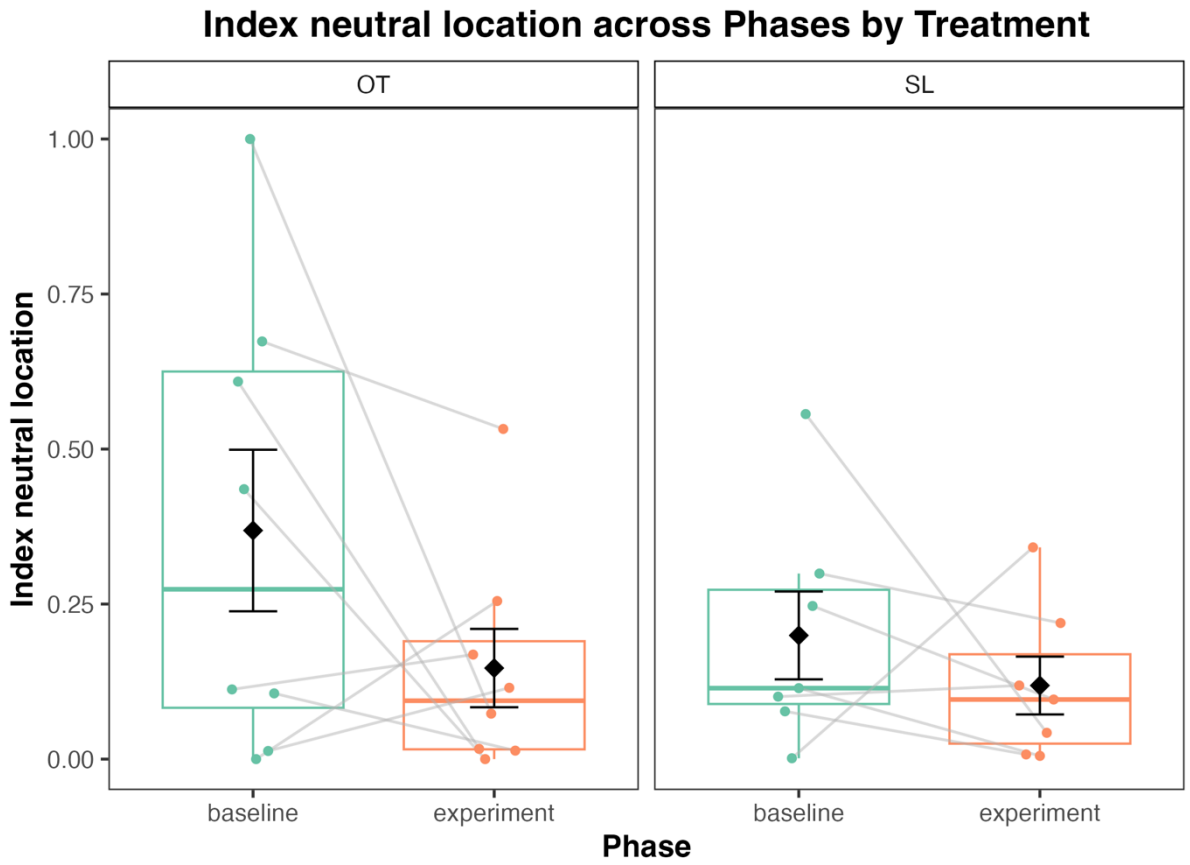

Figure 7: Illustration of the neutral location index across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The index neutral location indicates the proportion of time spent in the neutral zone, namely zone B, relative to the total time that the female was scored, i.e. time spent in zones A, B, and C. If the value is less than 0.5, the female spends more time next to one of the males than in the neutral zone, and if it is greater than 0.5, she spends more time in the neutral zone than next to the males. As shown in the figure, the females do not spend much time in the neutral

zone. Since the index value is well below 0,5, it indicates that females spend more time near other individuals than in the neutral zone throughout the entire experiment.
Statistical analyses confirmed these observations. A Welch ANOVA test was performed to investigate the effect of treatment and phase on the time spent in the neutral zone (zone B). There was no significant effect of treatment (p=0.266), phase (p=0.086), or their interaction on the index of neutral location, F (3, 14.1) = 1.13, p = 0.372. However, there is a phase trend suggesting a decrease in time spent by the female in neutral zone during experimental phase compared to baseline phase. Similarly, a Wilcoxon test revealed no significant treatment effect during the experimental phase (p=0.954).

3.3. Deplacement rate:

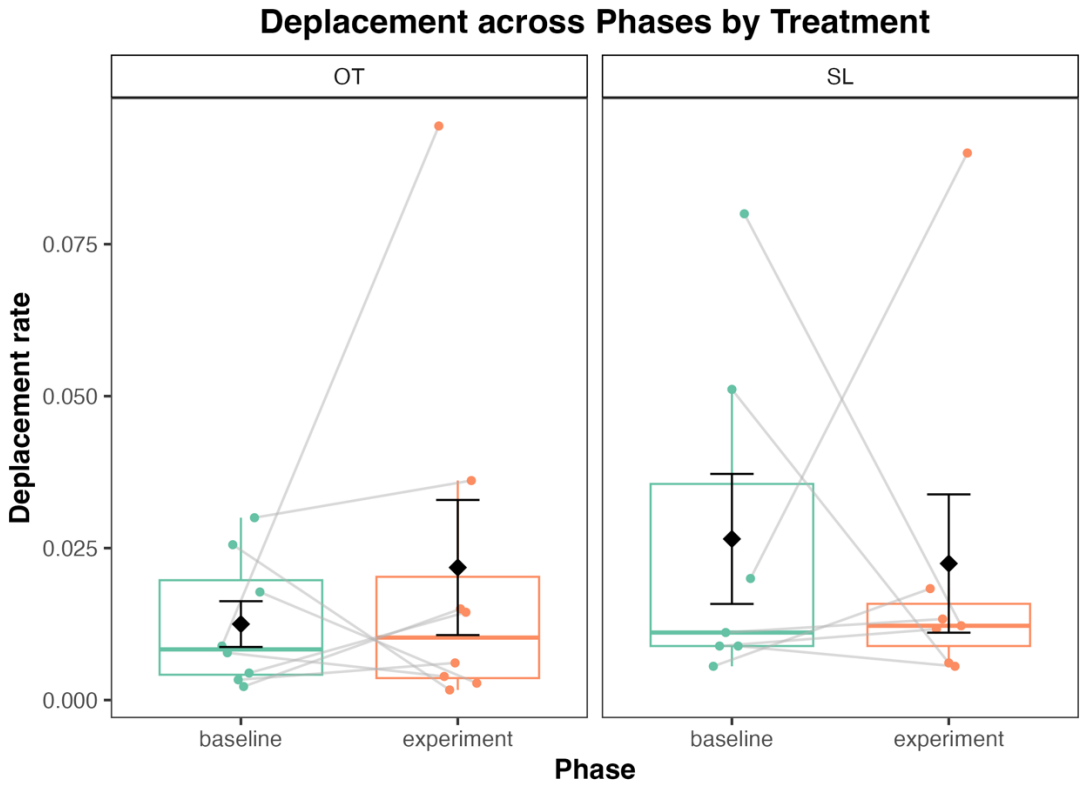

Figure 8: Illustration of the deplacement rate between zones across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

Deplacement rate is calculated by dividing the total number of times the female entered a zone (i.e. includes all zones, A, B, and C) by the phase duration in seconds. A higher value indicates more frequent movement between zones.

The value of the deplacement rate is low (<0.025 per second), indicating that, overall, the females did not frequently switch between zones in their cages.
A nonparametric longitudinal factorial test was performed to investigate the potential effects of treatment and phase on the deplacement rate. There is no evidence of a significant effect of treatment, WTS = 1.63, p = 0.20, indicating that the treatment does not affect the female's deplacement rate. Similarly, there was no effect of the phase, WTS = 6.77e-06, p = 0.997. Finally, there was no significant interaction between treatment and phase, WTS = 0.064, p = 0.80. Complementary, a Welch two sample t-test was conducted to investigate an effect of the treatment on the deplacement rate during the experiment phase. There was no significant effect of the treatment, p = 0.6854.

3.4. Index body posture directed toward partner:

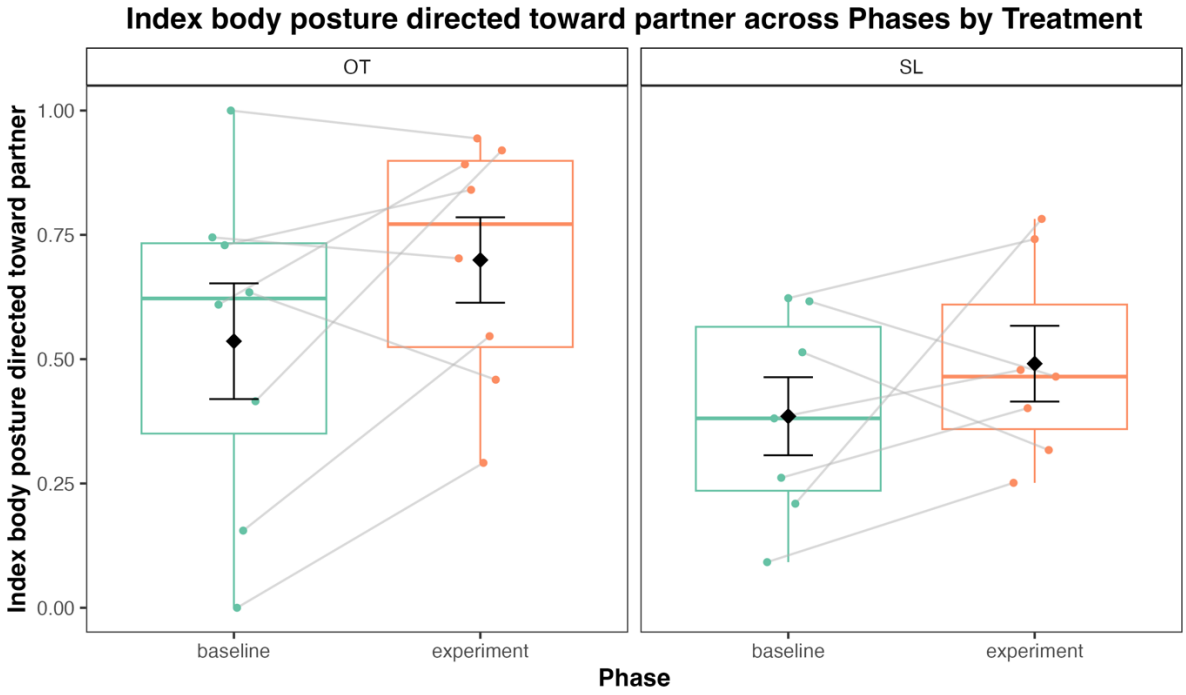

Figure 9: Illustration of the body posture directed towards partner index across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The index of body posture directed toward the partner is calculated by dividing the time the female's body is oriented toward her partner by the total time her body is directed either toward her partner or the unfamiliar male. If the value is less than 0.5, the female spends more time with her body facing the unfamiliar male, and if the value is over 0.5 the female spends more time with her body facing the partner compared to the unfamiliar male.

A two-way mixed ANOVA revealed no significant effect of treatment, $F(1,13) = 2.45$, $p = 0.142$, and no significant treatment x phase interaction, $F(1,13) = 0.20$, $p = 0.659$. The effect of phase, however, approached significance, $F(1,13) = 4.46$, $p = 0.055$, suggesting a possible trend toward increased body orientation toward the familiar male during the experimental phase as seen in figure 9. A complementary analyse supported this trend: a Welch two-sample t-test, conducted to examine the effect of the treatment on the body posture toward partner index, indicated a trend toward an increase in the time facing a familiar male ($p = 0.09264$).

3.5. Beak directed toward partner index:

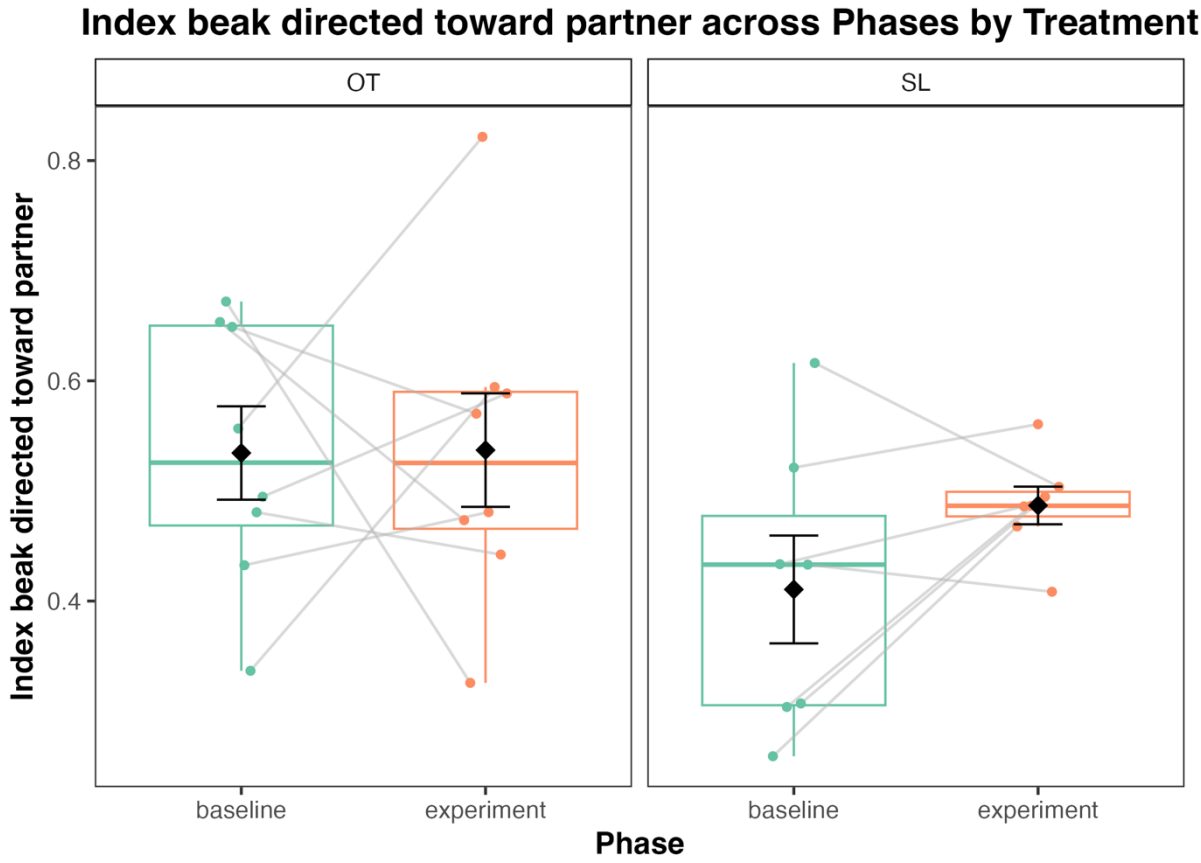

Figure 10: Illustration of the beak directed toward partner index across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The index of beak directed toward the partner is calculated by dividing the time the female's beak is oriented toward her partner by the total time her beak is directed either toward her partner or the unfamiliar male. If the value is less than 0.5, the female spend more time with

her beak facing the unfamiliar male, and if the value is over 0.5 the female spend more time with her beak facing the partner compared to the unfamiliar male.
A two way mixed ANOVA test was performed to investigate the effect of treatment and phase on the beak directed toward partner index. The impact of treatment approached significance, F (1, 13) = 4.49, p = 0.054, indicating a possible trend to more frequent beak directed toward partner index for OT than control females. There is no significant effect of the phase, F (1, 13) = 0.76, p = 0.399 and no significant interaction of treatment x phase, F (1, 13) = 066, p = 0.430. A complementary test confirmed these findings: a Welch two sample t-test indicated no significant differences between treatments in the beak directed toward partner index during the experiment phase, p = 0.380.

3.6. Proportion preening:

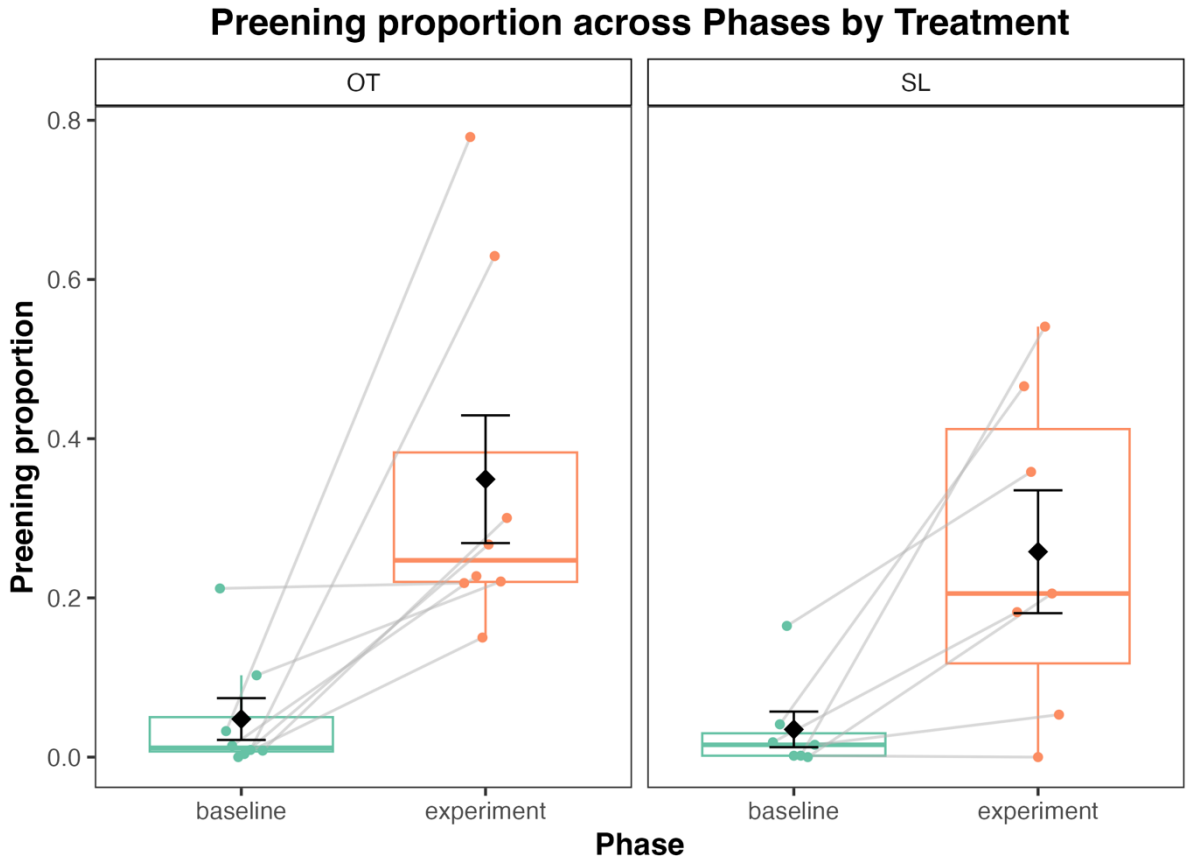

Figure 11: Illustration of the proportion of preening across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The preening proportion indicates the time, in seconds, that females spend preening divided by the phase duration in seconds. A high value shows that females preened more during the experiment; however, since the values are below 0.5, it means the females spend more time not preening than preening.
A nonparametric longitudinal factorial test was performed to examine the potential effect of treatment and phase on the proportion of preening in females. The results showed no effect of the treatment, $\chi^2(1) = 1.06$, $p = 0.303$, indicating that the treatment does not influence the amount of time females spend preening. There was a significant effect of the phase, $\chi^2(1) = 37.17$, $p < 0.001$, suggesting a strong impact of the phase: females preened more frequently during the experimental phase compared to the baseline phase, where the proportion of preening was nearly zero. Lastly, there was no significant interaction between treatment and phase, $\chi^2(1) = 0.82$, $p = 0.366$. A complementary Welch two sample t-test was conducted to investigate an effect of the treatment on the proportion of preening during the experiment phase. There were no significant differences between treatments in the proportion of preening during the experiment phase, $p = 0.3854$.

3.7. Proportion feeding:

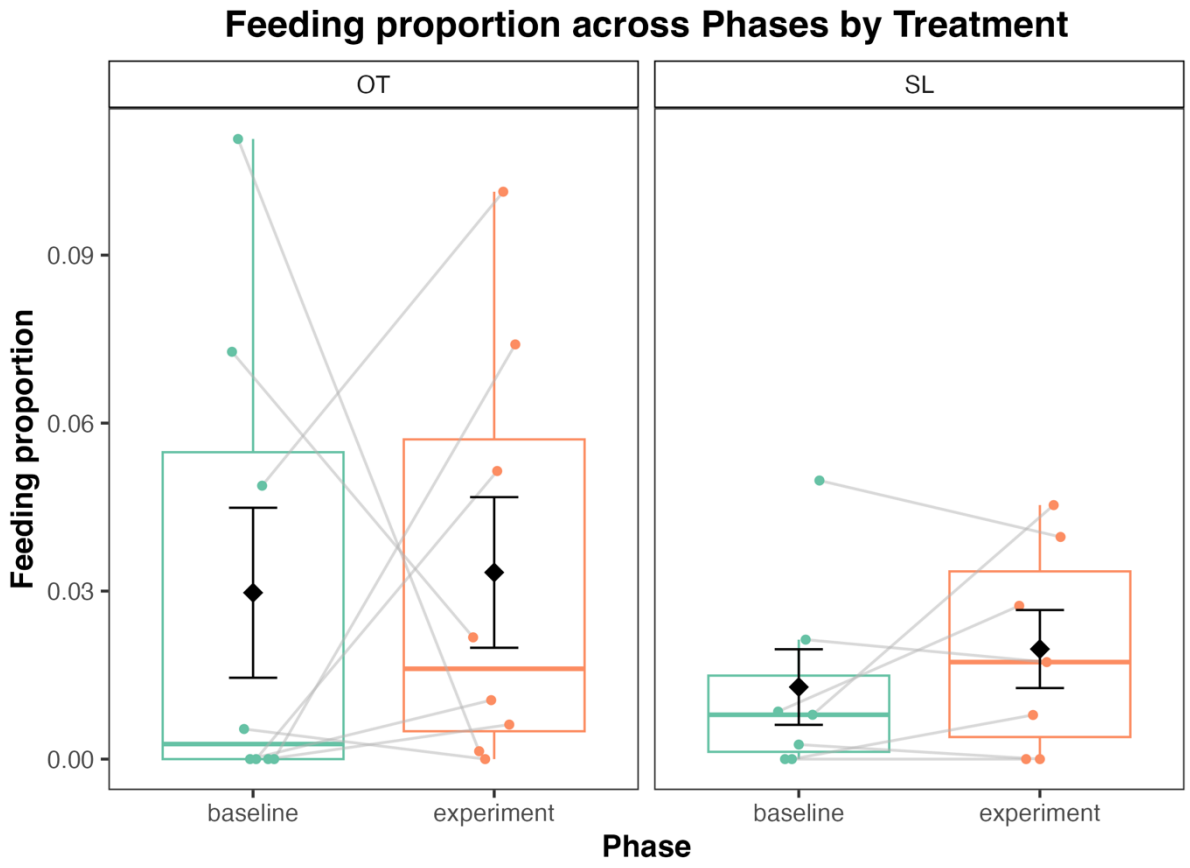

Figure 12: Illustration of the proportion of feeding/drinking across phases (baseline versus experiment) by treatment (Oxytocin versus Saline). Boxplots (green and orange) show medians (horizontal line), interquartile ranges (boxes) and whiskers showing the overall

range. Lines connect individual data points to represent repeated measures from the same subject over the two different phases. Black diamonds with error bar represent group means ± standard error (SE).

The feeding proportion represents the time, in seconds, the females spend feeding or drinking divided by the phase duration in seconds. The feeding proportion values are low, indicating that the females spend almost no time feeding or drinking during the experiment.
A nonparametric longitudinal factorial test was conducted to investigate a possible effect of treatment and phase in the proportion of feeding of the female. There was no effect of the treatment, $\chi^2(1) = 0.24$, $p = 0.622$ and no effect of the phase, $\chi^2(1) = 1.15$, $p = 0.284$. Finally, there was no significant interaction of between treatment and phase, $\chi^2(1) = 0.22$, $p = 0.640$. Complementary, a Welch two sample t-test was conducted to investigate an effect of the treatment on the proportion of feeding during the experiment phase. There were no significant differences between treatments in the proportion of feeding/drinking during the experiment phase, $p = 0.3871$.

3.8. Summary Tables

Table 3: Summary of the results from the ANOVA-type tests. Red indicates no significance, green and stars indicate significant differences, while blue shows trends.

Variable	Test type	Treatment effect (p)	Phase effect (p)	Interaction effect (p)
Index location partner	Two way mixed ANOVA	0.362	0.044***	0.923
Index beak direction partner	Two way mixed ANOVA	0.054 trend	0.399	0.430
Index body posture partner	Two way mixed ANOVA	0.142	0.055 trend	0.659
Index neutral location	Welch ANOVA	0.266	0.086	0.372
Deplacement rate	nparLD	0.442	0.796	0.515
Proportion of preening	nparLD	0.303	<0.001***	0.366
Proportion of feeding	nparLD	0.622	0.284	0.640

Table 4: Summary table of the results of the performed two-sample tests. Red indicates no significance and blue show trends.

Variable	Groups compared during experiment phase	Test type	p-value
Index location partner	MT vs SL	Welch two sample t-test	0.320
Index beak direction partner	MT vs SL	Welch two sample t-test	0.380

Index body posture partner	MT vs SL	Welch two sample t-test	0.092 trend
Index neutral location	MT vs SL	Wilcoxon rank-sum test	0.954
Deplacement rate	MT vs SL	Welch two sample t-test	0.685
Proportion of preening	MT vs SL	Welch two sample t-test	0.385
Proportion of feeding	MT vs SL	Welch two sample t-test	0.387

4.DISCUSSION:

The present study aimed to determine whether intranasal oxytocin administration influences early mate preference in female ring doves (*Streptopelia risoria*). Specifically, I examined whether females preferred a familiar male over an unfamiliar one during the initial stages of courtship (before mating) and whether oxytocin treatment would increase this preference. Against our expectations, females did not spend more time near the familiar male compared to the unfamiliar one. Contrary to my predictions, the results also did not suggest that OT affects mate preference at this very early stage of courtship. Overall, intranasal OT-administration did not increase the bias toward familiar males, as shown by various statistical tests across several behavioral indices.

Although no treatment effects were observed across most indices, phase effects emerged, indicating a significant difference between the two phases of our experiment: the baseline and experimental phases. For the location next to partner index, females spent more time near familiar males during the experimental phase compared to the baseline. Additionally, a significant phase effect was found for the proportion of preening, indicating more preening time during the experimental phase. Concerning the beak directed toward partner and body posture directed toward partner indices, no significant treatment or phase effects were detected; however, trends suggested a possible subtle OT-mediated influence. In fact, females in the OT group tended to spend more time facing their bodies and beak toward familiar males rather than unfamiliar ones, as shown by the two-way mixed ANOVA. There was also a trend of a phase effect for the body posture index, as revealed by the two-way mixed ANOVA. No significant results were found for the neutral location index, the displacement rate, or the feeding proportion.

The initial hypothesis predicted that females would prefer familiar males over unfamiliar ones and that OT administration would amplify this bias by increasing the preference. However, I did not observe a preference for the familiar male in either the control or oxytocin-treated group. These results suggest that, at least during the very early stages of courtship, OT does not influence female mate choice in ring doves. However, there are some methodologi-

cal issues influencing the present study, which will be discussed later. Nonetheless, the significant phase effect on the proportion of preening makes sense because preening is considered a social behavior in ring doves, and preening was more frequent during the experimental phase than the baseline phase, with mutual visibility between the birds being the only difference between these phases. This makes sense because preening functions as a social activity, encouraging bonding and communication among the doves during periods of mutual visibility.

Our findings are surprising because there is substantial evidence in the scientific literature linking OT with partner preference and familiarity across various taxa, including mammals and birds (Duque et al., 2018; Beery et al., 2018). In fact, prairie voles, which are socially monogamous, spend more time near their partner than near strangers (Williams et al.1992; Insel and Houlihan, 1995; Cho et al., 1999), and they also spend more time near familiar conspecifics than unfamiliar ones (Beery et al., 2018; DeVries et al., 1997; Lee et al., 2019), suggesting that familiarity plays a key role in social preferences in this species. The impact of familiarity in partner preference tests has also been observed in California mice, a truly socially monogamous species, where females show a clear preference for their partners by spending more time in their partner's cage compared to that of unfamiliar males (Kowalczyk et al., 2018). However, avian species also demonstrate familiarity-based partner preference. For instance, pinyon jays (*Gymnorhinus cynocephalus*) preferentially deliver food to familiar conspecifics rather than unfamiliar ones, and this behavior is enhanced after high oxytocin administration, indicating that OT influences prosociality when administrated in sufficient dose (Duque et al., 2018).

Next, I will discuss the methodological limitations that could explain the absence of significant OT effects in our results. First, the concentration of OT in the doses administered to the treatment group. In fact, the intranasal doses delivered through nebulizer spray in our study were much lower than those used in other publications with the same intranasal administration technique in birds (Duque et al., 2018; Duque et al., 2020), as we used 1 ml of oxytocin solution with a concentration of 10000 pg/ml (=10ng/ml). Duque and his colleagues (2018) used OT doses which are much higher (0.3 and 0.15 mg/ml) compared to the ones we used, and a decline in food-sharing ability, a prosocial behavior, was observed with lower OT doses (Duque et al., 2018). Contrarily, a too high dose of OT can lead to a decrease in activity, maybe due to receptor saturation in the system. We used a lower dose of OT because we wanted to be closer to the physiological level, as circulating peripheral OT concentrations have been quantified in Siberian jays (*Perisoreus infaustus*) (Lubrano and Canoine, non published) and ravens (*Corvus corax*) (Stocker et al., 2022). This prevented having side effects due to extreme concentration.

Second, the timing of intranasal administration is crucial. Our timing and dosage might not have matched the doves' neuroendocrine dynamics, but there are no other studies using this method on doves for comparison. We may have missed the optimal time window for behavioral effects after intranasal OT administration, as we administered OT right before the preference test. Additionally, in ravens, for example, peripheral OT levels peak during a specific period, namely the breeding season (Stocker et al., 2022). By extending the experiment due to diseases, we might have missed the reproductive window, which could have affected our results.

Third, the paired individuals have not yet completed a full courtship cycle, as they were unable to have physical contact during the two days of the habituation phase. This was done to test whether OT has an effect on mate preference during early courtship before mating. By doing this, we interfered with the classical courtship display by removing the physical stimulus, possibly interrupting the hormonal changes triggered by this stimulus, which led to subsequent behavioral changes (Lehrman, 1964; Lehrman, 1965). Nonetheless, we did not find any results confirming our hypothesis, which may suggest that during the initial stage of courtship, before mating, OT might not have a significant role. The neural links, implicated in bond formation and further in pair bonding, may not have been established due to the lack of a fully completed courtship. Another explanation could be that the habituation phase was not long enough to establish a strong bond between the two individuals in each habituation cage, possibly indicating that a longer bonding period is necessary for future studies, since we know that courtship displays can last several days (Lehrman, 1964, Lehrman, 1965, Silver et al., 1973).

Fourth, set up constraints made it challenging to observe and score the behaviors of the individuals in detail. One of the goals of this study was also to examine male behaviors because we know that one individual's behavior influences another's (Lehrman, 1964; Goodson, 2013); it is a dynamic relationship. However, this was not possible since the males were not fully visible throughout the entire video due to the cage design. In fact, the bottom part was not transparent (see Figure 4), making it impossible for me to see the male doves clearly when they were on the floor and not on their perch. Next, the room's lighting was also suboptimal, making it challenging to observe the birds, especially the males but also the females, when they were deep inside their cages. Next, the camera placement could have been improved, as it was hard to observe the animals clearly from that perspective (see Figure 4). Positioning the camera on top of the central cage with a fisheye lens to capture more detail could be an improvement for future studies. Another setup limitation was that the tested animals were not alone in the experiment room. In fact, all the other individuals tested in the same session were also present, making it impossible for me to determine which tested individual was producing calls or even whether it was one of the tested animals calling. Finally, the location of the feeding point in the female's cage was not optimal, as it was placed precisely at the boundary of zones B and C, possibly creating a bias in the location index of the female. Perhaps the feed point was unnecessary for the animals ' well-being at all since they all spent around 45 minutes in the partner preference test.

Fifth, the duration of the partner preference test may have been too short in our study. Most partner preference tests conducted in mammals and birds, especially voles and mice, last longer than our 30 minutes. In fact, the partner preference test duration in king quails is over an hour (Adkins-Regan, 2016), and in prairie voles, it can last even multiple hours (DeVries et al., 1997; Keebaugh and Young, 2011). Thirty minutes may not be sufficient to observe a preference for a partner, as the tested individuals could be exploring the cage and their neighbors during this short period. However, in a study performed with common waxbills (*Estrilda astrild*), the partner preference test lasted 15 minutes, with significant results (Trigo et al., 2023).

Sixth, the sample size could have influenced our results. In fact, we had seven female individuals in the control group and eight in the oxytocin-treated group, in total fifteen female indi-

viduals. Statistical underpower often results from a sample size that is too small and high variability in the data (Walum et al., 2016; Halpern et al., 2002). In our study, small sample size is definitely a factor, and some scored behaviors show high variability, such as preening and feeding times of the females. However, using a small sample size is normal for a pilot study. The underpowered nature of our research implies that the experiment might produce non-significant results, even if a biologically meaningful effect exists, because the study has a low probability of detecting a real effect.

Finally, the various behavioral parameters used to quantify partner preference, which were mainly spatial indices (Time near the partner, body posture, and beak orientation), may not fully capture avian partner preference. The spatial indices are commonly used indices to quantify the preference of individuals in rodents, as different studies have demonstrated it (Keebaugh and Young, 2011; DeVries et al., 1997). However, studies demonstrated that OT administration does not influence the proximity of paired birds (Duque et al., 2020), and the spatial proximity from dogs to their owner (Romero et al., 2014). In bird species, they used other indicators for partner preference as well, like allopreening, sharing food or huddling (Adkins-Regan, 2016, Duque et al., 2018). By not including these and not be able to score the males behavior, we might have missed subtle markers of bond formation during early courtship.

Nonetheless, other avian studies provide context for the results of the present study. For instance, a study in King Quail (*Coturnix chinensis*), a socially monogamous species, had mixed results: Untreated males did not statistically spend more time near familiar females than unfamiliar ones (Adkins-Regan, 2016). Nonetheless, males displayed aggressive behavior toward unfamiliar females, which was not observed with familiar females, demonstrating that there is a preference for a familiar individual without increased proximity toward familiar individual (Adkins-Regan, 2016). Furthermore, a study done on zebra finches, in which individuals were administered an OTA, showed a reduction in the time spent near familiar cagemates, highlighting that OT influences partner preference linked to familiarity in this species. However, OT administration in common waxbills led to a decrease in activity of the birds (Trigo et al., 2023). Additionally, OT administration tends to have a positive effect by increasing the time spent near familiar cagemates (Goodson et al., 2009). Taken together, these studies suggest that familiarity plays a crucial role in shaping social preferences in birds, although preference is not always expressed through spatial proximity, which is consistent with our own findings.

In conclusion, this study offers initial evidence that intranasal OT does not significantly affect female mate preferences during the early stages of courtship in ring doves. The lack of clear effects might be due to the timing of testing, the relatively low dose used, and the reliance on spatial measures instead of a broader array of other behaviors reflecting mate preference. Future research should incorporate higher and systematically varied oxytocin doses, extended habituation periods and lengthen the partner preference test to observe more consistent behavioral patterns. Methodological improvements such as better video and audio recording, optimized cage design, and more accurate behaviors for determining partner preference like allopreening, food sharing, huddling, and male displays will enable more detailed behavioral scoring in males and in females. Despite these limitations, the present study contributes to the understanding of the neuroendocrine mechanisms underlying social bonding in birds, especially in ring doves. The results suggest that oxytocin's role may emerge later in the courtship

process or may depend on specific contexts rather than on early mate preference alone. Addressing the methodological and conceptual challenges identified here will enable future studies to clarify how oxytocin influences the development and maintenance of pair bonds in avian species, as well as bridge the gap between mammalian and avian models of pair bonding.

Deutsche Zusammenfassung:
Das Ziel dieser Masterarbeit war, zu erforschen, wie intranasal verabreichtes Oxytocin (OT) die Partnerpräferenzen bei weiblichen Ringeltauben (*Streptopelia risoria*) beeinflusst. Es wurde erwartet, dass Weibchen vertraute Männchen gegenüber Unbekannten bevorzugen und dass diese Präferenz durch OT verstärkt wird. Das Experiment bestand aus einer zwei tägigen Gewöhnungsperiode zwischen einem Männchen und Weibchen, gefolgt von einem gefilmten Partnerpräferenztest zwischen vertrauenden und unvertrauten Männchen nach intranasaler Administrierung von OT durch das Weibchen. Die dadurch erlangten quantifizierten Parameter waren: Index für Position neben dem Partner, Index für neutrale Position, Bewegungsrate, Index für Körperhaltung zum Partner hin, Index für Schnabelausrichtung zum Partner, Fütterungsanteil und Gefiederpflegeanteil. Die Ergebnisse zeigten jedoch keine signifikanten Effekte: Weder in den meisten Verhaltensparametern noch in der Gesamtpräferenz konnte ein Einfluss von OT festgestellt werden. Lediglich Phaseneffekte, also Unterschiede zwischen experimentaler und Basisphase, traten auf, wie ein längerer Aufenthalt in der Nähe vertrauter Männchen sowie eine Zunahme des Gefiederpflegeverhaltens während der Experimentierphase. Die fehlenden Effekte lassen sich wahrscheinlich durch methodische Faktoren erklären, wie eine niedrige OT-Dosis, kurze Testdauer, unvollständige Brautwerbung und kleine Stichprobengröße. Dennoch liefern die Ergebnisse erste Hinweise, dass Oxytocin in der frühen Brautwerbung bei Ringeltauben keine klare Rolle spielt und erst in späteren Phasen der Paarbindung, möglicherweise nach der Paarung oder in bestimmten sozialen Situationen, relevant werden könnte. Damit trägt die Studie zum besseren Verständnis der neuroendokrinen Mechanismen sozialer Bindung bei Vögeln, insbesondere bei Ringeltauben, bei und gibt wichtige Anregungen für zukünftige Forschungen.

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Use of AI-Assisted Tools:

Throughout the writing process of this thesis, AI-based tools such as Grammarly were used to support language clarity, grammar correction, and sentence structure. Grammarly was primarily applied for proofreading and identifying minor grammatical errors. The content, analysis, and conclusions remain entirely original and were developed by the author. These tools were used solely to improve readability and linguistic accuracy, without influencing the interpretation of data or the research outcome.

6. APPENDIX:

	Behavior	Definition
Female	Preening (Duration)	If the beak is clearly visible, the start of the behavior is when the beak touches the 'preening zone' for the first time. The end of a preening behavior is when the dove lifts her head and stays heads-up for a minimum of 3 sec; then search for the frame where her beak touches her feathers for the last time (=stop frame). Nevertheless, in some cases the beak is not clearly visible. Use the head and the feathers movement as a cue. Watch how the head moves down, while the feathers start shaking. Then search for an appropriate frame where you are certain that the dove is preening and use this as start frame for the behavior.
Female	Visibility (Duration)	A dove is coded as *visible* if you can clearly see her head. This variable serves as an indicator for the head direction - if the dove is not *visible*, you will not be able to code the *head/beak direction* behavior.
Female	Beak_direction_R (Duration)	This behavior can only be coded if the head is clearly visible. When the dove is directly looking into the camera, the head direction is neutral, and you must imagine a hypothetical neutral line. During the video analysis you can search for the frame where the dove is directly looking at the camera; search on the following frames for the one where you can see the first deviation from the neutral line to start the coding of the behavior. Any deviation to the right of this ' hypothetical neutral line' will be coded as beak_direction_R.
Female	Beak_direction_L (Duration)	This behavior can only be coded if the head is clearly visible. When the dove is directly looking into the camera, the head direction is neutral, and you must imagine a hypothetical neutral line. During the video analysis you can search for the frame where the dove is directly looking at the camera; search on the following frames for the frame where you can see the first deviation from the neutral line to start the coding of the behavior. Any deviation to the left of this 'hypothetical neutral line' will be coded as beak_direction_L.
Female	Location_A (Duration)	Indicates that the female is located in zone A (see Figure 1). The behavior starts on a frame when more than half of the dove's body is in zone A and ends on a frame when less than half of her body is in zone A.
Female	Location_B (Duration)	Indicates that the female is located in zone B (see Figure 1). The behavior starts on a frame when more than half of the dove's body is in zone B and ends on a frame when less than half of her body is in zone B.

Female	Location_C (Duration)	Indicates that the female is located in zone C (seeFigure 1). The behavior starts on a frame when more than half of the dove's body is in zone C and ends on a frame when less than half of her body is in zone C.
Female	Body_posture_R (Duration)	Use the feet of the doves as a cue. The behavior *body_posture_R i*s coded whenever the dove's 3 forward toes are pointing towards the right. Search for the frame where you can clearly see the forward toes pointing towards the right and start coding. In case doves switch their body posture, the end of the body_posture behavior will happen when the forward toes are clearly pointing in the other direction and at this moment start the opposite body_posture behavior. If you cannot see the feet, use the longitudinal body gradient as a cue.
Female	Body_posture_L (Duration)	Same methodology as Body_posture_L except the direction is changing.
Female	Feeding (Duration)	In the female cage, there are two feeding areas - one for water and one for food. To code this behavior, wait until you are certain that the dove is feeding. Then go back with the frames and search for the first moment when you see the dove's head going down towards the feeding areas. Once you have found the appropriate frame, start coding. The end of this behavior is the first time the dove lifts her head up and stays head up for a minimum of 3sec after a feeding interval.
Unknown dove	Calls_coo (Event)	Unfortunately, the setup and the video quality did not allow me to be able to distinguish between the different sexual coos, that a dove can perform. For this reason, I decided to code the coos in general, without specific distinctions. The coo call sounds like this: "cooc-r-r-r-r-oooo". The calls_coo events are coded at the start of the second syllable, namely when you hear the 'r' for the first time during a call display. As the coo calls are typed as events, you don't need a stop frame for this behavior.
Unknown dove	Calls_laugh (Event)	This behavior refers to the sound made by the doves in various contexts, mainly when they are excited- but its meaning depends on the context and the dove's body language. It's a shorter sound than the coos and sounds like a human laugh (''hahaha''). This behavior will be coded at the moment you can hear the second syllable (same as for the coos).
Unknown dove	Calls_multiple_coos (Event)	This behavior refers to multiple coo calls being made simultaneously, by different individuals, as unfortunately there were other individuals in the experiment room. The coding of this behavior starts at the moment when you clearly can

		hear another coo being made before the first coo being heard stops. Search for the appropriate frame and use this one for the event frame.
Setup	Experimenter_leaving (Event)	To code this behavior, you must listen carefully. It will happen shortly after the screen removal. At some point you will hear the door closing and it's at this moment that you will have to press the button to code this 'behavior'. This behavior refers to the experimenter leaving the room – the doves are now alone in the room. It is important to code this, since we know that human presence can alter the behaviors of doves.
Setup	Final_frame (Event)	The final frame event is the very last frame of every video. It is important to have it for the reconstruction, since the original video was cut into 5 smaller pieces.
Setup	Screen_removal (Event)	The screens are placed in a small gap between the neighboring cages. The behavior *screen_removal* is coded when the screens are completely out of the gaps – search for the appropriate frame. Sometimes you will not be able to see this moment, because the experimenter will be blocking your view. In those cases, use the frame where you are certain that the animals are seeing each other for the first time.
Setup	Screen_removal_2 (Event)	Same as the behavior screen_removal. The only thing changing is that this one refers to the second screen, since there is one on each side.

R Script:

```
library(dplyr)
library(tidyr)
library(ggplot2)
library(afex)
library(ez)
library(car)
library(lme4)
library(lmerTest)
library(rstatix)
# define any constants
BL_DUR_S <- 15*60 # baseline duration is maximum 15 minutes (15 mins x 60 secs)
EXP_DUR_S <- 30*60 # experimental phase is maximum 30 minutes

expData <- read.csv('data/doves_experimentData_20250715.csv')

expData <- select(expData, ID, sex, partner, unfamiliar, treatment, admin_time, video_start)
# only interested in females:
expData <- filter(expData, sex=='f')
expData <- select(expData,-sex)
expData <- rename(expData, fNr=ID)

data <- read.csv('data/ethogram_95_OT_Dove_matepreference_ethogram_V2_28072025.csv')

# take info from filename
```

```
data <- separate(data,Scoring, into = c(NA,NA,NA,"mLeft","fNr","mRight","date","vidNr"), sep = "_", remove =
FALSE)
data$fNr  <- as.numeric(data$fNr)
data$mLeft  <- as.numeric(data$mLeft)
data$mRight  <- as.numeric(data$mRight)
data$vidNr <- as.numeric(substring(data$vidNr,1,1))

# reconstruct into a string to compare across video parts
data$trialName <- paste(data$date,data$mLeft,data$fNr,data$mRight,sep="_")

data <- select(data,trialName,mLeft,fNr,mRight,date,vidNr,Behaviour, Start, Start_Frame, Stop, Stop_Frame,
Subject, Value)


# add familiar/unfamiliar male location  into the experiment data:
expData$partnerL <- NA

for (e in 1:nrow(expData)) {
  f <- expData$fNr [e]
  p <- expData$partner[e]
  u <- expData$unfamiliar[e]
  # confirm this configuration in the scoring data
  frows <- which(data$fNr==f)
  if (length(frows)==0) {
    # no data, skip her
    next
  }
  mL <- unique(data$mLeft[frows])
  mR <- unique(data$mRight[frows])
  if (!all(sort(c(p,u))==sort(c(mL,mR)))) {
    print('Problem with male and female matching between expData and data')
  }
  expData$partnerL[e] <- p==mL
}

data$duration_s <- data$Stop- data$Start
data$duration_f <- data$Stop_Frame -data$Start_Frame

# add new column with times corrected for final frames, i.e. considering video pieces as full video
data$start_s <- NA
data$stop_s <- NA
data$start_fr <- NA
data$stop_fr <- NA
tnames <- unique(data$trialName)
for (v in tnames) { # for each complete video
  inds <- which(data$trialName==v)
  vids <- sort(unique(data$vidNr[inds])) # these are the chunks; all but 1 have 5, 1 has 4
  for (ch in 1:max(vids)) { # from second chunk
    # time of final frame of previous chunk
    if (ch==1) {
      FF_s <- 0 # previous end is zero and added consecutively for each video
      FF_fr <- 0 # previous end is zero and added consecutively for each video
    } else {
      FF_s <- FF_s + unique(data$Start[data$trialName==v & data$Value=='final_frame' & data$vidNr==(ch-1)]) #
was supposed to be scored in dove_unknown but also appears in female sometimes
      FF_fr <- FF_fr + unique(data$Start_Frame[data$trialName==v & data$Value=='final_frame' &
data$vidNr==(ch-1)])
    }
```

```
    # rows to be altered:
    inds <- which(data$trialName==v & data$vidNr==ch)
    data$start_s[inds] <- data$Start[inds] + FF_s
    data$stop_s[inds] <- data$Stop[inds] + FF_s # NA will remain NA
    data$start_fr[inds] <- data$Start_Frame[inds] + FF_fr
    data$stop_fr[inds] <- data$Stop_Frame[inds] + FF_fr # NA will remain NA
  }
}

# add column assigning each scored behaviour to baseline or experiment phase
data$phase <- NA
data$phase_duration_s <- NA
for (v in tnames) {
  bl_end <- data$start_s[data$trialName==v & data$Value=='screen_removal'] # time in seconds of screen re-
moval
  bl_start <- max(0,bl_end - BL_DUR_S) # set to a maximum of BL_DUR_S
  bl_duration <- bl_end - bl_start
  exp_start <- data$start_s[data$trialName==v & data$Value=='experimenter_leaving'] # time in seconds of ex-
perimenter leaving
  exp_end <- min(exp_start+EXP_DUR_S, max(data$start_s[data$trialName==v & data$Value=='final_frame'])) #
set to max of EXP_DUR_S
  exp_duration <- exp_end - exp_start

  inds <- which(data$trialName==v) # rows to be categorized

  # these are clearly in baseline/exp, i.e. start in the phase and (if they end) end in the phase:
  bl_inds <- (data$start_s[inds] >= bl_start & data$start_s[inds] <= bl_end) & (is.na(data$stop_s[inds]) |
data$stop_s[inds] <= bl_end) # events have NA as stop
  exp_inds <- (data$start_s[inds] >= exp_start & data$start_s[inds] <= exp_end) & (is.na(data$stop_s[inds]) |
data$stop_s[inds] <= exp_end)
  # these are clearly invalid:
  invalid_inds <- (data$start_s[inds] < bl_start & (is.na(data$stop_s[inds]) | data$stop_s[inds] < bl_start)) | #
started+ended before BL
    ((data$start_s[inds] > bl_end & data$start_s[inds] < exp_start) & (is.na(data$stop_s[inds]) | data$stop_s[inds]
< exp_start)) | # started+ended between BL and exp
    (data$start_s[inds] > exp_end) # started after exp ended

  # assign accordingly
  data$phase[inds[bl_inds]] <- 'baseline'
  data$phase_duration_s[inds[bl_inds]] <- bl_duration
  data$phase[inds[exp_inds]] <- 'experiment'
  data$phase_duration_s[inds[exp_inds]] <- exp_duration

  # deal with leftovers
  inds_ambiguous <- inds[!(bl_inds | exp_inds | invalid_inds)]

  if (length(inds_ambiguous)>0) { # if there are ambiguous ones left
    # move duration start before bl to bl_start
    bl_s_move_inds <- data$start_s[inds_ambiguous] < bl_start
    data$start_s[inds_ambiguous[bl_s_move_inds]] <- bl_start
    # move duration end after exp to exp_end
    exp_e_move_inds <- !is.na(data$stop_s[inds_ambiguous]) & data$stop_s[inds_ambiguous] > exp_end
    data$stop_s[inds_ambiguous[exp_e_move_inds]] <- exp_end
    # move duration start between bl and exp to exp_start
    exp_s_move_inds <- data$start_s[inds_ambiguous] > bl_end & data$start_s[inds_ambiguous] < exp_start
    data$start_s[inds_ambiguous[exp_s_move_inds]] <- exp_start
    # move duration end between bl and exp to bl_end
    bl_e_move_inds <- !is.na(data$stop_s[inds_ambiguous]) & data$stop_s[inds_ambiguous] > bl_end &
```

```
data$stop_s[inds_ambiguous] < exp_start
    data$stop_s[inds_ambiguous[bl_e_move_inds]] <- bl_end

    # assign again:
    # these are clearly in baseline/exp:
    bl_inds <- (data$start_s[inds_ambiguous] >= bl_start & data$start_s[inds_ambiguous] <= bl_end) &
(is.na(data$stop_s[inds_ambiguous]) | data$stop_s[inds_ambiguous] <= bl_end) # events have NA as stop
    exp_inds <- (data$start_s[inds_ambiguous] >= exp_start & data$start_s[inds_ambiguous] <= exp_end) &
(is.na(data$stop_s[inds_ambiguous]) | data$stop_s[inds_ambiguous] <= exp_end)
    # assign accordingly
    data$phase[inds_ambiguous[bl_inds]] <- 'baseline'
    data$phase_duration_s[inds_ambiguous[bl_inds]] <- bl_duration
    data$phase[inds_ambiguous[exp_inds]] <- 'experiment'
    data$phase_duration_s[inds_ambiguous[exp_inds]] <- exp_duration

    # and update the s duration of ambiguous behaviours
    data$duration_s[inds_ambiguous] <- data$stop_s[inds_ambiguous] - data$start_s[inds_ambiguous]

    # deal with final leftovers -> these ones start in the baseline and end in the experiment so need to be split
    to_be_split_inds <- inds_ambiguous[!(bl_inds | exp_inds)]

    if (length(to_be_split_inds)>0) {
      for (r in to_be_split_inds) {
        # confirm it is as expected
        if (!data$start_s[r]<bl_end & data$stop_s[r]>exp_start){
          print('PROBLEM!')
        }
        # make a copy for later
        row_copy_df <- data[r,]
        # original row is now ended in the baseline
        data$stop_s[r] <- bl_end
        data$phase[r] <- 'baseline'
        data$phase_duration_s[r] <- bl_duration
        # copy gets started in the experiment
        row_copy_df$start_s[1] <- exp_start
        row_copy_df$phase[1] <- 'experiment'
        row_copy_df$phase_duration_s[1] <- exp_duration
        # update duration of both
        data$duration_s[r] <- data$stop_s[r] - data$start_s[r]
        row_copy_df$duration_s[1] <- row_copy_df$stop_s[1] - row_copy_df$start_s[1]
        # append copy row to full data df
        data <- rbind(data,row_copy_df)
      }

    }

  }

}


# filtering the whole data for FEMALE BEHAVIOURS
female <- filter(data,Subject == 'female')%>%
  group_by(trialName)
doves_unknown <- filter(data, Subject == 'doves_unknown')%>%
  group_by(trialName)
female_preening <- data %>%
  filter(Subject== 'female' & Behaviour =='preening_dove' ) %>%
```

```
  group_by(trialName,fNr,mLeft,mRight, phase, phase_duration_s) %>%
  summarise(Time_preening_s= sum(duration_s),
            Preening_bouts= n())
narows <- which(is.na(female_preening$phase))
female_preening <- female_preening[-narows,]


# to filter the whole data into the 2 head_directions (L or R)
head_direction <- data %>%
  filter(Subject=='female' & Behaviour=='head_direction')
head_direction_R <- head_direction %>%
  filter(Value=='beak_direction_R') %>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s)%>%
  summarise(Time_beak_direction_R_s=sum(duration_s),
            beak_direction_R_nbr=n())
narows <- which(is.na(head_direction_R$phase))
head_direction_R <- head_direction_R[-narows,]

head_direction_L <- head_direction %>%
  filter(Value=='beak_direction_L')%>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s)%>%
  summarise(Time_beak_direction_L_s=sum(duration_s),
            beak_direction_L_nbr=n())
narows <- which(is.na(head_direction_L$phase))
head_direction_L <- head_direction_L[-narows,]
# merge head_direction_L and head_direction_R into one table
m_1 <- merge(head_direction_L, head_direction_R, by= c ('fNr','mLeft','mRight','trialName','phase', 'phase_dura-
tion_s'),all.y = TRUE)
# merge the created table with preening
m_2 <- merge(female_preening, m_1, by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_duration_s'),all.y =
TRUE)
## Locations of the female in the different zones
# filtering the whole data for ZONE A
location_female <- data %>%
  filter(Subject=='female' & Behaviour=='location_female')
Zone_A <- location_female %>%
  filter(Value=='location_A')%>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s)%>%
  summarise(Time_location_A_s=sum(duration_s),
            location_A_nbr=n())
narows <- which(is.na(Zone_A$phase))
Zone_A <- Zone_A[-narows,]
# filtering the whole data for ZONE B
Zone_B <- location_female %>%
  filter(Value=='location_B')%>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s)%>%
  summarise(Time_location_B_s= sum(duration_s),
            location_B_nbr=n())

narows <- which(is.na(Zone_B$phase))
Zone_B <- Zone_B[-narows,]
# merge the last table with Zone A
m_3 <- merge(Zone_A, m_2, by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_duration_s'),all.y=TRUE)
# merge with Zone C
m_4 <- merge(Zone_B, m_3, by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_duration_s'),all.y=TRUE)
#filtering the whole data for ZONE C
Zone_C <- location_female %>%
  filter(Value=='location_C')%>%
```

```
  group_by(trialName,fNr, mLeft, mRight,phase, phase_duration_s)%>%
  summarise(Time_location_C_s=sum(duration_s),
            location_C_nbr=n())
narows <- which(is.na(Zone_C$phase))
Zone_C <- Zone_C[-narows,]
# merge with zone C
m_5 <- merge(Zone_C, m_4,by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_duration_s'),all.y=TRUE)


# filtering the data for BODY POSTURE (left and right)
#to filter the data for BODY_POSTURE_R (right)
body_posture_R <- female%>%
  filter( Behaviour=='body_posture' & Value=='body_posture_R') %>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s) %>%
  summarise(Time_body_posture_R_s = sum(duration_s),
            Posture_R_nbr =n())
narows <- which(is.na(body_posture_R$phase))
body_posture_R <- body_posture_R[-narows,]
#merge with  body_posture_R
m_6 <- merge(body_posture_R, m_5, by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_dura-
tion_s'),all.y=TRUE)
#to filter the data for BODY_POSTURE_L (left)
body_posture_L <- female %>%
  filter(Behaviour=='body_posture' & Value=='body_posture_L') %>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s) %>%
  summarise(Time_body_posture_L_s = sum(duration_s),
            Posture_L_nbr =n())
narows <- which(is.na(body_posture_R$phase))
body_posture_R <- body_posture_R[-narows,]¨
#merge with body_posture_R
m_7 <- merge(body_posture_L, m_6, by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_dura-
tion_s'),all.y=TRUE)


# filter the data for VISIBILIY
visibility_female <- data %>%
  filter(Subject=='female' & Behaviour=='Visibility' & Value=='visible') %>%
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s) %>%
  summarise(Time_visible_s=sum(duration_s))
narows <- which(is.na(visibility_female$phase))
visibility_female <- visibility_female[-narows,]
# merge with the visibility of the female
m_8 <- merge(visibility_female, m_7,by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_dura-
tion_s'),all.y=TRUE)

# filter for FEEDING
feeding_female <- data %>%
  filter(Subject=='female' & Behaviour=='feedingg') I>
  group_by(trialName,fNr,mLeft,mRight,phase, phase_duration_s) I>
  summarise(Time_feedingg_s=sum(duration_s),
            Feedingg_nbr=n())
narows <- which(is.na(feeding_female$phase))
feeding_female<- feeding_female[-narows,]
# merge with feeding
m_9 <- merge(feeding_female, m_8,by=c ('fNr','mLeft','mRight','trialName','phase', 'phase_dura-
tion_s'),all.y=TRUE)
```

```
#filter for UNKNOWN DOVES CALLS
#filter for single coo calls
unknown_doves <- filter(data,Subject=='doves_unknown')
calls_coo <- unknown_doves %>%
  filter(Behaviour=='calls_dove' & Value=='calls_coo') %>%
  group_by(trialName,phase, phase_duration_s)%>%
  summarise(calls_coo_nbr=n())
narows <- which(is.na(calls_coo$phase))
calls_coo<- calls_coo[-narows,]
# merge with aingle coo calls
m_10 <- merge(calls_coo, m_9, by= c('trialName', 'phase', 'phase_duration_s'), all.y =TRUE)

# filter for laughs
calls_laugh <- unknown_doves %>%
  filter(Behaviour=='calls_dove' & Value=='calls_laugh')%>%
  group_by(trialName,phase, phase_duration_s)%>%
  summarise(calls_laugh_nbr=n())
narows <- which(is.na(calls_laugh$phase))
calls_laugh<- calls_laugh[-narows,]
# merge with laughs
m_11 <- merge(calls_laugh, m_10, by=c ('trialName','phase', 'phase_duration_s'),all.y=TRUE)


# filter for multiple coo calls (when you could hear different coos from different individuals at the same time)
calls_multiple <- unknown_doves %>%
  filter(Behaviour=='calls_dove'& Value=='calls_multiple_coos')%>%
  group_by(trialName,phase, phase_duration_s)%>%
  summarise(calls_multiple_nbr=n())
narows <- which(is.na(calls_multiple$phase))
calls_multiple<- calls_multiple[-narows,]
#merge with multiple coos
m_12 <- merge(calls_multiple, m_11, by=c ('trialName','phase', 'phase_duration_s'),all.y=TRUE)

# merge our crated table with the expüerimental data table
m_data <- merge(m_12, expData, by='fNr')

# replace every NA in the table by O
m_final_data <- m_data %>%
  replace_na(list(Time_location_A_s=0,
                  location_A_nbr =0,
                  Time_location_B_s=0,
                  location_B_nbr=0,
                  Time_location_C_s=0,
                  location_C_nbr=0,
                  Time_body_posture_L_s=0,
                  Posture_L_nbr=0,
                  Time_body_posture_R_s=0,
                  Posture_R_nbr=0,
                  Time_preening_s=0,
                  Preening_bouts=0,
                  proportion_feeding=0,
                  proportion_preening=0,
                  proportion_location_A=0,
                  proportion_location_B=0,
                  proportion_location_C=0,
                  proportion_body_posture_L=0,
                  proportion_body_posture_R=0,
                  Time_feedingg_s=0,
```

```
            Feedingg_nbr =0,
            calls_coo_nbr=0,
            calls_laugh_nbr =0,
            calls_multiple_nbr =0))
m_final_data_2 <- m_final_data %>%
  replace_na(list(index_location_unfamiliar=0,
            index_location_partner=0))

#  filter for SCREEN REMOVALS
screen_removal <- unknown_doves %>%
  filter(Behaviour=='Setup' & Value=='screen_removal')%>%
  group_by(trialName)%>%
  summarise(trialName,
        fNr,
        Start,
        Start_Frame)
screen_removal_2 <- unknown_doves %>%
  filter(Behaviour=='Setup' & Value=='screen_removal_2')%>%
  group_by(trialName)%>%
  summarise(trialName,
        fNr,
        Start,
        Start_Frame)

# filter for EXPERIMENTER LEAVING
exp_leaving <- unknown_doves %>%
  filter(Behaviour=='Setup' & Value=='experimenter_leaving')%>%
  group_by(trialName, phase, phase_duration_s)%>%
  summarise(trialName,fNr, Start, Start_Frame,phase, phase_duration_s)

# filter for FINAL FRAME
final_frame <- unknown_doves%>%
  filter(Behaviour=='Setup'& Value=='final_frame') %>%
  group_by(trialName,phase, phase_duration_s)%>%
  summarise(trialName,vidNr, Start, Start_Frame)

# Computations for different types of index
m_final_data$proportion_feeding <- m_final_data$Time_feedingg_s / m_final_data$phase_duration_s
m_final_data$proportion_preening <- m_final_data$Time_preening_s / m_final_data$phase_duration_s
m_final_data$proportion_location_A <- m_final_data$Time_location_A_s / m_final_data$phase_duration_s
m_final_data$proportion_location_C <- m_final_data$Time_location_C_s / m_final_data$phase_duration_s
m_final_data$proportion_beak_direction_L <- m_final_data$Time_beak_direction_L_s / m_final_data$phase_du-
ration_s
m_final_data$proportion_beak_direction_R <- m_final_data$Time_beak_direction_R_s / m_fi-
nal_data$phase_duration_s
m_final_data$proportion_body_posture_L <- m_final_data$Time_body_posture_L_s/ m_final_data$phase_dura-
tion_s
m_final_data$proportion_body_posture_R <- m_final_data$Time_body_posture_R_s/ m_final_data$phase_dura-
tion_s
m_final_data$proportion_location_A_nbr <- m_final_data$location_A_nbr / m_final_data$phase_duration_s
m_final_data$proportion_location_B_nbr <- m_final_data$location_B_nbr / m_final_data$phase_duration_s
m_final_data$proportion_location_C_nbr <- m_final_data$location_C_nbr / m_final_data$phase_duration_s

# the indices are relative to position of the partner, i.e. not split into L and R anymore
m_final_data$index_body_posture_partner <- NA
m_final_data$index_body_posture_partner[m_final_data$partnerL] <- m_final_data$Time_body_pos-
ture_L_s[m_final_data$partnerL] / (m_final_data$Time_body_posture_L_s[m_final_data$partnerL] + m_fi-
nal_data$Time_body_posture_R_s[m_final_data$partnerL])
```

```
m_final_data$index_body_posture_partner[!m_final_data$partnerL] <- m_final_data$Time_body_posture_R_s[!m_final_data$partnerL] / (m_final_data$Time_body_posture_L_s[!m_final_data$partnerL] + m_final_data$Time_body_posture_R_s[!m_final_data$partnerL])

m_final_data$index_beak_direction_partner <- NA
m_final_data$index_beak_direction_partner[m_final_data$partnerL] <- m_final_data$Time_beak_direction_L_s[m_final_data$partnerL] / (m_final_data$Time_beak_direction_L_s[m_final_data$partnerL]+m_final_data$Time_beak_direction_R_s[m_final_data$partnerL])
m_final_data$index_beak_direction_partner[!m_final_data$partnerL] <-m_final_data$Time_beak_direction_R_s[!m_final_data$partnerL] / (m_final_data$Time_beak_direction_L_s[!m_final_data$partnerL]+m_final_data$Time_beak_direction_R_s[!m_final_data$partnerL])

m_final_data$index_location_partner <- NA
m_final_data$index_location_partner[m_final_data$partnerL] <- m_final_data$Time_location_A_s[m_final_data$partnerL] / (m_final_data$Time_location_A_s[m_final_data$partnerL]+m_final_data$Time_location_C_s[m_final_data$partnerL])
m_final_data$index_location_partner[!m_final_data$partnerL] <- m_final_data$Time_location_C_s[!m_final_data$partnerL] / (m_final_data$Time_location_A_s[!m_final_data$partnerL]+m_final_data$Time_location_C_s[!m_final_data$partnerL])


m_final_data$index_location_unfamiliar <- (1- m_final_data$index_location_partner)
m_final_data$index_body_posture_unfamiliar <- (1- m_final_data$index_body_posture_partner)
m_final_data$index_beak_direction_unfamiliar <- (1- m_final_data$index_beak_direction_partner)

m_final_data$index_neutral_location <- m_final_data$Time_location_B_s / (m_final_data$Time_location_A_s+m_final_data$Time_location_B_s+m_final_data$Time_location_C_s)

m_final_data$deplacement_rate <- (m_final_data$location_A_nbr+m_final_data$location_B_nbr+m_final_data$location_C_nbr)/m_final_data$phase_duration_s



#creation of a dataset with only the experimental values; no baseline values
m_final_data_experiment <- m_final_data %>%
  filter(phase=='experiment')
#Creation of new data set for MT subjects
m_experiment_MT_data <- m_final_data %>%
  filter(phase=='experiment' & treatment=='MT')
m_baseline_MT_data <- m_final_data %>%
  filter(phase=='baseline' & treatment=='MT')
#Creation of new data set for SL subjects
m_experiment_SL_data <- m_final_data %>%
  filter(phase=='experiment'& treatment=='SL')
m_baseline_SL_data <- m_final_data %>%
  filter(phase=='baseline' & treatment=='SL')

###STATISTICS
## two way mixed ANOVA for index_location
m_final_data_long <- m_final_data_experiment %>%
  select(fNr, treatment, index_location_partner, index_location_unfamiliar) %>%
  pivot_longer(cols = starts_with('index_location'),
               names_to = 'familiarity',
               values_to ='index_location')%>%
  mutate(familiarity=ifelse(familiarity=='index_location_partner', 'Familiar', 'Unfamiliar'),
         treatment=factor(treatment),
         familiarity=factor(familiarity),
         fNr=factor(fNr))
```

```
anova_result_index_location <- aov_ez(id='fNr',
                    dv='index_location',
                    within = 'familiarity',
                    between = 'treatment',
                    data = m_final_data_long,
                    type = 3)
anova_result_index_location$anova_table
##two way mixed ANOVA fro index_location_partner with R package

aov_test_R_index_location_partner <- aov_car(index_location_partner~
                              treatment*phase +Error(fNr/phase),
                            data = m_final_data,
                            type = 3)
summary(aov_test_R_index_location_partner)
###ASSUMPTIONS
shapiro.test(residuals(aov_test_R_index_location_partner))
shapiro.test(residuals(anova_result_index_location))
leveneTest(index_location_partner ~ treatment * phase, data = m_final_data)

# two way mixed ANOVA for index_beak_direction
m_final_data_long_1 <- m_final_data_experiment %>%
  select(fNr, treatment, index_beak_direction_partner, index_beak_direction_unfamiliar) %>%
  pivot_longer(cols = starts_with('index_beak_direction'),
               names_to = 'familiarity',
               values_to ='index_beak_direction')%>%
  mutate(familiarity=ifelse(familiarity=='index_beak_direction_partner', 'Familiar', 'Unfamiliar'),
         treatment=factor(treatment),
         familiarity=factor(familiarity),
         fNr=factor(fNr))
anova_result_index_beak_direction <- aov_ez(id='fNr',
                    dv='index_beak_direction',
                    within = 'familiarity',
                    between = 'treatment',
                    data = m_final_data_long_1,
                    type = 3)
anova_result_index_beak_direction$anova_table
##two way mixed ANOVA fro index_beak_direction with R package
aov_test_R_index_beak_direction <- aov_car(index_beak_direction_partner~
                              treatment*phase +Error(fNr/phase),
                            data = m_final_data,
                            type = 3)
summary(aov_test_R_index_beak_direction)
##ASSUMPTIONS
shapiro.test(residuals(anova_result_index_beak_direction))
shapiro.test(residuals(aov_test_R_index_beak_direction))
leveneTest(index_beak_direction_partner ~ treatment * phase, data = m_final_data)

# two way mixed ANOVA for index_body_posture
m_final_data_long_2 <- m_final_data_experiment %>%
  select(fNr, treatment, index_beak_direction_partner, index_beak_direction_unfamiliar) %>%
  pivot_longer(cols = starts_with('index_body_posture_direction'),
               names_to = 'familiarity',
               values_to ='index_body_posture_direction')%>%
  mutate(familiarity=ifelse(familiarity=='index_body_posture_partner', 'Familiar', 'Unfamiliar'),
         treatment=factor(treatment),
         familiarity=factor(familiarity),
         fNr=factor(fNr))
anova_result_body_posture <- aov_ez(id='fNr',
```

```
                    dv='index_body_posture_direction',
                    within = 'familiarity',
                    between = 'treatment',
                    data = m_final_data,
                    type = 3)
anova_result_body_posture$anova_table

##two way mixed ANOVA fro index_body_posture with R package
aov_test_R_index_body_posture <- aov_car(index_body_posture_partner~
                                 treatment*phase +Error(fNr/phase),
                               data = m_final_data,
                               type = 3)
summary(aov_test_R_index_body_posture)

shapiro.test(residuals(aov_test_R_index_body_posture))
leveneTest(index_body_posture_partner ~ treatment * phase, data = m_final_data)

#  two way mixed ANOVA fro neutral_location
m_final_data_long_neutral_loaction <- m_final_data %>%
  select(fNr, treatment, index_neutral_location,phase)
anova_result_index_neutral_location <- aov_ez(id='fNr',
                                      dv='index_neutral_location',
                                      within = 'phase',
                                      between = 'treatment',
                                      data = m_final_data_long_neutral_loaction,
                                      type=3)

anova_result_index_neutral_location$anova_table

aov_test_R_index_neutral_location <- aov_car(index_neutral_location~
                                 treatment*phase +Error(fNr/phase),
                               data = m_final_data,
                               type = 3)
summary(aov_test_R_index_neutral_location)

shapiro.test(residuals(aov_test_R_index_neutral_location))
leveneTest(index_neutral_location~ treatment * phase, data = m_final_data)
# Welch ANOVA bc asssumptions were violated for two way mixed ANOVA
m_final_data %>%
  welch_anova_test(index_neutral_location ~ treatment * phase)
m_final_data %>% welch_anova_test(index_neutral_location ~ treatment)
m_final_data %>% welch_anova_test(index_neutral_location ~ phase)
m_final_data %>% welch_anova_test(index_neutral_location ~ interaction(treatment, phase))

# #  two way ANOVA for proportion_preening_s
aov_test_R_preening <- aov_car(proportion_preening~
                     treatment*phase +Error(fNr/phase),
                   data = m_final_data,
                   type = 3)
m_final_data$proportion_preening_log <- log(m_final_data$proportion_preening + 1)
aov_test_transformed <- aov(proportion_preening_log ~ treatment*phase + Error(fNr/phase),
                    data = m_final_data)

library(nparLD) # non parametric test

aov_trans <- nparLD(proportion_preening ~ treatment * phase,
            data = m_final_data,
            subject = "fNr",
```

```
                description = TRUE)

summary(aov_trans)

shapiro.test(residuals(aov_trans))
summary(aov_test_R_preening)
shapiro.test(residuals(aov_test_R_preening))
leveneTest(proportion_preening~ treatment * phase, data = m_final_data)
# #  two way ANOVA for proportion_feeding_s
aov_test_R_feeding <- aov_car(proportion_feeding~
                         treatment*phase +Error(fNr/phase),
                       data = m_final_data,
                       type = 3)
summary(aov_test_R_feeding)
aov_trans_feeding <- nparLD(proportion_feeding ~ treatment * phase,
                  data = m_final_data,
                  subject = "fNr",
                  description = TRUE)

summary(aov_trans_feeding)
shapiro.test(residuals(aov_test_R_feeding))
leveneTest(proportion_feeding~ treatment * phase, data = m_final_data)
# #  two way ANOVA for deplacement_index
aov_test_R_deplacement <- aov_car(deplacement_rate~
                           treatment*phase + Error(fNr/phase),
                         data = m_final_data,
                         type = 3)
summary(aov_test_R_deplacement)
non_parametric_test_result_trans_deplacement_rate <- nparLD(deplacement_rate ~ treatment * phase,
                         data = m_final_data,
                         subject = "fNr",
                         description = TRUE)

summary(non_parametric_test_result_trans_deplacement_rate)
shapiro.test(residuals(aov_test_R_deplacement))
leveneTest(deplacement_index~ treatment * phase, data = m_final_data)


aov_test_R_activity <- aov_car(
  activity_index ~ treatment * phase + Error(fNr/phase),
  data = m_final_data
)
summary(aov_test_R_activity)
shapiro.test(residuals(aov_test_R_activity))
non_parametric_test_result_trans_activity_index <- nparLD(activity_index ~ treatment * phase,
                                                  data = m_final_data,
                                                  subject = "fNr",
                                                  description = TRUE)
summary(non_parametric_test_result_trans_activity_index)


aov_test_R_activity_calls <- aov_car(
  activity_par_rapport_total_call_nbr ~ treatment * phase + Error(fNr/phase),
  data = m_final_data)
summary(aov_test_R_activity_calls)
shapiro.test(residuals(aov_test_R_activity_calls))
non_parametric_test_activity_calls <- nparLD(activity_par_rapport_total_call_nbr ~ treatment * phase,
                                             data = m_final_data,
```

```
                                        subject = "fNr",
                                        description = TRUE)
summary(non_parametric_test_activity_calls)


# # comaration des index entre groupe ; independent t-test
## index_location_partner
t.test(index_location_partner ~ treatment, data = m_final_data_experiment, var.equal = TRUE)
t.test(index_location_partner ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$index_location_partner[m_final_data_experiment$treatment == "MT"])
#VALIDADED
shapiro.test(m_final_data_experiment$index_location_partner[m_final_data_experiment$treatment == "SL"])
#VALIDADED
leveneTest(index_location_partner~ treatment, data = m_final_data_experiment) #VALID

#index_beak_direction_partner
t.test(index_beak_direction_partner ~ treatment, data = m_final_data_experiment, var.equal = TRUE)
t.test(index_beak_direction_partner ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$index_beak_direction_partner[m_final_data_experiment$treatment ==
"MT"]) #VALID
shapiro.test(m_final_data_experiment$index_beak_direction_partner[m_final_data_experiment$treatment ==
"SL"]) #VALID
leveneTest(index_beak_direction_partner~ treatment, data = m_final_data_experiment) #VALID

#index_body_posture_partner
t.test(index_body_posture_partner ~ treatment, data = m_final_data_experiment, var.equal = TRUE)
t.test(index_body_posture_partner ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$index_body_posture_partner[m_final_data_experiment$treatment ==
"MT"]) #VALIDADED
shapiro.test(m_final_data_experiment$index_body_posture_partner[m_final_data_experiment$treatment ==
"SL"]) #VALIDADED
leveneTest(index_body_posture_partner~ treatment, data = m_final_data_experiment) #VALID

#index neutral location
t.test(index_neutral_location ~ treatment, data = m_final_data_experiment, var.equal = TRUE)
t.test(index_neutral_location ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$index_neutral_location[m_final_data_experiment$treatment == "MT"])
#FAILED
shapiro.test(m_final_data_experiment$index_neutral_location[m_final_data_experiment$treatment == "SL"])
#FAILED
wilcox.test(index_neutral_location ~ treatment , data = m_final_data_experiment, exact = FALSE) #FAILED; NO
SIGNIFICANCE

# deplacement_index
t.test(deplacement_rate ~ treatment, data = m_final_data_experiment, var.equal = TRUE)
t.test(deplacement_rate ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$deplacement_rate[m_final_data_experiment$treatment == "MT"])
#FAILED
shapiro.test(m_final_data_experiment$deplacement_rate[m_final_data_experiment$treatment == "SL"])
#FAILED
wilcox.test(deplacement_rate ~ treatment , data = m_final_data_experiment, exact = FALSE) #FAILED; NO SIG-
NIFICANCE
```

```
#FEEDING
t.test(proportion_feeding ~ treatment, data = m_final_data, var.equal = TRUE)
t.test(proportion_feeding ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$proportion_feeding[m_final_data_experiment$treatment == "MT"]) #valid
shapiro.test(m_final_data_experiment$proportion_feeding[m_final_data_experiment$treatment == "SL"]) #valid

#PREENING
t.test(proportion_preening ~ treatment, data = m_final_data_experiment)
#ASSUMPTIONS
shapiro.test(m_final_data_experiment$proportion_preening[m_final_data_experiment$treatment == "MT"]) #valid
shapiro.test(m_final_data_experiment$proportion_preening[m_final_data_experiment$treatment == "SL"])
wilcox.test(proportion_preening~ treatment , data = m_final_data_experiment, exact = FALSE)

# paired t test
# Search for significance between Time near familiar mate and time near unfamiliar mate for MT subjects using
paired t-test
t.test(m_experiment_MT_data$index_location_partner, m_experiment_MT_data$index_location_unfamiliar,
paired = TRUE, mu=0, conf.level = 0.95)
m_experiment_MT_data$diff <- m_experiment_MT_data$index_location_partner - m_experiment_MT_data$in-
dex_location_unfamiliar
boxplot(m_experiment_MT_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_MT_data$diff)$out
print(outliers)
shapiro_test <- shapiro.test(m_experiment_MT_data$diff)
print(shapiro_test)

# Search for significance between Time near familiar mate and time near unfamiliar mate for SL(=control) sub-
jects using paired t-test
m_experiment_SL_data$diff <- m_experiment_SL_data$index_location_partner - m_experiment_SL_data$in-
dex_location_unfamiliar
boxplot(m_experiment_SL_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_SL_data$diff)$out
print(outliers)
shapiro_test <- shapiro.test(m_experiment_SL_data$diff)
print(shapiro_test)
t.test(m_experiment_SL_data$index_location_partner, m_experiment_SL_data$index_location_unfamiliar, paired
= TRUE, mu=0, conf.level = 0.95)

# search for significance between time body posture directed toward familiar male and unfamiliar male for MT
subjects using paired t-test
m_experiment_MT_data$diff <- m_experiment_MT_data$index_body_posture_partner - m_experi-
ment_MT_data$index_body_posture_unfamiliar
boxplot(m_experiment_MT_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_MT_data$diff)$out
print(outliers)
shapiro_test <- shapiro.test(m_experiment_MT_data$diff)
print(shapiro_test)
t.test(m_experiment_MT_data$index_body_posture_partner, m_experiment_MT_data$index_body_posture_un-
familiar, paired = TRUE, mu=0, conf.level = 0.95)

# search for significance between time body posture directed toward familiar male and unfamiliar male for SL
subjects using paired t-test
m_experiment_SL_data$diff <- m_experiment_SL_data$index_body_posture_partner - m_experi-
ment_SL_data$index_body_posture_unfamiliar
boxplot(m_experiment_SL_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_SL_data$diff)$out
```

```
print(outliers)
shapiro_test <- shapiro.test(m_experiment_SL_data$diff)
print(shapiro_test)
t.test(m_experiment_SL_data$index_body_posture_partner, m_experiment_SL_data$index_body_posture_unfa-
miliar, paired = TRUE, mu=0, conf.level = 0.95)

# search for significance between time beakdirected toward familiar male and unfamiliar male for MT subjects
using paired t-test
m_experiment_MT_data$diff <- m_experiment_MT_data$index_beak_direction_partner - m_experi-
ment_MT_data$index_beak_direction_unfamiliar
boxplot(m_experiment_MT_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_MT_data$diff)$out
print(outliers)
#bc normality violated
wilcox_result <- wilcox.test(m_experiment_MT_data$index_beak_direction_partner,
                        m_experiment_MT_data$index_beak_direction_unfamiliar,
                        paired = TRUE,
                        conf.level = 0.95,
                        conf.int = TRUE)
print(wilcox_result)
t.test(m_experiment_MT_data$index_beak_direction_partner, m_experiment_MT_data$index_beak_direc-
tion_unfamiliar, paired = TRUE, mu=0, conf.level = 0.95)

# search for significance between time beakdirected toward familiar male and unfamiliar male for SL subjects us-
ing paired t-test
m_experiment_SL_data$diff <- m_experiment_SL_data$index_beak_direction_partner - m_experi-
ment_SL_data$index_beak_direction_unfamiliar
boxplot(m_experiment_SL_data$diff, main ='outliercheck for differences')
outliers <- boxplot.stats(m_experiment_SL_data$diff)$out
print(outliers)
#bc normality violated
wilcox_result <- wilcox.test(m_experiment_SL_data$index_beak_direction_partner,
                        m_experiment_SL_data$index_beak_direction_unfamiliar,
                        paired = TRUE,
                        conf.level = 0.95,
                        conf.int = TRUE)
print(wilcox_result)
t.test(m_experiment_SL_data$index_beak_direction_partner, m_experiment_SL_data$index_beak_direction_un-
familiar, paired = TRUE, mu=0, conf.level = 0.95)



#Plots
ggplot(data = m_final_data_2, aes(phase,index_location_partner, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape = NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
```

```
  ) +
  labs(
    x = "Phase",
    y = "Index location next to partner",
    title = "Index location next to partner across Phases by Treatment"
  )
ggsave('Location next to partner 12.09.png')

ggplot(data = m_final_data_2, aes(phase,index_neutral_location, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index neutral location",
    title = "Index neutral location across Phases by Treatment"
  )
ggsave('Neutral location 12.09.png')

ggplot(data = m_final_data_2, aes(phase,deplacement_rate, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Deplacement rate",
    title = "Deplacement across Phases by Treatment")
ggsave('deplacement rate 12.09.png')
ggplot(data = m_final_data_2, aes(phase,index_body_posture_partner, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
```

```
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index body posture directed toward partner",
    title = "Index body posture directed toward partner across Phases by Treatment")
ggsave('index body posture 12.09.png')
ggplot(data = m_final_data_2, aes(phase,index_beak_direction_partner, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index beak directed toward partner",
    title = "Index beak directed toward partner across Phases by Treatment")

ggsave('index beak direction 12.09.png')
ggplot(data = m_final_data_2, aes(phase,proportion_preening, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Preening proportion",
    title = "Preening proportion across Phases by Treatment")
ggsave('proportion preening 12.09.png')
```

```
ggplot(data = m_final_data_2, aes(phase,proportion_feeding, fill = fNr, color = phase)) +
  geom_boxplot(outlier.shape =NA) +
  geom_line(aes(group=fNr), position = position_dodge(0.2),color = "grey70", alpha = 0.5, linewidth = 0.6) +
  geom_point(aes(fill=fNr,group=fNr), position = position_dodge(0.2)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 4, color = "black") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width = 0.2, color = "black") +
  facet_wrap(~ treatment)+
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Feeding proportion",
    title = "Feeding proportion across Phases by Treatment")

ggsave('proportion feeding 12.09.png')
ggplot(data = m_final_data_2, aes(x = phase, y = index_location_partner, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +
  geom_line(aes(group = fNr),position = position_dodge(0.2) , color = "grey60", alpha = 0.5, na.rm = TRUE) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index location next to partner",
    title = "Index location next to partner across Phases by Treatment"
  )
# index location partner plot
ggplot(data = m_final_data_2, aes(x = phase, y = index_location_partner, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_line(aes(group = fNr), color = "grey60", alpha = 0.5, na.rm = TRUE) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
```

```
    x = "Phase",
    y = "Index location next to partner",
    title = "Index location next to partner across Phases by Treatment"
  )
 ggsave('THESIS_INDEX_LOCATION_PARTNER.png')
ggplot(data = m_final_data_2, aes(x = phase, y = index_neutral_location, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index neutral location",
    title = "Index neutral location across Phases by Treatment"
  )
ggsave('THESIS_INDEX_NEUTRAL_LOCATION.png')

ggplot(data = m_final_data_2, aes(x = phase, y = deplacement_rate, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Deplacement rate",
    title = "Deplacement across Phases by Treatment"
  )
ggsave('THESIS_DEPLACEMENT_RATE.png')
ggplot(data = m_final_data_2, aes(x = phase, y = index_body_posture_partner, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
```

```
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index body posture directed toward partner",
    title = "Index body posture directed toward partner across Phases by Treatment"
  )
ggsave('THESIS_INDEX_BODY_POSTURE_PARTNER.png')
ggplot(data = m_final_data_2, aes(x = phase, y = index_beak_direction_partner, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Index beak directed toward partner",
    title = "Index beak directed toward partner across Phases by Treatment"
  )
ggsave('THESIS_INDEX_BEAK_DIRECTED_PARTNER.png')
ggplot(data = m_final_data_2, aes(x = phase, y = proportion_feeding, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Feeding proportion",
    title = "Feeding proportion across Phases by Treatment"
  )
ggsave('THESIS_FEEDING_PROPORTION.png')
ggplot(data = m_final_data_2, aes(x = phase, y = proportion_preening, color = phase)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3, width = 0.5) +
  geom_beeswarm(size = 2, alpha = 0.7, cex = 3, priority = "density") +  # colored points, all visible
  geom_path(aes(group = fNr), color = "grey60", alpha = 0.5) +
  facet_wrap(~ treatment) +
  scale_color_brewer(palette = "Set2") +   # nice clean palette
  theme_bw(base_size = 14) +
  theme(
    panel.grid = element_blank(),
    strip.background = element_rect(fill = "white", color = "black"),
```

```
    legend.position = "none",   # remove redundant legend
    axis.title = element_text(face = "bold"),
    plot.title = element_text(face = "bold", hjust = 0.5)
  ) +
  labs(
    x = "Phase",
    y = "Preening proportion",
    title = "Preening proportion across Phases by Treatment"
  )
ggsave('THESIS_PREENING_PROPORTION.png')
```