Manuscript ID: animals-526845
Manuscript Title: Changes in oxytocin and serotonin values in dialysis patients, after Animal Assisted Activities (AAA) with a dog. A preliminary study.

REPLY TO REVIEWER’S COMMENTS (The authors’ response is reported in bold)

REFEREE #1

General Remarks:
• This study is an interesting contribution to the literature on the effectiveness of AAA. The authors add to this literature by describing a study of the effectiveness of AAA among a novel sample of individuals—specifically, individuals experiencing end-stage renal failure and undergoing dialysis.

RE: We thank the reviewer for this appreciation.

• The major weakness of this study is that the authors did not include a treatment-as-usual comparison group. Thus, we do not know how serotonin and oxytocin levels might have changed over time among dialysis patients who did not participate in AAA.

RE: We thank the reviewer for this important comment. With the aim to explain and support our choice we added the following considerations in the discussion section:
“It is worth to highlight that this study is not a conventional treatment vs control study but a single group study, instead: as a matter of fact, as detailed in [68], single group studies can be performed in ‘pilot’ longitudinal cohort studies when an implicit comparison with respect to the lack of treatment is known. As there is no evidence in the literature that serotonin and oxytocin levels should change in time because of dialysis our implicit hypothesis was that serotonin and oxytocin levels should remain bounded by within-subject variability. Therefore, we estimated basal-level of variability and each subject has been considered as the ‘control’ of itself: variations of serotonin and oxytocin were observed with respect to basal-levels”.


Simple Summary:
• Page 1, lines 23-24: What is “game size”?
RE: We apologise for the lack of clarity in the manuscript. The sentence was rephrased as follows: “The dog, in particular, due to its ethological characteristics, allows the establishment through the play an active relationship, communication and interaction.”

1. Introduction
• There is a fair amount of research examining the benefits of AAA among individuals who experience diverse problems (e.g., alcohol/drug addiction, depression, impaired circulatory function, neurological disorders, and pain). The authors should provide a brief review (perhaps one paragraph?) of this literature. I have listed some helpful references below.


RE: Thank you for your suggestion, we have improved the introduction as follows: “AAIs are increasingly used both in health care and in the school context. In fact, they have been divided in Animal Assisted Therapy (AAT), Animal Assisted Activity (AAA) and Animal Assisted Education (AAE).[1] The human, like other mammals, develops a necessary bond of attachment that will condition his way of relating. Furthermore, emotional ties and attachment in relationships between people and different animal species have been studied extensively. [2-3-4]The dog is a main species involved in AAAs [2] and has an important competence which is the ability to read the non-verbal language of the human. This animal species has developed a particular preference for humans and the ability to recognize conspecifics seems to have played an important role towards these genetic changes [6]. In recent years, the effects of AAI have been subjected to a quantitative review [7] and systematic reviews, including randomized studies from 1990 to 2012 [8]. Further studies on the benefits of neurorehabilitation were published from 2001 to 2012 [9] and others related to biopsychosocial results in hospitalized children and adults [10-11-12-13]. Recently, the international scientific literature has shown the large beneficial effects deriving above from AAT involving dogs in a wide range of people with problems of psychophysical and mental health, such as well adults with Autism Spectrum Disorder [14], as adults with Alzheimer disease and the other dementias [15-16-17], but also in...
psychotherapy for adolescents [18]. Particularly, in the dementia care, pet robots have also been used to evaluate their effects on patients [19]. Moreover, the relationship turns out to be important on the effects of chronic and acute pain. Brown et al. 2003 [20] evaluated only human social support while later studies showed effects of the relationship with the dog [21] and more precisely: in the medication pain after replacement of articulation [22], in pediatric pain [23] and on perceived pain in patients with spinal cord injury [24]. Finally, these beneficial effects were also assessed in TAA with the involvement of other animal species such as the horse for post-traumatic stress disorder in veterans [25-26] and for young people at risk [27].

• Why did the authors schedule two meetings (after the AAA had taken place) without the dog? What was the purpose of these meetings?

RE: We apologise for the lack of clarity in the manuscript. As the prolongation of the effect of AAA is not known and is still under investigation, the purpose of the last two blood samplings without dog was to establish the return to the before-treatment state. As a matter of fact, an ANOVA test revealed that the serotonin/oxytocin levels in the last two sessions were different with respect to the basal levels. In the statistical methods section we added the following paragraph: “With the aim to investigate the return to basal levels, we performed an analysis of variance between the first and the last two sessions.” In the results section we added the following paragraph: “The ANOVA test between the first and the last two sessions revealed a significant difference in both serotonin/oxytocin levels.”

3.3 Statistical analyses

• Did the authors hypothesize that serotonin and oxytocin levels would increase from T0 to T1 within each meeting? If so, this hypothesis is not explicitly stated.

RE: We apologise for lack of clarity. For each session our null hypothesis was that no change took place between T1 and T2. This has been clarified in the text. In the statistical analysis section we added the following paragraph: “With the aim to test for within-session changes we used the Wilcoxon paired test (due to non-normality of data) between T1 and T2.”

• Did the authors have a hypothesis about how the levels of serotonin and oxytocin would change over the entire course of the study (i.e., all 14 weeks)? If so, this hypothesis is not explicitly stated.

RE: We apologise for lack of clarity. Our null hypothesis was that the level of serotonin and oxytocin remained unchanged during the treatment. After looking at the data, a biphasic behavior was hypothesised. Therefore, we hypothesized and compared three possible mixed effect models for each of the two phases: no level change; fixed slope, random intercept; both random slope and intercept. This has been clarified in the text. In the statistical analysis section we added the following paragraph: “Data inspection suggested a biphasic behavior from the 1st to the 11th session with dog. We tested the hypothesis of no change versus a biphasic behavior.”

• The authors used Wilcoxon paired tests to examine differences in serotonin and oxytocin concentrations from pre-AAA visit to post-AAA visit. Was this choice driven by the fact that the data were not normally distributed?
RE: We apologise for lack of clarity. We clarified in the text that we used a non-parametric test because of the non-normality of the sample.

4. Discussion

• In the first sentence of this section, the authors report that “results showed an increasing trend of both inter-intervention serotonin and oxytocin without any clear difference between the age of patients and between men and women.” They do not, however, report such analyses in the Statistical Analyses section. If the authors wish to note this finding in the Discussion section, they should report the results in the Statistical Analysis section—or at least reference the findings in a footnote.

RE: We apologise for lack of information. We added the following paragraph in the ‘Statistical analysis section’: “We tested for correlation between slopes and age; moreover, we tested for slope differences between men and women (Wilcoxon): no significant association (p>0.05) has been found in both cases”

• The authors state, “The results display an interesting trend that suggests a prolonged effect, also evident on the behaviour of the patients.” What patient behaviors did the authors observe?

RE: The new sentence has been inserted in the text: “They, in fact, increased sociality in the group and the demand for participation in the plays with dog. They became more colloquial among themselves and with the veterinarian and more feel care with the dog (someone brought from home prizes for dogs) In the interview they reported as pleasure of the hour of therapy in which the dog was in respect to the other hours, as an improvement in mood when they come back home in the day of AAA. (Lines 266 to 271, pag. 8)

• Do the authors have any explanation (or theory) about why the serotonin and oxytocin levels showed a biphasic pattern? In the Discussion, they note that “As already mentioned in another work the fifth meeting represents the critical point of the path. One could hypothesize that it represents the point from which the therapeutic alliance is triggered, as also indicated in many reports.” This finding might explain the increase in serotonin and oxytocin beginning during the fifth week. What might explain the drop in serotonin and oxytocin during the first four weeks of the study?

RE: We hypothesize that the initial decrease could be due to the novelty and to the necessary adaptation, but it is an aspect that we must still delve into with greater depth.

5. Conclusions

• In this section, the authors state, “From these preliminary results we can hypothesize that the AAAs with the dog induce a release of serotonin and oxytocin...” I’m not sure that the results of this study support such a strong statement—given that (1) during the first four weeks of the study, serotonin and oxytocin levels dropped and (2) there were no significant changes from T0 to T1. In addition, this study does not include a treatment-as-usual comparison group. Thus, we do not know how serotonin and oxytocin levels might have changed over time among dialysis patients who did not participate in AAA.

RE: Thank you for your suggestion, we improved the text as follows: “From these preliminary results, we observed that the AAAs with the dog induce a release of serotonin and oxytocin after an initial decrease, that we hypothesized as due to the novelty and the necessary adaptation. A significant increase of serotonin and oxytocin, was then observed in the subsequent sessions. However, no significant difference was detected between the beginning and the end of the same session, possibly due to individual variable levels. Finally, the increase of serotonin and oxytocin was not detected in the determinations carried out after the termination of the
activities, as serotonin and oxytocin levels settled approximatively on the initial values. Further studies are necessary to deeply evaluate the results obtained.” (lines 293 to 301, Pg 9)

Figure 1 and Figure 2
• In the Blood Sampling section, the pretest values of serotonin and oxytocin are referred to as T0; the posttest values are referred to as T1. Figures 1 and 2 refer to them as T1 and T2. The authors should revise either the Blood Sampling section or Figures 1 and 2 to make the labels consistent.
RE: We apologize for the error, we have changed in the text, therefore T0 is now T1 and T1 is now T2.

Figure 2
• What does the abbreviation “pz” stand for? Patient?
RE: We apologise for the lack of clarity: we added the meaning of “pz” in the figure captions.