

Intranasal oxytocin studies on dogs might not be double-blind

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ABSTRACT

A single dose of intranasal oxytocin has been shown to affect family dogs' behaviour across a series of social tasks and situations. These studies have all been conducted using a double-blind placebo controlled design, which is the gold-standard in the field of drug-effect research. However, it has not been investigated whether intranasal oxytocin studies in dogs are truly double-blind, meaning that participants are indeed unaware of the treatment(s) dogs have received. Here we used unpublished data from three already published studies assessing the effects of intranasal oxytocin (compared to placebo) administration on dogs' behaviour. Owners in these studies were asked in the end to make a guess about the treatment received, which is analysed in the present paper. Results show that in two out of the three datasets owners were above chance with their guesses when dogs had received placebo treatment; and in one out of the three datasets they were also overall significantly above chance when looking at data from both the oxytocin and placebo groups. Owners' guesses were not related to dogs' performance in the variables measured in any of the three behavioural tasks. These results clearly indicate that caution should be taken regarding the blindness of intranasal oxytocin experiments in dogs. However, we also conclude that the previously reported oxytocin effects on dogs' behaviour (at least in these three studies) were not caused by the owners' beliefs.

1. Introduction

There has been a steadily growing interest in the effect of oxytocin on dogs' human-directed social behaviour (reviewed in Kis et al., 2017a). It has been shown across a range of studies that a single dose of intranasal oxytocin treatment changes dogs' behaviour in tasks measuring capabilities such as pointing following (Oliva et al., 2015), reaction to threatening approach (Hernádi et al., 2015), looking at human faces (Dzik et al., 2020; Kis et al., 2017b; Somppi et al., 2017), inequity aversion (Romero et al., 2019) and contagious yawning (Kis et al., 2020) among others. The most common method for administering exogenous oxytocin is the use of nasal spray. Although this technique has been shown to yield considerable variance in dogs' oxytocin uptake (compared to using a vaporizer mask (Schaebs et al., 2020)), the method is still considered valid based on physiological evidence; e.g. proving elevated blood and urine oxytocin levels (Temesi et al., 2017) as well as decreased heart-rate and increased heart-rate variance (Kis et al., 2014) following intranasal oxytocin administration.

The gold standard for intranasal oxytocin experiments (in both dogs and humans) is the double blind placebo controlled design, which means that neither the participants nor the experimenter have knowledge

about the treatment received. Special attention was directed towards this double blind design in intranasal oxytocin research when Lane et al. (2015) reported that they were unable to replicate their own previous study about the effect of oxytocin on a human trust measure. The only thing that was changed between the original and the replication study was that they switched from a single-blind design to a double blind design (in the original study the experimenter was aware of the treatment). It is claimed by others as well (Gilder and Heerey, 2018), that the experimenter's beliefs can unintentionally affect research outcomes in psychology. This is a phenomena well-known to comparative cognition research under the term "Clever Hans effect". Hans was a horse that was able to answer mathematical questions by tapping his hoof and was later found to rely on subconscious cues of the experimenter, such as their head jerks or body orientation (Pfungst, 1911). Potential effects of owners' voluntary and/or involuntary cues (e.g. facial or postural communicative signals) have also been studied in dogs. Scent detection, for instance, has been shown to be influenced by handler beliefs (Lit et al., 2011), whereas owners could not influence their dogs' performance in a point-following task even if they were instructed to gently push the dog to the correct direction (Hegedüs et al., 2013; Schmidjell et al., 2012).

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The blindness of participants regarding the treatment received is also important. It has been shown (Eisenegger et al., 2010) that human subjects (women) who believed that they had received a testosterone treatment – regardless of whether they actually received it or not – behaved much more unfairly in a bargaining situation than those who believed that they had been treated with placebo. Thus, the folk hypothesis suggesting that testosterone induces antisocial, egoistic, or even aggressive human behaviours seems to generate a strong negative association between subjects' beliefs and the fairness of their offers. This is despite the fact that it was found in the same dataset that testosterone in fact caused a substantial increase in fair bargaining behaviour in the very same subjects.

The neurohormone oxytocin also has several folk-theories attached to it. Media appearances labelling it e.g. love-hormone (Pedersen, 2004) make people believe that oxytocin administration would make them more social. Thus it is unavoidable to take the participants' beliefs into account in this field as well. Human studies using double blind design in oxytocin research have reported that participants were unable to guess the treatment received (Kis et al., 2013). However, it has not yet been investigated, whether in dog oxytocin research participating owners are able to make correct guesses or not and if their guesses could influence the research outcome.

The current study uses data from our three previously published dog oxytocin studies (Kis et al., 2022, 2015; Kovács et al., 2016) complemented with data about owners' guesses in order to test if they were correct about the treatment their dogs had received and if their guesses were related to the dogs' performance in the given tests.

2. Methods

2.1. Ethics statement

We did not collect new experimental data for the purpose of the current study. The original studies, which resulted in the datasets analysed here, were approved by the National Animal Experimentation Ethics Committee (Ref No. XIV-I-001/531-4-2012) and by the National Research Ethics Committee (PE/EA/55-4/2019) for dog experiments with owner participation. Research was done in accordance with the Hungarian regulations on human and animal experimentation and the guidelines for the use of animals in research, described by the Association for the Study of Animal Behaviour (ASAB) as well as carried out in accordance with the Declaration of Helsinki.

2.2. General procedure

Here we analyse unpublished data from our three previous studies, which have investigated the effect of intranasal oxytocin versus placebo administration in dogs. *Dataset 1* relates to a study on cognitive bias (Kis et al., 2015), *Dataset 2* relates to a study on biological motion perception (Kovács et al., 2016), and *Dataset 3* relates to a study of social susceptibility measured by a food preference task (Kis et al., 2022).

In all three studies dogs received 3 puffs of oxytocin (12 IU, Syntocinon-Spray, Novartis) or saline (isotonic sodium chloride 0.9 % solution) nasal spray in a double blind design (i.e. both the owner and the experimenter were unaware of the treatment). Before drug/placebo administration all owners provided an informed consent, and were thus aware of the possible effects of oxytocin, including its effects on social behaviour, also knowing that the administered dose was relatively small and thus any behavioural changes will likely be undetected. Nasal spray administration was followed by a 40-minute-long waiting period to ensure that oxytocin level reach a plateau by the time the experiment is carried out. During this time, the owners went for an on-leash walk with their dogs for 25–30 min, then quietly rested in a room for 10–15 min. This was followed by a behavioural test (depending on the specific aims of the study). In the end, the owners were asked to make a guess about which treatment (oxytocin or placebo) they think their dog received.

2.3. Subjects

Dataset 1 (Kis et al., 2015) was comprised of $N = 52$ dogs (out of the $N = 64$ original participants, data about owner guess was missing for 12 individuals), 22 males, 30 females, mean $\pm$ SD age = 4.4 ± 2.7 years, who participated in a single test occasion.

Dataset 2 (Kovács et al., 2016) was comprised of $N = 33$ dogs 14 males, 19 females, mean $\pm$ SD age = 4.5 ± 2.4 years that participated in two test occasions, receiving both oxytocin and placebo in a random order (data for the second occasion was only available for $N = 27$ dogs; the original sample size for this study was $N = 39$, owner guesses for 6 dogs were missing for both occasions). Owners in this design knew in advance that their dog would receive both oxytocin and placebo, thus they had access to the information that the two treatments would be different, but they did not get any feedback about their guess after the first occasion.

Dataset 3 (Kis et al., 2022) was comprised of $N = 35$ dogs (out of the $N = 37$ of the original study) 17 males, 16 females, mean $\pm$ SD age = 4.3 ± 2.6 years, that participated in a single test occasion.

(See the original publications for more details.)

Importantly subjects were eligible for inclusion in the above studies (both the oxytocin and the placebo treatment sessions) if they were not pregnant, not lactating and not in heat.

2.4. Statistical analysis

For *Datasets 1–3* binomial tests (test proportion 0.5) were conducted for the oxytocin-treated and placebo-treated dogs separately to test if owners could correctly guess the treatment received at a probability higher than chance. Correct guesses were scored as “1” and incorrect guesses were scored as “0”.

Next, χ^2 tests were conducted for *Datasets 1–3* to test if owners of oxytocin- versus placebo-treated dogs differed in their likelihood of oxytocin / placebo responses.

The behaviour of dogs whose owners made an oxytocin versus a placebo guess was also compared. For this purpose the main outcome variables were used, based on the original studies, which had been shown to significantly differ between oxytocin- versus placebo-treated dogs (see Kis et al., 2022, 2015; Kovács et al., 2016 for further details on the specifics of the tests and behaviour coding). For *Dataset 1* the variable used was the Cognitive Bias (%), and the two owner-guess groups were compared using independent-samples t-test (data normally distributed and continuous). For *Dataset 2* the variable used was the Biological Motion Preference score, and the two owner-guess groups were compared using independent-samples t-test (data normally distributed and continuous). For *Dataset 3* the variable used was Change-In-Bias score, and the two owner-guess groups were compared using Mann-Whitney test (data not continuous).

3. Results

3.1. Owners' guesses for oxytocin and placebo treatment

The number of oxytocin versus placebo guesses were compared in the two treatment groups contrasting to the 50 % chance level as well as between actual treatment received (Fig. 1). In *Dataset 1*, owners of placebo-treated dogs ($N = 25$) guessed correctly in 84 % of the cases, which is significantly above chance (Binomial test, $p < 0.001$). Owners of oxytocin-treated dogs ($N = 27$), however, were only correct in 41 % of the cases, which is at chance (Binomial test, $p = 0.442$). Overall, owners were better than chance (69 %, Binomial test, $p = 0.036$) guessing the treatment their dog received. Also owners of oxytocin-treated dogs were more likely to make an oxytocin guess compared to owners of placebo-treated dogs ($\chi^2 = 3.871$, $p = 0.049$).

In *Dataset 2*, on the first occasion owners of placebo-treated dogs ($N = 18$) guessed correctly in 67 % of the cases, which is at chance

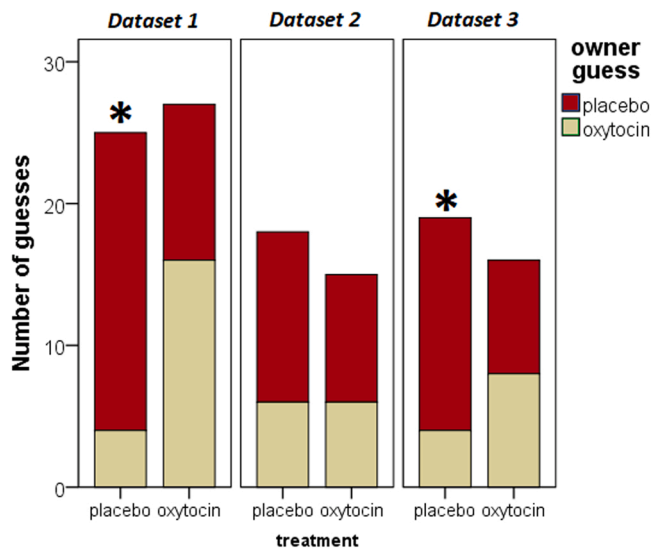


Fig. 1. Number of owners across Datasets 1–3 making “oxytocin” versus “placebo” guesses in the two treatment groups. Asterisks (*) denote significantly above chance (50 %) correct guesses.

(Binomial test, $p = 0.238$). Owners of oxytocin-treated dogs ($N = 15$) were also at chance on the first occasion (40 %, Binomial test, $p = 0.607$). Owners of both treatments were at chance on the second occasion as well (placebo: $N = 11$, 55 % correct, Binomial test, $p = 1.000$; oxytocin: $N = 16$, 50 % correct, Binomial test, $p = 1.000$). Out of the $N = 27$ owners that made guesses on both occasions, $N = 18$ made different guesses for the two, while $N = 9$ made the same guess for both occasions. (Some owners might have forgotten their previous guess and the experimenter did not provide this information to them.)

In Dataset 3, owners of placebo-treated dogs ($N = 19$) guessed correctly in 79 % of the cases, which is significantly above chance (Binomial test, $p = 0.019$). Owners of oxytocin-treated dogs ($N = 16$), however, were only correct in 50 % of the cases, which is at chance (Binomial test, $p = 1.000$). Overall, owners were not significantly above chance (65 %, Binomial test, $p = 0.090$) with their guesses regarding the treatment their dog received. The difference between owner guesses of oxytocin- versus placebo treated dogs, also did not reach significance ($\chi^2 = 3.230$, $p = 0.072$).

3.2. Dogs' performance in relation to owner guesses

For Dataset 1 (Kis et al., 2015) we found that dog's Cognitive Bias score (the measure influenced by oxytocin treatment) was not different between dogs whose owners guessed oxytocin versus placebo (independent samples t-test, $t_{(48)} = 1.098$, $p = 0.277$; Fig. 2). Similarly, in Dataset 2 (Kovács et al., 2016) the Biological Motion Preference score (for non-masked stimuli presented on the first occasion, which was affected by oxytocin treatment) was not different between owners guessing oxytocin or placebo ($t_{(30)} = 0.953$, $p = 0.348$). Data for masked stimuli (presented on the second occasion) was not analysed here, as the original study did not find any effect of oxytocin treatment. Also in Dataset 3 (Kis et al., 2022) dogs' Change in Bias score (which was affected by oxytocin treatment and reflects dogs' willingness to conform to the experimenters choice of smaller food quantity compared to their original choice) was not different depending on owners' guesses (Mann-Whitney test, $U = 66.5$, $p = 0.343$).

4. Discussion

The present study found evidence that certain owners were able to guess the treatment their dogs' had received in double-blind placebo

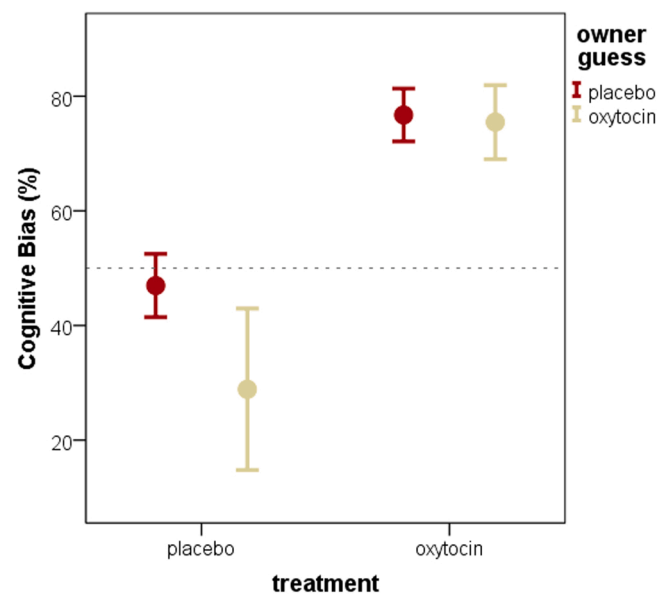


Fig. 2. Cognitive Bias scores (Dataset 1) as a function of treatment received and owner guesses. Values above the dashed line indicate an “optimistic” bias, values below the dashed line indicate a “pessimistic” bias as calculated from latencies to reach a food bowl at the “positive” (always baited), the “negative” (never baited) and the “ambivalent” (positioned in between the positive and negative) locations.

controlled studies involving intranasal oxytocin administration. Specifically results show that owners of placebo-treated dogs were correctly guessing the treatment in two out of the three studies. Owners of oxytocin-treated dogs, however, were guessing randomly in all three studies. This suggests that there might be a general tendency for owners to expect more drastic changes in their dogs' behaviour following the oxytocin treatment and thus they more readily made placebo guesses when no such changes could be detected.

One obvious difference between Dataset 2 (where owners could not guess the placebo treatment either) and the other two datasets is that in this one case dogs participated in two test occasions and owners likely overcomplicated their guesses trying to compare the two. Although we did observe that some owners made the same guess on both occasions. Another interesting pattern that can be observed in Datasets 1 & 3 is that owners are consistently better at guessing placebo treatment compared to oxytocin treatment suggesting that they are confident in reporting that their dogs behave the same way as they usually do, but they are uncertain to detect the small changes caused by the oxytocin treatment.

The definition of double-blind entails that the treatment received is unknown to both the participant as well as the experimenter. We have to note that the fact that owners were able to guess the treatment does not directly invalidate this criterion, since dogs (who were the subjects of the experiment) were still most probably unaware of the nature of the treatment received. Human studies using placebo-controlled design to test the effect of intranasal oxytocin have reported that participants are unable to guess the treatment received (Kis et al., 2013) which is in contrast with the current findings on dog owners. This raises the question that it might be easier to correctly guess as an outside observer than for the self. A similar line of arguments can be found in clinical psychology research where for multiple conditions diagnostic criteria include both self-reports and expert ratings as the two complement each other (e.g. for personality disorders (Dinger et al., 2014) or binge eating (De Zwaan et al., 1993)). An important implication of this for dog oxytocin research would be that research staff conducting the experiment, being outside observers themselves, might involuntarily and unknowingly have a correct guess about the participants' treatment despite the double blind design. This holds true not only for dogs, but also for

human experiments and needs further investigations to confirm. It is possible e.g. that in order to make correct oxytocin / placebo guesses, the guesser needs to know the participant closely (as is the case for dog owners). Unfortunately, we do not have data about experimenter guesses, thus cannot settle this debate. However, we do argue that the question needs attention, especially since it has been demonstrated that the experimenters' expectation alone can determine the research outcome (Klein et al., 2012).

Regarding the implications of owners' beliefs about the treatment, we did not find evidence in any of the three datasets that owner guesses would have an impact on dogs' behavioural performance. This suggests that at least in the three paradigms investigated here (cognitive bias, biological motion perception, social susceptibility), the positive effect of oxytocin treatment holds true even if owners were not entirely blind. There have been previous attempts at determining if and to what extent the owner's knowledge of correct response can influence dogs' behavioural performance. The results contradict in this regard, suggesting that such effects are highly dependent on the task itself. In two pointing following tasks, no effect of owner influence was found (Hegedüs et al., 2013; Schmidjell et al., 2012), while dogs were influenced by handler beliefs in a scent detection task (Lit et al., 2011). Previous research in (human) psychopharmacological studies have already raised the problem that placebo-controlled drug studies might not be truly double blind. For example it was reported (Margraf et al., 1991) that in double-blind comparison of alprazolam, imipramine, and placebo, the great majority of panic disorder patients and their physicians were able to rate accurately whether active drug or placebo had been given; moreover, physicians could distinguish between the two types of active drugs. Some suggest that active placebo should be used instead of an inert one (Fisher and Greenberg, 1993), while others argue that a deceptive administration of placebo (when all subjects are told that they receive the active drug) is favourable compared to the double-blind design (Kirsch and Weixel, 1988).

Taken together our results suggest that caution should be taken about the blindness of intranasal oxytocin experiments in dogs, and owner guesses should always be included as a confounding variable in the analysis. Within-subject designs (with dogs randomly receiving both oxytocin and placebo on two separate occasions) might be favourable with respect to treatment-blindness.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability

All data are stored safely in the institutional database (NAS server) of the Research Centre for Natural Sciences and is available upon request from the authors.

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Author contributions

József Topál: Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Anna Kis:** Conceptualization, Methodology, Statistical analysis, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Henrietta Bolló:** Conceptualization, Methodology, Writing – review & editing. **Anna Gergely:** Conceptualization, Methodology, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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