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The influence of oxytocin on dogs' attentiveness to human communicative cues

DIPLOMA THESIS
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Abstract

Oxytocin, a neuropeptide known for its role in social bonding and communication, has been shown to enhance dogs' responsiveness to human social cues. Previous research demonstrates a significantly enhanced performance in object choice tasks in dogs after intranasal Oxytocin administration. This study investigated whether a novel nebulized Oxytocin administration method, that requires no prior training and is delivered with food rewards, would improve dogs' pointing comprehension.

Twenty-six pet dogs (ages one to twelve years, 10–40kg) participated in a within-subject, double-blind crossover design, receiving either nebulized Oxytocin or placebo in two sessions separated by at least ten days. Urinary Oxytocin concentrations confirmed systemic absorption. Dogs completed an object-choice task in which the experimenter indicated hidden food using momentary, proximal, ipsilateral pointing with ostensive cues. Performance was measured by proportion of correct choices and odor control trials were included to ensure dogs were using the pointing cue rather than olfactory information and olfactory perception would not be influenced by the administration of Oxytocin.

Contrary to our hypothesis, generalized linear mixed models revealed no significant effect of post-treatment urinary Oxytocin concentrations on pointing test performance ($\beta = 0.15$, $SE = 0.18$, $z = 0.83$, $p = 0.409$). Dogs performed significantly above chance in both Oxytocin and placebo conditions, demonstrating robust pointing comprehension regardless of treatment. Performance in odor control trials did not differ from chance, confirming that dogs were utilizing the pointing gesture rather than olfactory cues.

We interpret these null finding as resulting from a ceiling effect: our pointing gesture was sufficiently easy, resulting in nearly optimal performance even under placebo condition, leaving minimal room for Oxytocin-induced improvement. Our findings highlight the importance of task difficulty in Oxytocin research, suggesting Oxytocin's effects on canine social cognition are most efficient in moderately challenging tasks. This study successfully demonstrates the feasibility of the novel nebulized administration method, which may prove valuable for future stress-free Oxytocin delivery in research and clinical applications.

Zusammenfassung

Oxytocin, ein Neuropeptid mit zentraler Bedeutung für soziale Bindung und Kommunikation, erhöht nachweislich die Sensitivität von Hunden gegenüber menschlichen Hinweisreizen und erhöht ihre Leistung in Objektauswahlverfahren (Pointing Test) nach intranasaler Verabreichung. Ziel dieser Studie war es zu untersuchen, ob eine neuartige, vernebelte OT-Applikationsmethode ohne vorheriges Training, die mit Futterbelohnungen kombiniert wird, das Verständnis von menschlichen Zeigegesten bei Hunden verbessert.

Sechszwanzig privat gehaltene Haushunde (ein bis zwölf Jahre alt, 10–40kg) nahmen an dieser doppelblinden Within-Subject-Crossover-Studie teil. Sie erhielten in zwei Sitzungen (Intervall $\geq$ zehn Tage) entweder vernebeltes Oxytocin oder ein Placebo. Die systemische OT-Aufnahme wurde durch Untersuchung der Urin-Oxytocin-Konzentration bestätigt. Im Anschluss wurde der Pointing Test durchgeführt, in dem mittels kurzer, ipsilateraler Zeigegeste und ostensiven Hinweisen auf ein verstecktes Futterstück gezeigt wurde. Die Leistung wurde anhand der Anzahl richtiger Entscheidungen der Hunde gemessen und durch Geruchskontrollen abgesichert.

Entgegen unserer Hypothese zeigten generalisierte lineare Mischmodelle keinen signifikanten Effekt der Urin-Oxytocin-Konzentration nach Oxytocin-Applikation auf die Leistung im Pointing Test ($\beta = 0.15$, $SE = 0.18$, $z = 0.83$, $p = 0.409$). Die Hunde schnitten sowohl in Oxytocin- als auch in Placebo-Bedingungen signifikant über Zufallsniveau ab, was auf ein gutes Verständnis der Zeigegesten unabhängig von der Behandlung hinweist. Die Leistung in den Geruchskontrollen unterschied sich nicht vom Zufallsniveau, was bestätigt, dass sich die Hunde an der Zeigegeste und nicht an Gerüchen orientierten.

Wir interpretieren diese Nullbefunde als Folge eines Deckeneffekts: Die verwendete Zeigegeste war hinreichend einfach, sodass bereits unter Placebo-Bedingung eine nahezu optimale Leistung erzielt wurde und somit nur wenig Spielraum für eine Oxytocin-induzierte Leistungssteigerung blieb. Unsere Ergebnisse unterstreichen damit die Wichtigkeit der Aufgabenschwierigkeit und legen nahe, dass die Effekte von Oxytocin auf die soziale Kognition von Hunden insbesondere bei moderat anspruchsvollen Aufgaben auftreten. Darüber hinaus zeigt die vorliegende Studie erfolgreich die Durchführbarkeit der neuartigen Applikationsmethode, die sich künftig als wertvoll für stressfreie Oxytocin-Verabreichung in Forschung und Klinik erweisen könnte.

Index of Abbreviations

OT		Oxytocin
E1		Experimenter 1
E2		Experimenter 2
C		Caretaker
NaCl		Natrium Chloride
IU		International Unit
PL		Placebo
Po		Pointing Test
SE		Standard Error
Un		Unsolvable Task
GLMM		Generalized Linear Mixed Models
Pre_OTM		Pre-Treatment Urinary OT Concentration
Pos_OTM		Post-Treatment Urinary OT Concentration
TreatOrder		Treatment Order

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1. Introduction

1.1. The Role of Dogs in Social Cognition Research

Dogs have a unique position as models for understanding human social cognition and behavior. Their ability to interpret human communicative signals has made them central to discover the evolutionary origins of social cognition (1). Unlike primates that share evolutionary homology with humans, dogs offer insights into social cognitive abilities that evolved independently during the domestication process in the human environment. This convergent evolution, together with their development in the human environment, makes dogs valuable for understanding principles of social cognition, as their abilities developed in response to similar social pressures faced by humans rather than shared ancestry (1, 2, 3).

Over 15,000 years of domestication has equipped dogs with abilities to interpret and respond to human social cues (3, 4). Dogs demonstrate a range of social cognitive skills that enable them to navigate human social environments, including gaze following (5, 6), understanding human attentional states (5, 6, 7), and responsiveness to different forms of human communication (4, 8, 9, 10). Comparative studies between dogs and wolves reveal the extent of dogs' specialization: while hand-reared wolves can eventually learn to follow human pointing gestures and other social cues, dogs demonstrate earlier ability to do so while also showing a greater proficiency in utilizing these cues (4, 11). This enhanced responsiveness appears to result from both evolutionary changes during domestication as well as developmental experiences with humans during the critical early life period (3, 5, 12, 13).

Beside their evolutionary relationship with humans, dogs have a unique position as social cognition models because of their practical advantages as research objects. They can be studied in naturalistic settings that mirror real-world social interactions, are easily accessible and highly motivated to participate in cognition research (13, 14, 15). This practical accessibility combined with their evolutionary relevance makes dogs ideal subjects for investigating behavioral mechanisms across diverse experimental paradigms and research contexts (1, 12, 13).

1.2. Cognitive Mechanisms of Canine Social Cognition

Dogs appear to possess highly refined attention mechanisms that allow them to orient to and process human social signals, including body postures, facial expressions, and ostensive cues (5, 7, 16, 17). They are flexible in these abilities, allowing them to adjust their focus based on the relevance of social information and contextual demands (5, 7).

Research has shown that dogs can form expectations about human behavior (18), adjust their behavior based on past experiences (17, 18), and remember individual humans and their behavioral patterns (17). There is also evidence implicating dogs understand aspects of theory of mind in humans, including what humans can and cannot see (6, 19). The extent of their ability to do so remains a subject of ongoing research (20).

Recent research in canine neuroscience reveals information about the neural foundations underlying dogs' social cognitive abilities by providing insights into the brain systems that support interspecies communication and social behavior. Studies using fMRI for Neuroimaging have identified brain areas in dogs that show activation to human faces and social stimuli (12, 21, 22). Some of these regions may be analogous to the human social brain network (12, 21), suggesting that the evolution of social cognition in dogs involved modifications to neural circuits that process social information (12). Regions associated with attention, reward processing, and memory during social cognitive tasks have been identified, suggesting social cognition in dogs involves coordination between multiple brain systems (12, 21). The ability to adapt to different social partners and contexts throughout a dog's life may be supported by neural plasticity mechanisms. The similarities between the dog and human social brain network suggest that dogs may also serve as models for researching psychiatric and neurological conditions that affect social cognition (12). The continued development of non-invasive neuroimaging techniques for dogs promises to further explore the neural foundations of their social cognition (12, 21).

1.3. The Pointing Test as a Paradigm

The pointing test was initially developed to examine whether non-human animals could utilize human gestural cues to locate hidden rewards. Rooted in developmental psychology, where pointing represents a pre-linguistic milestone in human infants, Michael Tomasello's 1990's research demonstrated that non-human primates failed pointing comprehension tasks,

suggesting this skill was uniquely human (23). In 1998, two independent groups simultaneously challenged this view. In 1998, Hare et al. demonstrated that dogs successfully used human communicative signals by observing Hares own dogs while working in Tomasello's laboratory (24). At the same time, Miklósi et al. (1998) showed that dogs could utilize human gestural cues to locate hidden food (25). These seminal publications catalyzed follow up studies showing dogs outperformed wolves and chimpanzees in following human pointing gestures (3), supporting the domestication hypothesis that dogs evolved enhanced abilities to read human social cues as an adaptive advantage in the human-dominated environment (3, 26). The pointing test has since evolved into a fundamental model in comparative cognition research (26, 27).

The methodological diversity within pointing test research has revealed crucial differences in how gestural variants influence dogs' performance (28). Extensive research has examined several key differences of pointing protocols, including spatial proximity (distal versus proximal pointing), temporal duration (momentary versus sustained pointing), communicative context (ostensive versus non-ostensive cues) and multimodal combinations, each resulting in distinct patterns of behavioral responses across dog populations (29, 30).

Distal versus Proximal Pointing

The spatial dimension of pointing gestures is one of the most investigated aspects of canine gesture comprehension. Research shows that the distance between the pointing hand and the target object significantly influences task difficulty and overall performance (10). The greater the distance between the pointing hand and the target object, the more challenging the task was for dogs compared to proximal pointing, where the gesture is delivered close to or touching the target object (10, 31). Most dogs perform better if the pointing gesture is performed with the same side hand (ipsilateral), rather than a cross-body (contralateral) pointing gesture (32).

Research with free-ranging dog populations showed that they could utilize distal pointing to locate hidden food better than proximal pointing cues, indicating different comprehension of the pointing gestures in pet dog vs. non-pet dog populations (15). Cross-species comparative research revealed that dogs outperformed cats across all pointing types, especially regarding distal pointing cues (33).

Momentary versus Sustained Pointing Cues

Dogs' performance in the Pointing Test varies depending on the duration and repetitions of pointing cues. Sustained pointing protocols have consistently demonstrated higher detectability and reduced between-subject variance compared to momentary pointing (30, 34). Many successful protocols also involve repeating the pointing signal until the dog makes a choice (35).

Ostensive versus Non-Ostensive Cues

Including ostensive signals to the protocol dramatically affects dogs' responsiveness and comprehension. Research shows that dogs show enhanced performance when pointing occurs within an ostensive cue including eye contact, attention-getting behaviors, and verbal address (36, 37, 38). Gaze-following behavior is specifically tuned to human communicative signals, with ostensive cues serving to activate attention and create expectations of communicative relevance (39).

Overall, ostensively framed, proximal/ipsilateral cues with a salient component (e.g. radiant colored sleeve worn by experimenter) produce highest success rates (35, 36), while distal/contralateral, non-ostensive, low-salience cues reduce performance and might result in dogs using simpler heuristics (9, 32).

Familiarity with the human pointer and breed characteristics reveal individual differences in pointing comprehension abilities, as shown in studies comparing working and non-working dogs (40, 41). Service dogs and therapy dogs consistently outperform pet dogs on complex pointing cues, although this likely results from enhanced impulse control and attention rather than superior understanding of communicative cues (42). Age-related differences in pointing comprehension have been documented and show that, while older cats showed improved performance compared to young cats, dogs maintained superior performance across age groups (33). Nevertheless, either cognitive maturation or learning experience is necessary for dogs to gain full competence, as puppies under 12 weeks demonstrate limited ability to comprehend pointing cues (2, 4). While dogs raised in environments with limited human

contact during socialization period (4-14 weeks of age) show reduced sensitivity to pointing gestures, this can be improved through socialization training (43).

The effectiveness of different pointing gestures appears to be influenced by several methodological factors, including gesture duration, reinforcement, and testing context (30, 34). Methodological heterogeneity across studies, as well as individual traits of each dog, complicate direct quantitative comparisons (32, 44).

1.4. Oxytocin and Social Behavior

Oxytocin (OT) is a neuropeptide that functions as a neurotransmitter in the central nervous system and as a hormone in the periphery (45). It is synthesized in the hypothalamus, with its release happening both centrally into the brain networks and peripherally through the posterior pituitary (45). This dual function allows OT to coordinate physiological responses such as childbirth and lactation with central regulation of social and stress-related neural networks (45). In humans, OT influences attention, social information processing and approach-avoidance decisions by enhancing sensitivity to social cues and increasing the importance of social stimuli (46). Neuroimaging studies demonstrate that OT can enhance recognition of emotional expressions and alter gaze behavior toward socially informative features such as the eye region (47). While OT promotes affiliative bonding and prosocial behavior it is important to consider that it can also promote defensive or competitive behaviors toward outgroups (48).

Given OT's role in enhancing social cue sensitivity in humans, canine cognition research has investigated whether OT similarly influences dogs' ability to interpret human social cues, engage in prosocial behavior, and form bonds by employing exogenous administration and endogenous measurement approaches (49, 50). OT promotes selective social bonds by enhancing positive social experiences and reducing stress responses during human-dog interactions (51). Dogs with higher baseline OT levels present increased attachment-seeking behaviors, separation stress and reunion behaviors with their owners (50, 51). Research shows that both human and dog experience synchronized hormonal responses during bonding behaviors like sustained eye contact and physical contact (51, 52). Furthermore, OT strengthens the human-dog bond by its ability to modulate dogs' sensitivity to human emotional states (53).

Gaze behavior in dogs is characterized by a bidirectional positive feedback loop: Mutual gaze between dogs and owners leads to increased endogenous OT levels, which in turn promotes

further gazing behavior (52). Additionally, exogenous OT administration promotes gazing behavior, supporting the role of OT in this bonding mechanism (52). OT reduces dogs' aversion to human gaze cues (53) and alters dogs' face-scanning behaviors, reducing their attention to angry facial expressions and a reduction in arousal to threatening social stimuli (54). Additionally, higher urinary OT concentrations are associated with proximity-seeking behavior toward owners (50). Similarly, food rewards have been shown to increase urinary OT levels in dogs, suggesting that positive social interactions involving food also activates the secretion of OT (55). The effects of OT are not uniform across all populations, as pet dogs show greater social responsiveness compared to shelter dogs, implying that the hormone's influence varies based on individual traits and prior experience (56).

Several studies imply that the effects of OT on dogs' prosocial and affiliative behaviors are context-dependent and influenced by individual differences (57, 58, 59). In dogs with high baseline social performance, Turcsán et al. (2022) found that OT administration potentially leads to more avoidant behaviors toward unfamiliar humans in a social cognitive test battery, while dogs with low baseline performance showed increased approach behaviors, reduced avoidance, and more affiliative responses (e.g., body contact, proximity-seeking) toward strangers after receiving OT (57). Some studies report the possibility of antisocial effects such as reduced affiliative behavior and context-dependent increases in aggression or avoidance in certain individuals (60) and less proximity seeking to their owners after OT administration than after receiving placebo (saline) (58). These findings show the importance of interpreting the effects of OT in dogs relative to context, baseline behavior and individual traits (57, 58).

1.5. The Influence of Oxytocin Administration on Dogs' Performance in the Pointing Test Paradigm

Previous studies in human social cognition research show that OT enhances detection sensitivity for biological motion, which is a critical component of gesture comprehension, and therefore increases salience for socially meaningful gestural cues (61). OT increases viewing time directed to objects an actor looked at or pointed to, showing enhanced sensitivity to pointing and gaze cues in healthy adults (62) and improves reliance on nonverbal information during social-communicative judgments, indicating better integration of nonverbal referential cues into communication processing (63).

In dogs, similar OT-mediated enhancements in communicative signal processing have been documented: A handful of studies show that intranasal OT administration improves dogs' performance in pointing-based object choice tasks, indicating enhanced processing of human communicative signals (64, 65, 66). It is hypothesized that, because of OTs' modulation of emotional and reward-processing systems, when administered OT, dogs treat human gestures as more valuable and informative, and therefore, utilize pointing cues to guide choice behavior (64, 65, 66).

In 2015, Oliva et al. were the first to document the effects of intranasal OT on dogs' pointing cue comprehension: 62 dogs received 40 IU of OT, 45 minutes before the start of an object choice task in one of their visits, while receiving 0.09 % saline in another visit. They conducted a momentary distal pointing cue with the ipsilateral arm while keeping the head straight throughout the pointing gesture for a total of 10 trials in both OT and placebo condition. The dogs' performed significantly better after receiving intranasal OT (83.8 % correct choices) compared to saline (74.1 % correct choices) (64).

In a study by Macchitella et al. in 2016 with a different methodological approach, similar findings could be observed: 14 dogs received intranasal OT calculated based on their individual weight (2 IU/kg) in one visit, while receiving a placebo in another. After 15–17 minutes, 20 experimental trials conducting a momentary distal pointing cue with the ipsilateral arm while looking at the baited subject were carried out, showing a significantly better performance in OT conditions (72.1 % correct choices) compared to placebo conditions (65.7 % correct choices) (65).

In the most recent study observing the effect of OT on dogs pointing cue comprehension, Oliva et al. in 2019 compared the number of correct choices between a group of dogs which received 24 IU intranasal OT to a placebo group (0.09 % saline) in a between-groups experimental design. The pointing gesture conducted was a momentary distal pointing cue with the ipsilateral arm while keeping the head straight throughout the pointing gesture. Only the dogs who chose 10 or more times out of possible 20 trials were included in the analysis, scoring 82.05 % (N= 8) correct choices in the OT group compared to 61.80 % (N= 12) in the placebo group (66). Furthermore, this study demonstrated that OT increased the percentage of correct choices in a pointing task without increasing the number of attempts, suggesting that OT enhances decision accuracy rather than motivation or persistence (66). These findings indicate

that OT may improve the quality of social information processing rather than simply increasing engagement with a task (66).

In all three studies mentioned, odor control trials were conducted. The procedure was the same as in the test trials, but a pointing gesture was not performed. These control trials were implemented to ensure that the dogs would not rely on olfaction to locate hidden food, that OT does not alter olfactory perception and performance improvements after OT administration were specific to social cue trials. These three independent studies found that the dogs did perform below or at chance level in the odor control trials in both OT and placebo conditions, therefore providing strong validation that the observed effects of OT on pointing comprehension are genuine effects on social communicative processing rather than artifacts due to olfactory discrimination (64, 65, 66).

Overall, there is strong evidence that supports enhanced pointing performance in dogs after OT administration, likely mediated through increased positive expectancy regarding human cues and altered emotional valuation. However, questions remain regarding optimal dosing, timing parameters, experimental conditions, and individual variables under which these enhances occur, highlighting the necessity for further research of OTs' role in canine social cognition (64, 65, 66).

1.6. Methodological Advances in Oxytocin Administration

Approaches to administer OT in animal cognitive research have evolved from highly invasive surgical interventions to more gentle, less invasive techniques (67). The route of administration significantly impacts central nervous system penetration, peripheral exposure levels, and stress responses that can alter behavioral observations (68).

Invasive techniques like insertion of cannulae into brain ventricles to administer OT directly into the cerebrospinal fluid may provide a reliable delivery to the central nervous system, but considering the degree of invasiveness, risk of infection and overall stress on the animal (69), this is not a reasonable approach for cognitive research. Intravenous and subcutaneous injection of OT faces challenges crossing the blood-brain barrier. Previous research observed peripheral physiological effects (changes in heart rate, blood pressure, and stress hormone levels) with each of these routes, while stress associated with restraint can alter treatment effects by activation of endogenous OT release (70, 71). These factors and overall

consideration of animal welfare make invasive administration techniques suboptimal for cognitive research.

Currently, intranasal OT administration by nasal spray or droplet application is the mostly used method for cognitive research due to its non-invasive nature and effective central nervous system penetration. With this approach OT transfers from the olfactory epithelium directly through the olfactory and trigeminal nerve into the brain (67). This method requires only a brief manual restraint and, therefore, reduces unwanted physiological stress-responses (72). However, intranasal delivery faces limitations including peripheral spillover, leading to elevated plasma oxytocin levels, and variable absorption between subjects due to different nasal cavity anatomy, individual respiratory patterns, and mucus production (68, 73). Despite these limitations, intranasal administration offers an acceptable balance between experimental practicability, central delivery, and animal welfare considerations.

Recently, the approach to administer OT by nebulized and aerosol delivery systems have been introduced, potentially offering reduced peripheral exposure and improved drug deposition compared to traditional intranasal routes. For this, devices producing fine aerosol particles, typically between 1 – 5µm in diameter, are being used. Research on rhesus macaques have shown the advances of this technique by scoring similar cerebrospinal fluid OT levels compared to intranasal sprays but significantly reduced peripheral plasma concentration after administration (68).

Despite theoretical advantages of nebulized OT administration, there are no recorded studies that utilize nebulized OT in relation to the pointing test. Schaebs and colleagues (2020) investigated the efficacy of intranasal spray versus vaporized OT administration to identify a reliable non-invasive method for OT administration in dogs (N = 20). In this study a vaporizer mask was used for the aerolized treatment after the dogs received training to get them used to comfortably placing their snout in the mask. Results show that urinary OT concentrations were significantly elevated in all dogs when using the vaporizer, but not when using the nasal spray (74). These findings suggests that vaporized OT application may be the preferred method for future canine cognitive research on the effects of OT in dogs.

Hattori and colleagues (2024) invented a novel approach to administer OT without previous training by providing a continuous stream of food inside a container that's connected to a nebulizer. In this study, pet cats received high-quality cat food through a syringe to keep their snout voluntarily in the container while receiving the treatment (75). Similar administration

methods of OT have been employed in chimpanzees, bonobos (76) and horses (77). This method offers advantages regarding minimal stress levels related to inhibitory control and minimal time expenditure, unlike previously described training methods.

1.7. Study Aims and Hypotheses

The present study employs the novel method of Hattori et al. (2024) of nebulized OT administration that eliminates the need for mask training. This approach offers various advantages compared to previously used methods: it requires no prior training, doesn't cause adverse reactions caused by restraint and presents treatment in a positive context associated with a food reward. Adapting this method to dogs can be a significant methodological advancement, as it's non-invasive and low-stress for the dogs, while also being easy and time-efficient for researchers.

The primary aim of this study is to investigate whether nebulized OT administration, delivered via this novel method, enhances dogs' performance on the pointing test compared to placebo (0.9% NaCl). Specifically, this thesis focuses on evaluating the influence of OT on dogs' ability to perceive human pointing cues, measured by choice (correct/incorrect) as a binominal variable using a within-subject, double-blind experimental design.

This thesis represents one component of a larger research project. Other aspects of this project, covered in separate theses, include the validation of the nebulized administration method through urinary OT level analysis and the examination of OT's effect on dogs' gazing behavior toward humans using the unsolvable task paradigm.

Furthermore, based on established literature demonstrating OT's effects on canine social cognition we formulate the following hypotheses:

Dogs will demonstrate significantly better performance in the pointing test after receiving nebulized OT compared to placebo (sodium chloride (NaCl) 0.9%). This hypothesis is grounded in previous studies showing that OT treatment improves dogs' accuracy in interpreting human pointing cues (64, 65, 66), and assumes that the novel administration method will achieve comparable results.

2. Materials and Methods

2.1. Recruitment of participants

For the participation in this project caretakers were recruited through multiple channels. The main component of the recruitment process involved actively reaching out to caretakers who already participated in other projects in the Clever Dog Lab with their dog.

In addition to direct outreach, posts on social media platforms (Facebook, Instagram) were shared to make caretakers aware of the project. These posts contained general information about the project, as well as the inclusion criteria. Furthermore, information about the project was featured on the Clever Dog Labs' homepage. Caretakers who reached out to express interest in the study received an informational document explaining the procedure.

2.2. Test subjects

Dogs that were between one and twelve years old, weigh between 10 and 40 kg, and were generally in good health were eligible for participation. Other criteria were a high food drive, as well as no existing allergies or sensitivities to the food that was used in this project. Intact female dogs were not allowed to participate while in heat to prevent distraction to other dogs in the Clever Dog Lab.

A total of 43 dogs participated in the project. The dogs had a mean age of 6.4 ± 3.1 years. However, only 26 dogs were included in the final data analysis, as urinary samples could not be obtained from 14 dogs, two dogs did not pass the pre-test training, and one dog was excluded due to owner interference. A detailed list of participants can be found in the appendix.

2.3. Study design

All dogs were tested in a within-subject design. They came in for two appointments and were tested once in each condition (OT or placebo) with at least ten days between sessions. The median value of the number of days that passed between the two appointments was 16 (range: 10–43 days). This project implemented a double-blind procedure: neither the caretakers nor the experimenters were aware of the treatment condition.

Before each visit, caretakers were instructed not to feed their dogs for at least two hours. At arrival at Vetmeduni Vienna a urine sample was collected from each dog before entering the Clever Dog Lab. During the appointment, caretakers remained in the room with their dog but were instructed not to interact with them unless they were asked to by the experimenter, including avoiding eye contact or giving food. Before treatment began, the owners completed a consent form. After receiving the treatment, the caregivers were asked to wait with their dogs for 40 minutes, as this is the estimated time required for OT to reach peak levels in the brain (78). The dogs then participated in two behavioral tests: The Pointing Test and The Unsolvable Task. The order of treatments and behavioral tests were counterbalanced across dogs. After the behavioral tests were conducted, a second urine sample was collected from the dogs. The urine samples were prepared and stored for further testing immediately after collection. To measure urinary OT concentrations a commercial Enzyme Immunoassay Kit (Arbor Assays, Ann Arbor) that has been validated by G. Wirobski et al. was used (79). The analyses have been conducted at the hormone lab of Virgine Canoine at the University of Vienna. Given the recognized effect giving food on the secretion of endogenous OT, which potentially boosts its release (55), during the experiment all dogs received an equal amount of food.

In this diploma thesis I will specifically thematize the Pointing Test. A detailed description of the other topics within this project is available in diploma and master theses of other students that participated in this project. Five experimenters were involved in this project (Shany Dror, PhD., Tereza Benešová, Nicole Rudi, Leonie Belsky, Vanessa Matt).

2.4. Treatment

In this project 24 international Units (IU) OT (4084-v by Peptide Institute, Inc. + 0.15 ml NaCl 0.9% by Brown) and a placebo (0.15 ml NaCl 0.9% by Braun) were administered. The OT used in this study is Oxytocin 4084-v manufactured by Peptide Institute. This specific substance was chosen because it is completely odorless. The treatment solutions were prepared by a person who was not involved in the study otherwise.

Apparatus

The apparatus used for the nebulized treatment was purpose-built in the workshop of the Messerli Research Institute by Markus Fitzka for this project. It was attached to a wooden

board for stability, and the height was adjustable to the dog's size (Fig. 1). The circular transparent plastic container at the top was designed to fit the dogs' snout. Two openings were cut out at the back of the container: the lower opening allowed for the attachment of a syringe filled with wet food, while the upper opening was used to connect the nebulizer. The mesh nebulizer used in this project was the model MESH – S600C by MedPath, Germany. It is a portable nebulizer, originally designed for administering nebulized medication to children.



Figure 1: Apparatus

Familiarization

In a familiarization phase, each dog was encouraged to explore the nebulizer and apparatus while being fed small pieces of food. The food used in this project was Activa Friends Mini Sticks. At first the experimenter fed the dog with one hand while handling the nebulizer with the other hand (operating it with NaCl), turning it on and off at the lowest setting and gradually rotating it toward the dog to make the mist visible. Once the dog was comfortable with the clicking sound from turning the nebulizer on and off and the mist near their face, food was placed inside the plastic container to encourage the dog to insert the snout. When the dog was comfortable with this, the nebulizer was attached to the apparatus at the lowest setting and the

dog continued receiving food while keeping its snout inside the container. During the familiarization phase, every dog received 20 pieces of food.

Administration

For treatment the nebulizer, now filled with either OT or placebo, and a syringe filled with wet food (activa CLASSIC Leberwurst Light) were attached to the apparatus. Once the dog was comfortable placing its snout inside to eat from the syringe, the nebulizer was turned on at the lowest setting of 0.15 ml/minute, as shown in Figure 2. At this point, the stopwatch was started. Timing was paused whenever the dog removed its snout from the container and resumed when the snout was placed back in. After 60 seconds had elapsed, the dog received the entire treatment of 24 IU and the nebulizer was turned off. By then, the dog also received 15 ml of wet food from the syringe.



Figure 2: Administration of Treatment

During the waiting time, the experimenter explained the upcoming tests and the caretaker's role in them, which consisted of releasing and recalling their dog on cue. Depending on which behavioral test was conducted first, the time between nebulized treatment and the start of the pointing test was either 40 or 55 minutes.

2.5. Pointing test

2.5.1. Experimental set up

The setup used in this project was based on the design of the Many Dogs 1 study (37). The project took place in one of the rooms (approximately 19.2 m²) at the Clever Dog Lab at the Veterinary University Vienna (Fig. 3). The room was always equipped with a water bowl and a blanket for the dogs during the waiting period between treatment and behavioral tests. Four ceiling mounted cameras recorded the entire procedure.

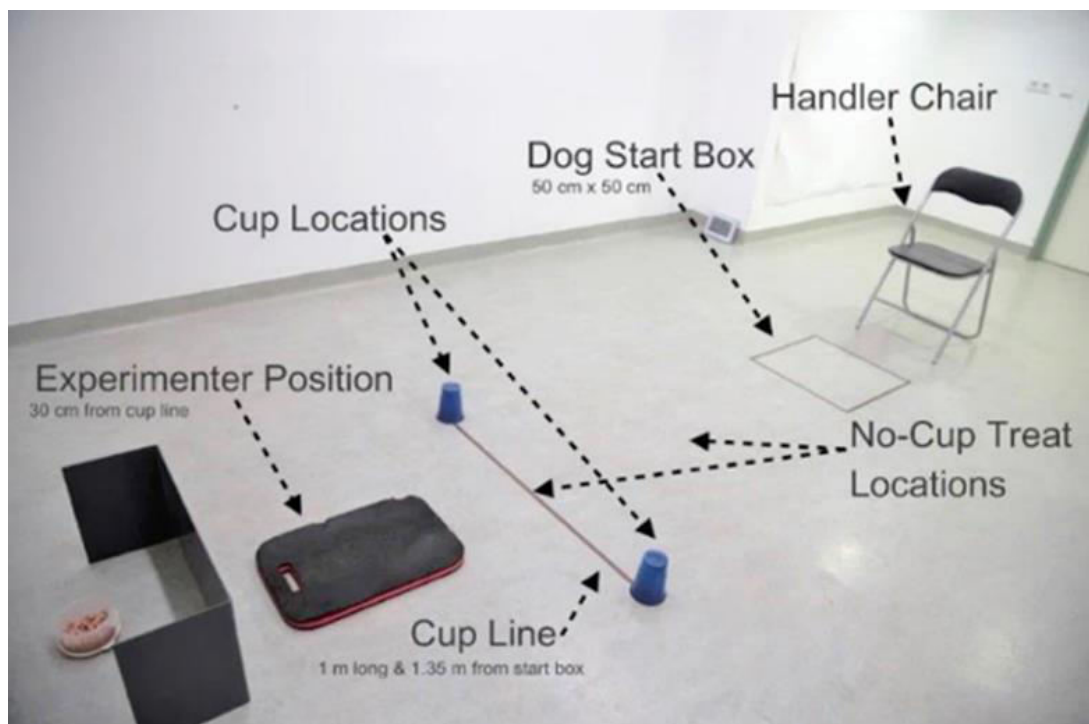


Figure 3: Experimental set up (ManyDogs Project 2023)

Using the pointing test the caretaker was seated on a chair and handed a leash to prevent the dog from roaming freely in the room. They also received a baseball cap and were

instructed to keep their head facing downward to avoid seeing the location to which experimenter points (as seen in Figure 4). This ensured that caretakers could not provide unintentional cues to their dogs. Marked on the floor in front of the caretakers' chair was a "starting box" (50 x 50 cm) from which the dog began each trial. The caretaker was responsible for keeping their dog in this starting position, as well as releasing and recalling it on cue.

The distance between the start box and the cup line was 1.35 m. The cups were placed along this line with 1 m between them. 30 cm behind the cup line, Experimenter 1 (E1) was seated on a sitting pad on the floor. E1 wore wireless headphone to communicate with Experimenter 2 (E2), who provided instructions regarding side alternations from outside the room. When at rest, E1 got in a neutral position with hands placed on the thighs and a gaze downward. E1 was responsible for handling the cups, presenting treats, and giving cues to the caretaker while ensuring that no unintentional cues were provided to the dogs. The treat pouch was placed on E1s back, out of the dogs' sight, to prevent distraction from the cups. An occluder was used to block the dog's view when food was hidden under the cups and was stored behind E1s back once it was not needed. A second chair, placed behind E1, was used for storing the cups.



Figure 4: a) Caretaker and dog in starting position b) View from behind E1.

For this project two separate pairs of plastic cups were used:

1) Cups for warm-up phase and test trials

A false bottom was created by stacking two cups together. One cup was sham baited, as a treat was lodged in the false bottom. Small holes were poked into the bottom to enable the dogs to smell the treat. The use of the sham baited cups was employed to ensure that the dogs could not rely on olfactory cues when choosing a cup.

2) Cups for odor control trials

The odor control trials were conducted to monitor if any potential OT-induced changes in the dog's sense of smell or a change of strategy to rely on olfactory, once a pointing cue was not conducted, can be observed. Therefore, while these cups visually resembled the other set of cups (containing a false bottom), they were not sham baited.

2.5.2. Warm up phase

Before the test trials, a warm-up phase was carried out to ensure that the dogs understood the concept of food being hidden under the cups and that they can receive it by approaching and touching the cup. These two phases were based on the warm-up phase protocol in ManyDogs Project (2023).

One-Cup Alternating Trials

In this warm-up phase only one cup was used (Fig. 5). The dog was placed in the starting position. E1 presented a piece of food while making eye contact with the dog and calling its name. The food was then hidden under the cup while the dog observed the baiting procedure. Afterward, E1 got into a neutral position with hands behind the back and a gaze lowered and gave the verbal cue "Okay" to indicate to C that the dog could be released. The position of the cup alternated between left and right side on the cup line.

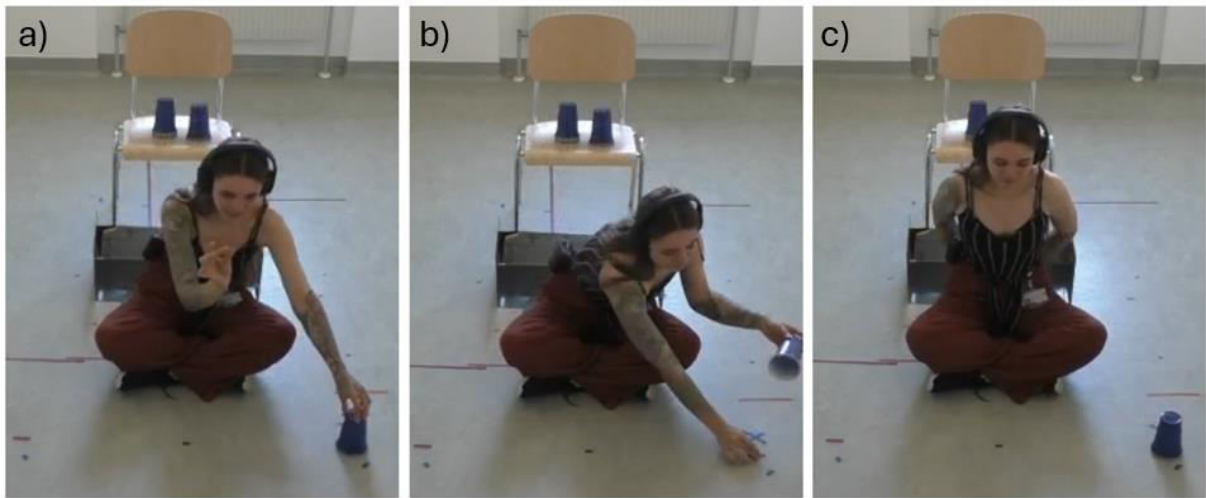


Figure 5: *Performing the One-Cup Alternating Trials:*
 a) Showing the piece of food while calling the dogs' name
 b) hiding the food under a non-baited cup
 c) getting back into neutral position, giving the releasing signal

If the dog did not approach the cup within 25 seconds, it was called back into the starting position and the trial was repeated on the same side up to three times. The criteria for passing this phase was for the dog to approach the cup four consecutive times in a row in a total of 7 trials. If this criteria was not achieved, the dog would have been excluded. In case the dog did not need all seven trials to reach this passing criteria, the remaining food was given to them at the end of this phase.

Two Cup Visible Placement

For this phase a sham baited cup was added. This cup contained a piece of food hedged in the false bottom. The baiting procedure was performed in the same manner as in the “one cup-alternating trials”. The dog observed as the experimenter placed a piece of food under the non-baited cup. The baiting position alternated sides between left and right.

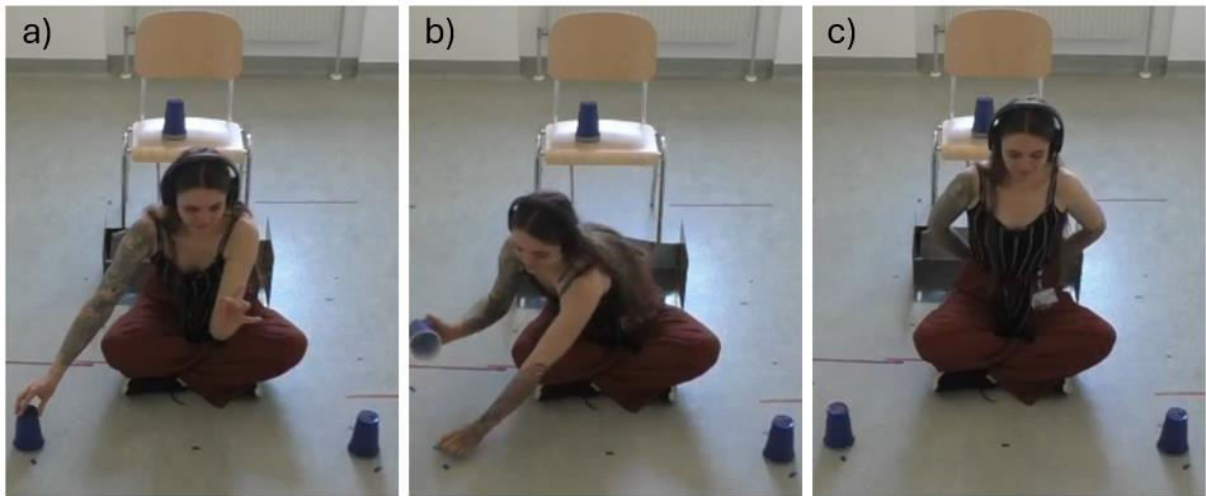


Figure 6: *Performing the Two Cup Visible Placement:*
 a) Showing the piece of food while calling the dogs' name
 b) hiding the food under a non-baited cup
 c) getting back into neutral position, giving the releasing signal

If the dog did not approach the cup within 25 seconds or chose the wrong cup, it got send back into the starting position and the trial was repeated on the same side up to three times. In case the dog failed the same trial three times, a correction trial was performed: The false baited cup was removed, and the trial was performed with only one cup. The passing criteria for this phase was the same as in the previous phase.

2.5.3. Test trials

The test trials started with the dog in its starting position (Fig. 7). E1 set up the occluder in front of them to hide the food under one of the cups without the dog being able to see the baiting. While one of the cups was false baited, the non-baited cup was used to hide the food under.

E1 removed the occluder and placed it behind them. To ensure that the position of the occluder did not influence the dogs' choices, E1 alternated between turning right and left when placing the occluder behind them.

After the occluder was removed, E1 slid the cups to their final position simultaneously. With both hands behind their back, E1 made eye contact with the dog while saying their name to get the dogs attention.

The pointing was performed with the same side hand to the baited location while gazing at it for 1–2 seconds with the other hand to the back. This section of the protocol deviated from the one used in ManyDogs Project (2023), where the experimenter pointed with the contralateral hand and maintained eye contact with the dog while performing the pointing gesture (instead of looking at the baiting location).

E1 then went into a neutral position with both hands behind their back and head facing down saying “Okay”. The dog was then released by C and was allowed to choose a cup within 25 seconds.



Figure 7: Test trials procedure:

- a) Baiting the cup behind the occluder*
- b) Putting the occluder behind the back*
- c) Sliding the cups to their position simultaneously*
- d) Making eye contact, calling the dogs' name*
- e) Performing the pointing cue for 1-2 seconds*
- f) Going into a neutral position with lowered gaze, giving the releasing signal*

If the dog made an incorrect choice, E1 told C to recall the dog. Once it was back in the starting position, the piece of food was tossed to them. This was to ensure that all the dogs received the same amount of food by the end of the trial.

Overall, ten trials were performed. Based on a semi-randomly determined order, the food was either hid under the left or right cup, five trials per each side. The same side was not used more than twice in a row to prevent side bias. After trial five and trial ten, an odor control trial was performed, one per each side.

Odor control

The odor control trials were executed exactly like the test trials with the two differences: a set of new cups was used (see description in chapter 2.5.1.) and the pointing cue was not performed. Overall, each dog received two odor trials.

We ensured that by the end of the test, regardless of their performance, all the dogs received twelve pieces of food (ten for test trials, two for odor controls).

2.6. Data collection

While the pointing test was performed, E2 gave instructions regarding side alternations and watched the procedure through cameras from outside the testing room. Everything was documented by E2 in an excel sheet. This sheet contained the experimenters name, order of side alternations, time stamps for the beginning of each phase and odor controls, and the dog's choices in each trial (1= correct, 0 = incorrect).

2.7. Data analysis

To assess if OT influenced dogs' performance in the pointing test trials a Generalized Linear Mixed Model (GLMM) was fitted, using the `glmer()` function from the `lme4` package (80) with a binominal error distribution and logit link function. Model optimization was performed using the `bobyqa` optimizer with a maximum of 200.000 iterations to ensure convergence.

Model for Test Trials

- Response Variable: Choices (binary): Correct (1) or incorrect (0) selection of baited location
- Test predictor:
 - Post-treatment urinary OT concentration (Pos_OTM): Log-transformed and z-standardized. We chose to use the posttreatment urinary OT levels as the test predictor, instead of the treatment, as we hoped it would better account for the individual variability in the dog's physiological reaction to the treatment (for a similar approach see Pedretti et al., 2021).
- Fixed Effects:
 - Pre-treatment urinary OT concentration (Pre_OTM): Log-transformed and z-standardized
 - Test order (TestOrder): Binary factor indicating whether the pointing test (Po) or unsalvable task (Un) was conducted first.
 - Treatment order (TreatOrder): Binary factor indicating whether OT or placebo (PL) was administered first.
 - Sex: Binary factor (male/female)
 - Age: z-transformed
 - Visit number (Visit): Binary factor (Visit 1 or Visit 2)

We included random intercepts and slopes to account for individual variation and repeated measurements: Random intercepts for each dog (Subject), random slopes for z-standardized and log-transformed post-treatment OT (z.log.Pos), z-standardized and log-transformed pre-treatment OT (z.log.Pre) as well as random slope for visit number (Visit).

Model Formula:

choices ~

z.log.Pos_OTM +

z.log.Pre_OTM + TestOrder + TreatOrder + Sex z.Age + Visit +

(1 + Visit + z.log.Pre + z.log.Post || Subject)

The model met all the necessary assumptions, including normal distribution of Best Linear Unbiased Predictors (BLUPs), sufficient number of observations per estimated effect, and

absence of collinearity. Model stability was assessed by comparing the model's estimates with those from models where each random effect was excluded one at a time. This assessment confirmed good model stability.

For visualization purposes, we generated 95% confidence intervals using a parametric bootstrap procedure with 1000 resamples applied to the final GLMMs. All predictors were standardized, and binary predictors centered. Predicted values were computed across the observed range of post-test OT concentrations and plotted with bootstrapped confidence intervals to visualize the relationship between treatment effects and performance.

Model for Odor Control Trials

We used the same model structure as in the test trial's GLMM. All model assumptions were satisfied, and model stability was assessed as moderate.

Above-Chance Performance Analysis

We conducted analyses for each combination of trial type and treatment condition, where we fitted an intercept-only binominal GLMM with choice as the response variable and a random intercept for Subject to account for repeated measurements within individual dogs.

Model Formula: $\text{choices} \sim 1 + (1 \mid \text{Subject})$

We used emmeans package (81) to derive response estimates (probabilities) from intercept-only models, reflecting the general likelihood of making correct choices in test trials or identifying the baited location in odor control trials.

3. Results

Results of Test Trial Model

The analysis of the full model revealed that post-treatment OT concentrations did not significantly influence dogs' choices in the pointing test trials ($\beta = 0.15$, $SE = 0.18$, $z = 0.83$, $p = 0.409$). Among the control predictors, none of the predictors reached statistical significance, except for sex, with males performing less accurately than females ($\beta = -0.66$, $SE = 0.32$, $z = -2.06$, $p = 0.04$; see Table 1).

Table 1: Results of GLMM examining the influence of post-treatment urinary OT concentrations on the dogs' choices in the pointing test trials (SE = Standard Error), N = 26.

Predictor	Estimate (β)	SE	z	p
(intercept)	2.44	0.35	-	-
z.log.Pos_OTM	0.15	0.18	0.83	0.409
z.log.Pre_OTM	-0.16	0.27	-0.58	0.564
Treatment order (PL > OT)	0.36	0.31	1.15	0.249
Test order (Un > Po)	-0.50	0.33	-1.54	0.122
Sex (male)	-0.66	0.32	-2.06	0.040
z.Age	-0.09	0.17	-0.53	0.595
Visit	-0.2	0.14	-0.15	0.880

The relationship between post-test OT concentrations and performance is shown in Figure 8 with 95% confidence intervals.

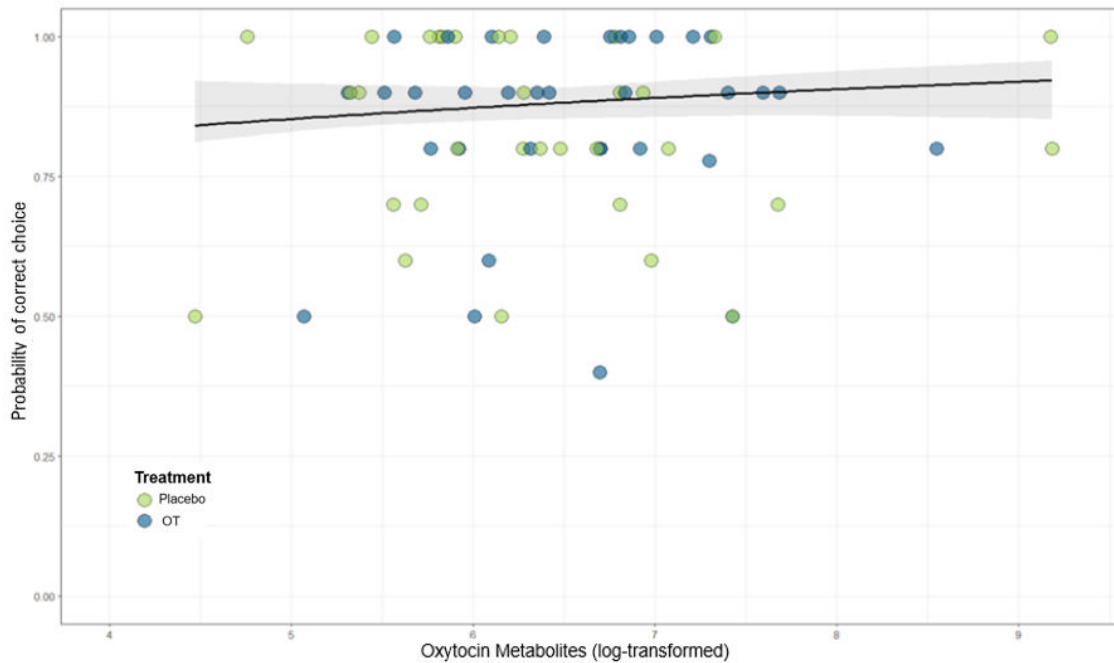


Fig 8: The probability of correct choices plotted against post-test oxytocin concentrations. Fitted model with 95% confidence intervals plotted on top of the raw data (green dots = performance in placebo conditions, blue dots = performance in OT conditions).

Results of Odor Control Model

In the odor control trials, an examination of the used model revealed that post-treatment OT concentrations did not significantly influence the dogs' choices ($\beta = -0.03$, $SE = 0.22$, $z = -0.15$, $p = 0.885$; see Table 2). Additionally, none of the control predictors had a significant influence on the dog's choices.

Table 2: Results of GLMM examining the influence of post-treatment urinary OT concentrations on the dogs' choices in the odor control Trials (SE = Standard Error).

Predictor	Estimate (β)	SE	z	p
(intercept)	0.55	0.38	-	-
z.log.Pos_OTM	-0.03	0.22	-0.15	0.885
z.log.Pre_OTM	0.07	0.24	0.28	0.777
Treatment order (PL > OT)	0.19	0.38	0.48	0.629
Test order (Un > Po)	-0.51	0.36	-1.41	0.158
Sex (male)	-0.28	0.38	-0.75	0.453
z.Age	0.08	0.20	0.37	0.709
Visit	-0.05	0.18	-0.25	0.803

Results of Above-Chance Performance Analysis

The model used to determine above-chance performance showed that dogs performed above chance in both OT and placebo conditions, while in the odor control trials, their performance did not differ from chance (see Table 3). Table 4 shows the calculated response scale outcomes for the intercept-only models.

Table 3: Results of the intercept-only models, assessing whether dogs performed above chance with the different treatments in the different trial types.

Trial type	Treatment	Est. (β)	SE	z	p
Test trials	OT	2.17	0.27	8.08	<.001
	PL	1.88	0.23	7.97	<.001
Odor control	OT	-0.03	0.23	-0.11	0.91
	PL	0.37	0.23	1.59	0.11

Table 4: Results in a response scale for the intercept-only models, assessing whether the dogs performed above chance in different trial types and treatment conditions.

Trial type	Treatment	Probability	SE	Upper CI	Lower CI
Test trials	OT	0.89	0.02	0.83	0.94
	PL	0.87	0.23	0.81	0.91
Odor control	OT	0.49	0.06	0.38	0.61
	PL	0.59	0.06	0.48	0.69

4. Discussion

This study investigated the effects of nebulized oxytocin administered to dogs in a pointing task. Dogs performed above chance in both OT and placebo conditions, demonstrating robust pointing comprehension regardless of treatment. Contrary to our initial hypothesis, we found no significant difference in pointing test performance between the OT and placebo conditions. The absence of significant effect stands in contrast with several previous studies that reported improvements in dogs' use of pointing cues after OT administration (64, 65, 66).

Methodological Considerations: Pointing Paradigm and Ceiling Effects

Three previous studies have demonstrated that intranasal OT administration enhances dogs' performance in pointing tasks. Oliva et al. (2015) were among the first to show that intranasal OT significantly improved dogs' performance. In their study they employed a momentary distal pointing cue with their ipsilateral arm and used ostensive signals (gazing at baited bowl while conducting pointing gesture). The dogs that received OT achieved 84 % correct choices, compared to 74 % for the dog's receiving saline (64). This finding was replicated by Macchitella et al. (2016) by using a similar pointing cue configuration. The tested dogs achieved 72.1 % correct choices in OT conditions, compared to 65.7 % correct choices in placebo conditions (65). More recently, Oliva et al. (2019) showed an improved performance after OT administration in a pointing task with the same pointing cue configuration, resulting in 82.05 % correct choices compared to 61.80 % correct choices after placebo was administered (66).

In a large-scale research project (N = 704), the ManyDogs Project 2023 investigated dogs' pointing comprehension using pointing cues that turned out to be even more challenging: they utilized a contralateral momentary pointing cue without gazing to the target and compared the performance conducting ostensive cues (getting the dogs attention by calling their name and maintaining eye contact with the dog while pointing at the target) and non-ostensive pointing gestures. Although the dogs performed above chance with this protocol, their performance in the ostensive condition was only 53%, which is relatively low compared to the previously mentioned pointing studies.

We suggest that the absence of a significant treatment effect in our study can be attributed to the specific pointing cue configuration used (proximal, momentary, ipsilateral pointing cue with ostensive signals), which allowed dogs to perform near-optimally even in placebo condition, resulting in a ceiling effect. The concept of a ceiling effect is central to understanding our null

findings. It occurs when task performance is so high in the baseline or control condition that there is minimal room for an experimental manipulation to produce a measurable improvement, likely masking any potential enhancement that OT might have provided. A critical difference between the mentioned studies and ours lies in the baseline performance levels and the nature of the pointing cues employed.

Although our experimental setup was based on the setup used in the ManyDogs Project 2023, we deliberately deviated from their conducted pointing gesture. We created a simpler spatial relationship between the pointing gesture and the target by employing ipsilateral rather than contralateral pointing. Additionally, we included ostensive cues in all trials rather than manipulating them as an experimental variable. Contrary to mentioned studies investigating the effect of intranasal OT to pointing comprehension, we conducted a proximal pointing cue, which provides clearer spatial information than distal pointing (55). These choices were made with the intention of establishing a sufficiently high baseline performance that would allow detection of OT-induced changes in either direction – both improvements and potential decline in performance.

However, in retrospect, our methodological choices appear to have created a task that was too easy, resulting in a ceiling effect that limited our ability to detect OT-induced changes in performance. The high baseline performance in our placebo condition suggests that our dogs' performance was near their maximum capacity for this task, leaving little room for OT to produce measurable improvements.

Methodological Considerations: Study Design and Timing

We employed a within-subject design with at least ten days between treatment conditions and counterbalanced treatment orders across subjects. While this design offers advantages in statistical power, it also has the potential for carryover effects. Pharmacokinetic studies indicate that serum OT peaks 15 minutes after intranasal administration and returns to baseline by 45 minutes, while urinary OT peaks later at 45–60 minutes and declines slower (57). Based on these reports, our washout period appears to be sufficient. However, behavioral effects of intranasal OT have been reported to persist for 5–15 days after a single dose (64). This discrepancy between pharmacokinetic clearance and behavioral effects raises the possibility of behaviorally mediated carryover effects.

The pointing test was conducted 40 or 55 minutes after OT administration, depending on the order of behavioral tests. This testing window aligned with standard protocols in the literature indicating that OT reaches peak levels in the brain (78) and behavioral improvements in object-choice tasks are reliably observed when testing occurs approximately 40 minutes after administration (64). However, the interindividual variability in peripheral OT peaks and behavioral changes (57) means that our standardized testing time may have captured different states across subjects, with some dogs at peak effect and others already declining, especially considering that the testing window varied (40 or 55 minutes after OT administration).

Limitations

A limitation of this study is that while we measured urinary OT concentrations using a validated enzyme immunoassay (79) to confirm systemic absorption, peripheral measurements alone cannot definitely prove that OT crossed the blood-brain barrier. Some research has reported improved task performance after intranasal OT without detectable plasma elevation (66), suggesting that behavioral effects can occur even without measurable peripheral change. But, overall, the evidence of peripheral and central OT correspondence remains limited in canine research. Only direct cerebrospinal fluid sampling could confirm central penetration, which was not feasible in this study with pet dogs.

Although our main analysis was focused on group-level effects, individual dogs exhibited significant variability in baseline pointing understanding and OT treatment responses, but our sample size ($N = 26$) limited the ability to conduct sufficiently powered tests on individual differences. This variability aligns with growing evidence that dogs' behavioral responses to intranasal OT depend on multiple factors including breed, sex and neuter status, baseline social competence and owner attachment style (57, 59, 83). Notably, we observed a sex effect, with males performing less accurately than females. This pattern is consistent with some prior research showing sex differences in social cognition and OT responsiveness (59, 82). Practically, this indicates that OT-based research may need to be tailored to individual dogs' profiles rather than applied uniformly to a sample of varying breeds, ages, and sex/neuter status.

Another limitation to consider is that this thesis is focused on choice accuracy only in the pointing test. Considering OT's effect on gaze patterns and persistence (52, 53), more sensitive measures might have detected subtle OT effects even when accuracy was at ceiling. However, while this thesis specifically addresses the performance in the pointing test after OT

administration, another behavioral test (the Unsolvable Task) was conducted in this project. In other theses within this project, the effects of OT on gaze behavior are addressed.

Conclusion and Future Directions

This study investigated whether nebulized OT enhances dogs' pointing comprehension using a within-subject design with 26 pet dogs. Contrary to our hypothesis and previous findings (64, 65, 66), we found no significant difference between OT and placebo conditions, with dogs performing high above chance in both conditions. We attribute this null finding primarily to a ceiling effect resulting from our methodological choices: the combination of ipsilateral, proximal pointing cues with ostensive signals created a task that was too easy, allowing dogs to perform near-optimally in the placebo condition, leaving minimal room for OT-induced improvements. Additional limitations include the inability to confirm that OT crossed the blood-brain barrier, the focus on choice accuracy only, and individual variability that our sample size could not adequately address.

These findings highlight several directions for future research. Future studies should carefully adjust task difficulty to avoid ceiling effects by using more challenging pointing configurations such as distal or contralateral cues or modifying ostensive cues. Second, research should take individual difference moderators like breed, sex, neuter status, and baseline social competence into consideration when comparing the performance of OT and placebo conditions in object choice tasks. Third, studies should incorporate multiple dependent measures beyond choice accuracy, which may detect subtle OT effects even when accuracy reaches ceiling. Finally, research should aim to clarify questions regarding central nervous system penetration after intranasal administration, discrepancy between pharmacokinetic clearance and prolonged behavioral effects and individual variability in absorption and peak response times of OT.

In conclusion, while this study did not detect significant OT effects on pointing comprehension, it provides valuable methodological insights for further research, underscoring the importance of task difficulty and multi-method approaches in pharmacological research on canine social cognition.

5. Appendix

Table 1: List of all dogs that participated in this project

** excluded from data analysis due to failed urine collection*

*** excluded from data analysis due to failed pre-testing phase or owner interference during test trials*

Dog ID	Breed	Age in years	Sex	Castrated	Days between visits	Included in data analysis
1	Mixed Breed	2.69	F	Yes	18	Yes
2	Border Collie	11.11	M	Yes	17	No*
3	Border Collie	7.21	F	Yes	11	No *
4	Mixed Breed	1.97	F	Yes	14	No*
5	Romagna Waterdog	7.55	M	Yes	11	Yes
6	Border Collie	11.12	M	Yes	13	Yes
7	Australian Kelpie	3.27	M	No	12	Yes
8	Mixed Breed	7.31	M	Yes	28	Yes
9	Mixed Breed	10.92	M	Yes	14	Yes
10	Mixed Breed	7.04	M	Yes	10	Yes
11	Mixed Breed	4.21	M	Yes	20	Yes
12	Mixed Breed	8.25	M	Yes	15	Yes
15	Golden Retriever	9.81	F	No	34	No*
16	Golden Retriever	2.77	F	No	34	Yes
17	Mixed Breed	5.93	F	Yes	12	Yes
18	Golden Retriever	10.97	M	Yes	16	Yes
19	Mixed Breed	9.18	M	Yes	11	Yes
20	Basset Hound	6.19	M	Yes	27	No**
21	Australian Shepherd	7.76	M	Yes	31	Yes
22	Fox Terrier	8.05	M	Yes	27	No**
23	Welsh Corgi Pembroke	3.92	F	Yes	23	No*
24	Mixed Breed	5.13	F	Yes	17	No*
25	Podenco	10.67	M	Yes	40	Yes
26	Podenco	5.67	M	Yes	17	No*
27	Mixed Breed	4.68	M	Yes	15	No*
28	Golden Retriever	2.38	F	No	43	Yes
29	Labrador Retriever	6.31	M	No	17	No
30	Australian Shepherd	2.24	F	No	26	Yes
31	Schnauzer	11.11	F	Yes	16	Yes
32	Eurasier	4.15	M	No	15	No*
33	Labrador Retriever	10.04	F	Yes	18	Yes
34	Labrador Retriever	3.72	M	Yes	12	Yes
35	Mixed Breed	2.88	M	Yes	13	No**
36	Kooikerhondje	7.80	M	No	25	No*
38	Labrador Retriever	7.75	F	Yes	13	Yes
39	Welsh Corgi Pembroke	6.05	M	Yes	14	Yes
40	Mixed Breed	7.43	F	No	21	Yes
41	Golden Retriever	2.48	F	No	11	Yes
42	Mixed Breed	3.05	M	Yes	11	No*
43	Welsh Corgi Pembroke	6.08	M	Yes	13	No*
44	Serbian Hound	10.00	F	Yes	22	No*
45	Mixed Breed	5.99	M	Yes	15	Yes
47	Labrador Retriever	10.09	F	Yes	14	Yes

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Appendix:

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7. References

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