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MEASURING SOCIAL SYNCHRONY AND STRESS IN THE HANDLER-DOG DYAD DURING ANIMAL ASSISTED ACTIVITIES: A PILOT STUDY

Federica Pirrone[a], Alessandra Ripamonti[a], Elena C. Garoni[b], Sabrina Stradiotti[b], and Mariangela Albertini[a]

[a]Department of Veterinary Medicine, University of Milan, via Celoria 10, 20133 Milan, Italy
federica.pirrone@unimi.it
aleripa@fastwebnet.it
mariangela.albertini@unimi.it

[b]Focus Dog s.r.l., Milan, Italy
elenagaroni@libero.it
sabrina.stradiotti@tiscali.it

Corresponding Author:

Federica Pirrone
Department of Veterinary Medicine, via Celoria 10, Milan 20133, Italy
Telephone: +39 02 50318129
Fax: +39 02 50318135
E-mail: federica.pirrone@unimi.it

Abstract

Synchrony – the coordination of behavior between interacting partners – is a complex phenomenon requiring the perception and integration of multimodal communicative signals. Originally conceptualized by developmental psychologists to study the human-human relationship, it could also apply to cross-species interactions. Here we examined synchrony patterns as a potentially important mechanism to evaluate human-dog interactions during animal assisted activities (AAA). Four dog handler-dog dyads were videotaped before (T_0), during (T_1) and after (T_2) 45-minute sessions of AAA and coded for the following synchrony patterns: gaze synchrony (GS), joint attention (JA), and touch synchrony (TS). Both partners' salivary cortisol and heart rate, and dogs' behaviors were measured to identify any signs of stress which would lower levels of synchrony. All dyads showed synchronous behaviors in T_0 and T_1, while these were absent in T_2. On average, the highest frequency was recorded in T_1 ($P < 0.05$), particularly as regards JA. All dogs fulfilled the majority of their handler's cues (74%, $P < 0.05$) while working with a patient, showing appropriate levels of cooperation. No stress-related signs were detected in either the dogs or their human handlers. These findings highlight the human-dog bonding as one prototypical context for studying the biological basis of cross-species SS. This may also generate evidence-based knowledge that can help strengthen the scientific foundation of current canine assisted intervention practices.

Keywords: SS; human-dog bond; animal assisted activities; salivary cortisol

Introduction

Human studies have shown that social synchrony (SS) – the coordination of nonverbal behaviors between interactive partners (Feldman, 2007) – is an experience learned within the caregiver-infant bond (Atzil et al., 2014). SS provides a unique exemplar of patterned behavior that is deeply rooted in mammalian biology and underlines the capacity of social species to be empathic and collaborative (Feldman, 2012). Synchrony implies the following (Delaherche et al., 2012): 1) behaviors include communicative and emotional signals (e.g., gestures, postures, facial displays, vocalizations and gazes); 2) interactions entail coordination between partners and ability to respond each other using different modalities (Vandenberg, 2006); 3) it builds on familiarity with the partner's behavioral repertoire (Leclère et al., 2014). All these components appear "within the dog-human social dyad" (Miklósi et al., 2004; Kerepesi et al., 2015; Duranton and Gaunet, 2015). Moreover, as for the caregiver-infant bond, the relationship between a dog and his caregiver is bidirectional in nature (Kaminski and Marshall-Pescini, 2014) and involves similar attachment bonds (Serpell, 1996a; Mariti et al., 2013). Given these common prerogatives, we strongly believe in the crucial value of studying synchrony within the dog-human social dyad. The SS construct could bring a relevant contribution to understanding the nature and quality of human-dog interactions, which have a great effect on dogs' social, emotional and cognitive well-being (Pirrone et al., 2015).

Although much is now known about SS in human social cognition, there has been relatively little investigation into SS in dogs and across species. A recent study by Palagi et al. (2015) revealed the presence of rapid mimicry - that is an involuntary, automatic and fast response through which individuals mimic others' expressions (Iacoboni, 2009) - in dogs under the playful context. Rapid mimicry may facilitate communicative exchanges and behavioral coordination in the sequence of actions (Mancini et al., 2013), but SS is supported by a coordinated behavioral matching that requires both automatic and mental processing, and, thus, implicates a sort of dialogue that goes beyond simple mimicry (Harrist and Waugh, 2002). The difference between mimicry and SS has been well explained in human caregiver-infant interactions by Harrist and Waugh (2002): if caregiver adjustments to infant behavior are in the same modality and the same behavioral form as the infant's (e.g., when an infant's smile elicits the caregiver's smile), interactions are akin to mimicry (Stern et al., 1985). In this case, the interactional process can be thought of as contagion and would not necessarily lead to a state of dyadic synchrony (Harrist and Waugh, 2002).

In this framework, a systematic account of the role of SS in human-dog reciprocal understanding is still missing in the current literature. The present paper aims to bridge the gap by examining whether SS may be one mechanism through which humans and dogs engage in cooperative interactions during animal assisted activities (AAA). AAA is a specific type of animal assisted intervention that is delivered spontaneously, lacks a previously defined goal and provides opportunities for motivation, education, or recreation to enhance quality of life (Kruger and Serpell, 2006).

We chose the context of AAA for two major reasons. First, according to Fiebich and Gallangher (2013), in humans, successful cooperation cannot be achieved without SS which acts as the window to the social relationship of the interacting partners (Kochanska, 1997; Hartup, 2006). In our opinion, it is likely that this is the case also in human-dog cooperation. However, more than a decade ago, Naderi et al. (2002) related dogs' innate ability for cooperation with humans to training rather than to the relationship with the owner. AAA is a salient setting of cooperation within participating dog-handler dyads, in which success is closely dependent on their affiliative and trust-building bond converging on joint activities with AAA's clients (Kirchengast and Haubenhofer, 2007). This makes AAA an ideal field for investigating SS behaviors within the human-dog dyadic exchange, possibly shedding new light on their inter-specific cooperation. Second, analysis of inter-specific communication and interaction would give insight into some of the positive health benefits of AAA (Franklin et al., 2007). Previous research focused on the human side of this interaction (Franklin et al., 2007). However, the deciphering of both sides of the dialogue may quantify new aspects of communication that will not only explain the real nature of the interaction itself, but also will provide guidance for AAA strategy planning in order to make them more effective, suitable and respectful of the welfare of all participants.

Finally, distress lowers levels of synchrony (Weinberg et al., 2006) and work may be stressful for handler-dog teams who deliver AAA in health care service (Hatch, 2007; Kirchengast and Haubenhofer, 2007). Therefore, along with SS, we analyzed dogs' stress-related behaviors, dogs' responsiveness to handler's cues and physiological reactions (measured by saliva cortisol sampling and heart rate measurement) of both dog handlers and dogs.

Materials and Methods

Participants

Four handler-dog dyads regularly delivering AAA programs in one adult health care facility in Italy were recruited on a voluntary basis. To avoid bias due to either the working method or experience, all dyads had been awarded an AAA certificate after attending the same *Pet - Handler Operator* course organized by the SpazioperNoi Association (Alzate Brianza, Como, Italy) and exhibited exactly 1 year of working experience. Handlers were women aged between 28-39 years (34.8 ± 2.4, Mean ± S.E.) and had different occupations. In three dyads the handler was the owner of the dog and lived with the animal, while in the remaining one (dyad n. 2) the handler was a familiar caregiver, though not a member of the dog's household. A card registry was compiled for each animal using demographic data, which included breed, age, sex, weight, provenance and experience in AAA (Table 1). Two dogs were spayed females, one was an intact male, one was a neutered male and the dogs were either pure or mixed breed. Dogs were between 3 and 8 years old (4.5 ± 1.2, Mean ± S.E.) and weighed between 22 and 35 kg (28.5 ± 2.7, Mean ± S.E.) at the time of the sampling period. As reported in a similarly designed study (Glenk et al., 2014), to be eligible for participation in the AAA program, the dogs were required to be in good clinical health (i.e., free from pain, external and internal parasites, and immunized) and subjected to regular health screening and behavioral monitoring by two veterinarians with expertise in animal behavior.

Study design

Handler-dog dyads were assessed while involved in weekly group sessions of AAA delivered to 2-5 adults suffering from different diseases, such as senile dementia and degenerative and/or congenital psychomotor dysfunctions. Sampling was carried out during 5 subsequent AAA sessions per dog, that is, 20 AAA sessions in total. Each session was 55 minutes in length, with a 10-min pause at the middle (actual working time: 45 minutes). Thus, during a session, dogs were working for about 25 minutes, then they took a 10-min break, and worked again for another 20 minutes. There was only one experimenter (female) in this study who attended three AAA sessions for each dyad prior to data collection so that the animal handlers, dogs and patients were already familiar with her presence. A video camera was set up on a tripod, and left running continuously. The experimenter switched the camera on just before the session started, and switched it off when the session ended. In order to be less distracting for the dogs, during the sessions she usually sat on a sofa. The pre-study phase also enabled handler-dog teams to become familiar with the environment. Sessions were performed in common spaces at the facility in the presence of staff members, as shown in Fig. 1. In more detail, at AAA sessions, two visiting dogs, two dog handlers, two health care professionals, and one experimenter were always present. During each session, one of the visiting dogs was guided by the handler to interact with the patients at regular turns (Fig. 1a). Due to a severe neuromuscular deficit, two patients needed individual interactions which were carried out concurrently when they were lying down (Fig. 1b). In this case, the second dog was also involved and all other patients were allowed to stay in the room, even if they were not directly involved in the activities. As part of their participation in the AAA certificate training program, all these dogs were thoroughly trained to ignore environmental distractions, including the presence of another dog.

Informed consent was obtained from all participants (or their legally authorized representatives), who were previously advised by the facility staff members of an experimenter's presence for the videotaping procedure. All patient-animal contact in this study was guided by an experienced dog handler and based exclusively on positive reinforcement and gentle handling. Patient–animal interaction behaviors included verbal contact, where a patient talked to the dog to praise him/her, and/or tactile contact, where a patient softly touched and/or groomed the dog. For ethical reasons, dogs were never forced into positions and were able to lie down, drink water, or leave the AAA room at any time (Glenk et al., 2014). The handlers were aware of the aim of the study. They knew that we would have evaluated the dog's behavior and interaction with the handler in the context of AAA, but they were not informed in detail about the SS patterns we were trying to investigate. This precisely to avoid the awareness of being studied and related consequences for behavior. However, after completing the study, the handlers received a full explanation of what we expected to find.

As detailed below, dogs' behaviors were assessed at three time points: 15 minutes after arrival at the facility (T_0), during each 45-min AAA session (T_1) and 22 minutes after (T_2). Both handlers' and dogs' physiological parameters were also measured at T_0 and T_2.

Behavioral assessments

The behavior of each dog was videotaped by the experimenter and subsequently analyzed in T_0 and T_2 (video length: 3 minutes), as well as during each entire 55-min trial (T_1), except for the 10 minutes break. At T_0 and T_2, dogs were with their handlers in the yard of the healthcare facility. Animals were off leash at all three time points and the handlers were asked to act spontaneously by the experimenter. Analysis of behavior was carried out with focal animal sampling and continuous recording using the Observer XT software package (Noldus Information Technology, 6702 EA Wageningen, The Netherlands). To preserve the anonymity of participants, video recordings were stored in the principal investigator's computer at the Department of Veterinary Medicine at the University of Milan.

In the preliminary phase we identified behaviors that could be reliably recognized (Table 2), and defined them on the basis of a literature review (Beerda et al., 1999; Haverbeke et al., 2008, and Pastore et al., 2011). Intra-observer reliability of the experimenter who analyzed the videos was computed by coding of independent samples of videotaped sessions twice several weeks apart and calculating the percentage of agreement (Landis and Koch, 1977; Fleiss, 1971), which revealed a kappa value of 0.85 (95% CI: 0.81-0.92).

The following synchronous variables were used: gaze synchrony (GS), joint attention (JA), and touch synchrony (TS). These behavioral variables were adapted from the study by Feldman et al. (2014) assessing SS between caregivers and children through nonverbal interactions.
According to the AAA protocol, behaviors in T_2 were divided into 2 categories of activity: solitary activities (SA), periods in which dogs were not involved in the handlers' work with patients, and guided interactions (GI), periods in which dogs were instead involved, guided by the handler, in the activity with patients, thus allowing for analysis of SS. Dogs had their own carpet to lay on during SA, though they were free to choose their location and act spontaneously in the room. We measured dogs' responsiveness to instructions of the handler, coding the dogs' performance based on the number of correct responses during GI. We only considered the immediate readiness to respond, thus a correct response meant the dog performing the corresponding behavior within 5 seconds to the first request issued by the handler.
Behavioral variables were measured in terms of relative frequency (the number of occurrences per minute) and/or duration (time spent on a behavior, expressed in seconds) of occurrence during each observation period. Duration of SS behaviors was expressed in semi-quantitative categories (< 1 sec, 1-5 sec, > 5 sec). We chose these time frames because, based on the pre-study observations, they appeared to be the most realistic and appropriate.

Physiological parameter assessments

Salivary cortisol concentration (ng/mL) and heart rate (HR, bpm) were assessed either on dogs and handlers at T_0 and T_2, in order to evaluate physiological responses to work in both species. Blood cortisol concentrations rise approximately 20 minutes after a dog encounters a stressor (Vincent and Mitchell 1992) and 20–40 minutes after a human encounters a stressor (Nicolson, 2008). Changes in plasma and salivary cortisol levels are closely synchronized: after injections of cortisol, salivary levels increase within 1 minute, and peak concentrations in blood are seen 2 to 3 minutes later in saliva (Kirschbaum and Hellhammer, 2000). Thus, saliva samples from both handlers and dogs that were taken 22 minutes after the end of each session (T_2) captured postsession levels, that correspond to the time during AAA sessions. The handlers were given a demonstration by the experimenter how to take saliva samples from themselves and their dog using Salivette® Cortisol tubes (Sarstedt, Nümbrecht, Germany). All samples were taken by the handler herself. First the handler put the oral swab under her tongue and then she took the sample from the dog at the same time. The swab was gently placed into the cheek pouch or under the tongue of the dog for approximately 30-50 seconds, without restraint of the animal. The dog's salivation was stimulated by smelling food treats. The dog received a food treat only after the saliva sample was taken

(Bennett and Hayssen, 2010; Ligout, 2010). Each sample was replaced in the device tube and closed with a plastic stopper to avoid evaporation. The collected material was refrigerated at -4°C and then stored at -20°C immediately after it arrived at the laboratory. At the time of analysis, the samples were thawed at room temperature and centrifuged (3,500 rpm for 15 minutes). Analysis was performed using a multispecies Cortisol Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN), according to the protocol for salivary samples. The intra-assay and interassay coefficients of variation were 6.9% and 13.6%, respectively. The minimum detectable dose of cortisol ranged from 0.030 to 0.111 ng/mL; the mean minimum detectable dose was 0.071 ng/mL. Prednisolone, Reichstein's substance S, progesterone, cortisone, 4-androstene-3,17-dione, corticosterone, deoxycorticosterone, estradiol, and prednisone were assayed for cross-reactivity, and no significant interference was observed (R&D Systems Inc., Minneapolis, MN).
HR was tested non-invasively, to assess arousal levels (Beerda et al., 1998), by each handler 2 minutes after saliva sampling, through radial artery pulse palpation (femoral artery in dogs). The healthcare professionals informed us that some of the patients might have uncontrollable and excessive gripping, thus dogs could not wear any elastic chest bands during the session. In order to standardize the analysis of dogs' behaviors, making them more comparable across the three time points, we decided to completely avoid the use of a telemetric device.
In addition, handlers collected saliva and heart rate at similar times as in AAA days (8:30 a.m and 11:30 a.m.) during two non-consecutive control days from themselves and their dog. To avoid potential effects of food or exercise on home baseline cortisol and HR levels, handlers were advised not to eat and feed their dogs at least 1 hour before sampling and to avoid any hard or unusual exercise on that day (Glenk et al., 2014).

Statistical analysis

Data were analyzed through nonparametric statistical tests. Differences in physiological parameters and behaviors between time points and dog handler-dog dyads were analyzed using Pearson's χ^2 test of independence in 2x2 contingency tables, Kruskal–Wallis test for multiple comparisons, Mann-Whitney U-test for comparing two groups and Wilcoxon signed-rank test for one sample. Post-hoc Mann-Whitney *U* tests with the Bonferroni correction followed Kruskal–Wallis test in case a significant effect was detected. The Spearman rank correlation test was used to measure the degree of association between frequency of SS variables and rates of correct responses to the handler's cues.

Cortisol concentrations, heart rate, duration and relative frequency of behaviors are presented as Mean ± S.E.. P values ≤ 0.05 were deemed statistically significant. Statistical analyses were performed with SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Behavioral assessment

As shown in Fig. 2, SS was absent in T_2 and significant differences were found in the exhibition of GS and JA between T_0 and T_1 for all working teams ($P < 0.05$, Kruskal-Wallis test). During $T_{1,}$ JA was the most frequent behavior ($P < 0.05$, Kruskal-Wallis test). Overall, differences were found in SS behavior durations (Table 3): episodes of GS and JA lasting either less than 1 second or up to 5 seconds were observed significantly more often than those longer than 5 seconds ($P < 0.05$, Kruskal-Wallis test). The majority of TS episodes lasted less than 1 sec ($P < 0.05$, Kruskal-Wallis test).

During T_1 (GI), dogs received on average 28 ± 3.9 E.S. cues each session, which did not differ across dogs ($P > 0.05$, Kruskal-Wallis test). Dogs fulfilled a high percentage (74% ± 4.9, Mean ± S.E.) of handler's requests ($P < 0.05$, Wilcoxon Signed-Rank test), mostly when these only concerned a patient's physical proximity (67% ± 9.4, Mean ± S.E.) rather than physical touch (23% ± 7.6, Mean ± S.E.) by the dog ($P < 0.05$, Wilcoxon Signed-Rank test). Compared to the other dogs, the dog in dyad 4 showed the lowest responsiveness to the handler's request for physical touch with patients (47.6% ± 5.7, *vs* 77.5% ± 5.1 dog 1, 87.9% ± 6.2 dog 2, 89.6% ± 5.9 dog 3, Mean ± S.E., $P < 0.05$ Kruskal-Wallis test). This dog also showed a lesser (although not significantly) mean frequency of spontaneous physical contact-seeking with patients (Table 4).

Analysis of the degree of association between frequency of SS and correct responses of dogs revealed no significant results.

The four dogs exhibited significantly longer resting (sitting, standing, and lying down) than active periods at all the time points (Fig. 3, Kolmogorov-Smirnov for one sample test, $P < 0.05$). Summing the behavioral signs of stress, we found no significant differences in terms of relative frequency among the three phases ($T_0 = 1.4 \pm 0.3$, $T_1 = 2.0 \pm 0.4$ and $T_2 = 0.8 \pm 0.2$, Mean ± S.E.). The looking behavior was the most frequent behavior in T_1 (SA) ($P < 0.05$). Most times the dog was looking at the handler rather than the patient (63.7% ± 4.1 *vs* 36.3% ± 4.1, Mean ± S.E., $P < 0.05$, Wilcoxon Signed-Rank test).

Physiological Parameters

Handlers had higher salivary cortisol levels in T_0 than in T_2 ($P < 0.05$, Wilcoxon Signed-Rank test) both during activity and control days (Table 5). The same trend was detected in dogs, but was not statistically significant. No difference was observed between handlers' cortisol values during AAA compared to control days. Among the dogs, two had significantly higher salivary cortisol levels than the others in T_0, and one of them also in $T_{2,}$ both in AAA and control days (Kruskal-Wallis test) (Table 6). As shown in Table 5, heart rate was higher in dogs during AAA days than in control days ($P < 0.05$, Mann-Whitney U test). There was no statistically significant difference in the handlers' mean heart rate values. We did not find time-dependent differences in cortisol and heart rate in handlers or in dogs across 5 subsequent AAA sessions.

Discussion

The aim of this pilot study was to use a field-based methodology to explore whether there is evidence for the construct of human caregiver-infant synchrony in human-dog dyads and how this synchrony might be expressed in a context that requires close cooperation between the handler and the dog. We observed patterns of SS within all four dyads involved in AAA and, as we had initially hypothesized, the highest rates of SS were recorded when the handler and the dog engaged in shared activities with patients. Joint attention and, to a lesser extent, gaze synchrony were the most frequent patterns in this phase. Notoriously, the eyes have a dual function - to perceive information and also to signal intentions – that make them a remarkable indicator for social interaction (Gobel, 2015). Joint attention is typically defined as a social-communicative skill in which two subjects use gestures and gaze to share attention with respect to interesting targets (Jones and Carr, 2004). Joint attention is very important for social animals because they reveal an adaptive social-cognitive skill for vicariously detecting food, predators, but also important social interactions among group members (Itakura, 2004). It arises from coordination, and coordination is probably the most crucial component of joint attention – the part that makes joint attention joint, rather than just parallel, attention (Carpenter, 2012). In nonverbal communication, gaze is an important aspect of establishing common ground, which is a mutual belief that the communicants understand one another (Clark and Brennan, 1991). Previous studies reported on the ability to follow human's gaze (Miklósi et al., 1998; Hare et al., 2002) and to read the visual information conveyed by human gaze (Hare et al., 2002) in dogs, whose evolution has been largely shaped by humans. Moreover, contrary to wolves, dogs develop the ability to exploit these basic human social cues as puppies, without requiring extensive exposure to humans (Hare et al., 2002; Riedel et al., 2008). Thus, it is very likely that domestication has influenced dogs' abilities to read inter-specific social cues in a cooperative context, even early in development (Hare and Tomasello, 2005). As a result of this early predisposition to interact cooperatively with humans, dogs may then develop other cognitive social skills (e.g., social-emotional sharing and co-regulation with a human referent), which could resemble what is argued for the development of human social cognition in children (Wobber and Hare, 2009). In the present study, dog attention seemed to be contingent upon handler attention. Attention was a reciprocated behavior: handlers were attentive to the dogs and dogs became attentive to their handlers. Human attention (Gácsi et al., 2004), the experience of the dog handler (Lynge and Ladewig, 2005) and familiarity of the dog with his/her handler (Coutellier, 2006; Lefebvre et al., 2007) can affect dog behavior such as their obedience. The type of training received by these dogs may also have contributed to increased GS during guided interactions. Although dogs were not explicitly trained for attention on command (e.g., watch me) they were stimulated to negotiate the collaborative activities to be carried out using eye gaze. This process of mutual negotiation may have also led to the observed patterns of mutually cooperative actions. In line with this assumption, aside from reaching the highest levels of SS, the handler-dog teams in our study obtained successful cooperation while working with patients, which was reflected in the high rate of correct responses of the dogs to the handlers' signals. SS might be one mechanism through which dogs decode human social information, and this may help them perform better when dealing with shared tasks and become collaborative dyad members.

The fact that non-touching eye behaviors prevailed over touching patterns is not surprising, mostly because touch synchrony – the coordination of the handler affiliative touch with the handler's and dog's social gaze – requires physical proximity between social partners and a handler's free hands. In our study, instead, during guided interactions handler and dog were often not so close to each other and/or the handler was already using hands to manipulate an object or interact with a patient. On the other hand, touching is the most powerful and influential mode of nonverbal communication, more invasive than other nonverbal behaviors (Huwer, 2003). We cannot know for certain whether the low levels of touch synchrony were due to emotional rather than physical and/or spatial barriers. Lag sequential analysis, a widely used method for evaluation of communication sequences that contribute to improve team performance (Bowers et al., 1998), would help explore both handler and dog-initiated patterns and optimal sequences of touch synchrony, as well as other synchrony types. It is thus strongly recommended for the future research agenda.

Both before and after working sessions dogs and handlers sought little (T_0) or no (T_2) interaction with each other. At the arrival to the facility, before they started an AAA session, handlers and dogs showed some eye gaze social exchanges (with or without touch), which may have helped them establish a common ground to work collaboratively immediately afterwards. This is in line with the function of eye contact, that serves not only to monitor each other's state of attention (e.g. gaze direction) and emotion (e.g. facial expressions), but also to temporally synchronize interactions and to establish mutual acknowledgment (Gobel, 2015). Probably, this reciprocal engagement was not more necessary at the end of a session, and this might explain why we observed no SS after each AAA session in all four dyads. Handlers and dogs spent most of that time on their own, as if they both needed to switch off after working.

In our study, dog 1 showed statistically more spontaneous contact-seeking to the patient, which was maintained mostly by leaning the muzzle or the body against the patient's legs. It could be possible that this dog was more comfortable than the others with unfamiliar people. Its greater willingness to seek physical interactions with patients could suggest a higher level of affiliation and engagement (Fine, 2015). The dog in dyad 4 refused to engage in guided physical contact with patients more than the others, and spontaneously sought a patient's physical contact less often. According to a recent study analyzing defensive behavior in shelter dogs (Kocis and Ţibru, 2015), a general mild lack of trust may have contributed to this dog's less liking of a stranger's physical contact. Insecurity may be associated with cortisol reactivity (Bernard and Dozier, 2010), and it may therefore not be a coincidence that this dog showed higher, although within the normal range, salivary cortisol values compared to the other three dogs. A recent review by Glenk (2017) discussed the challenge and validity related to interpreting salivary cortisol of shelter dogs in AAIs, and concluded that it may not be a suitable marker to investigate the intervention effect in these dogs.

Much more research is needed to understand the reported inter-individual variability in patterns of SS and cooperation between a handler and a dog, that may impact performance (Beebe et al., 2016) and should therefore be taken into account when planning an animal-assisted intervention.

Gender and the dyadic gender combination appears to influence social interactions in humans (Ben-Ner et al., 2004). In general, same-gender parent-infant dyads seem to experience more synchrony (Leclère et al., 2014). Dogs can probably discriminate human gender and may adapt their behavior according to the owner gender, so that minor variations in the owners´ interaction styles may have distinct effects on the dogs´ physiological and behavioral responses (Hennessy et al., 1998; Schöberl et al., 2017). Unfortunately, the sample is presently too small to produce a meaningful result that is worth exploring in future research.

Dogs are sensitive to their handlers´ emotional states (Müller et al., 2015) and emotional contagion between owners and dogs is possible (Yong and Ruffman, 2014) contributing to the level of emotional disturbance experienced. Thus, dogs may mirror the anxiety and negative expectations of handlers in their cortisol levels and this could actually happen in the context of AAA, as therapeutic work affects handler-dog teams who work in animal-assisted health care service both emotionally and physiologically (Kirchengast and Haubenhofer, 2007). This is the reason why we decided to monitor both the dyadic members for signs of stress. In our study, nor dogs nor the handlers showed physiological changes indicating stress. Overall, findings suggest that this particular AAA, or expectation itself, did not negatively impact the welfare of both these handlers and dogs. This was likely because activities were predictable and controllable. No physiological or behavioral indicators of stress were observed, and salivary cortisol levels were determined to be no different between home and AAA settings. Dogs' and handlers' levels of salivary cortisol were higher before the AAA session than after, but this trend was observed on both AAA and control days and values remained always within the physiological range (human: 3-10 ng/mL, dog: 0.70-3.40 ng/mL) (Sandri et al., 2015). This outcome was likely due to the normal circadian rhythmicity of cortisol secretion (Dreschel, 2007; Beerda et al., 1999). It is worth remembering that dog 4 showed higher cortisol levels than the other dogs, and values were even higher at control than at AAA. This might suggest that this dog was less confident in general, not just related to the physical contact with the patient.

HR was higher in dogs in AAA days than in control days, but always within physiological range, so found values can be ascribed to positive arousal (Ng et al., 2014). There was no statistically significant difference in any physiologic parameter over the five subsequent sessions, and so the chance of chronic stress accumulation effect may be excluded.

This study has several limitations. First, the small study size: in future studies a larger sample covering more AAA sessions may be needed for more generalized results. A larger sample size will also enable us to explore the possible role of factors, such as handler's gender, dog's sex and story in the experience of synchrony. Second, the video-recorded assessment was coded for the occurrence of handler-dog eye gaze synchrony. However, coding from video may be not optimal for precise determination of one's looking targets. Eye-tracking assessments of SS may provide more precise spatial and temporal information than face-to-face assessments. Third, synchrony is shown to depend on physiological mechanisms supporting bond formation in mammals—particularly such as those involving the hormone oxytocin. Measurement of this hormone would be needed to evaluate the robustness of our findings. Although peripheral oxytocin is commonly used to approximate central concentrations, the validity of this experimental approach has yet to be established (Valstad et al., 2017). Because of the limitations of this study already named and others (only female dog handlers participating, effect of patient familiarity not explored), cause-effect relationships could not be inferred and we cannot generalize the results of our study to other handler-dog teams. Further research is needed to clarify whether the overall lack of distress facilitates synchrony and cooperation of a dog with the human handler or, *vice versa*, the absence of stress results from the creation of synchronous handler-dog dynamics.

Conclusions

Synchrony is a key feature of mother-infant interactions. Touch, eye contact, and joint attention are fundamental behaviors that maintain child-caregivers interactions and establish a basis for their emotional capacity to respond each other (Feldman, 2007). Based on the assumption that attachment-related interaction styles are displayed in interactions with dogs in the same way as they are expected to be in interaction with humans (Mariti et al., 2013), we sought to explore whether and how a synchronous interaction is displayed within handler-dog dyads. All in all, our findings suggest that a handler and a dog engage in synchronous interactions, that may be well observed while they are jointly committed under unstressed working conditions. SS underlies the development of affiliative bonds and, thus, its detection in social contexts may be important for bond formation and, consequently, for adequate social functioning (Atzil et al., 2014). Understanding the dynamics of human-dog interactions and identifying synchronic patterns within human-dog dyads are therefore important to promoting healthy relationships, and might also shed useful light on some of the mechanisms by which the human-dog partnership is able to impact upon AAA sessions, thereby positively influencing the outcome of an intervention. Our ongoing studies have been designed to consider more physiological indicators of human-dog bonding in a larger and more varied population of dyads, so as to provide a reasonable demonstration of the SS construct at the inter-specific level.

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Conflict of interest statement

The authors declare no conflicts of interest.

Ethical Approval

This study was approved by the Ethics Committee of the RSD Luigi and Dario Villa – Stefania Foundation, Muggiò, Italy. Research was based on voluntary participation and informed consent with the institution, patients (or their legally authorized representatives), and animal handlers. This research complies with the current Italian laws on animal welfare.

Authorship

The idea for the paper was conceived by Federica Pirrone. The experimental protocol was designed by all authors. The data were statistically analyzed by Federica Pirrone and discussed by all authors. The paper was written by Federica Pirrone.

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Table 1 Information about the dogs participating in this study

Dyad code	Breed	Age	Sex	Weight	Source	Experience with AAA
1	Briard	3 years	Female (spayed)	30 kg	Official breeder	1 year
2	Golden retriever	3 years	Female (spayed)	27 kg	Official breeder	1 year
3	Mix	4 years	Male (intact)	22 kg	Shelter	1 year
4	German shepherd	8 years	Male (neutered)	35 kg	Shelter	1 year

Table 2 List of behaviors and definition used in the study

Behaviors	**Description**	**Measured values (F/D)**
Social synchrony behavior		
Gaze synchrony	Dog and dog handler engage in simultaneous social gaze (dog looks at handler, handler looks at dog)	F, D
Joint attention	Dog and handler attend to the same target	F, D
Touch synchrony	Handler provides affectionate touch while handler and dog look at each other	F, D
Responsiveness to a handler's cue		
Fulfilled	The dog fulfils the handler's cue within 5 sec	F
Not fulfilled	The dog doesn't fulfil the handler's cue within 5 sec	F
Others		
Ears plastered back	Backward positioning of ears	F
Looking at	Looking to either the handler or patient	F
Lips/nose licking	Part of the tongue is shown and moves along the upper lip and/or nose	F
Yawning	Slow and deep inhalation with forced and involuntary jaws and mouth opening	F
Paw lifting	A fore paw is lifted from the ground, flexed into a position of approximately 45°	F
Tail down	Lowered position of tail	F
Vocalizing	Any form of vocalization, including barking, whining, growling, howling.	F
Standing	Upright static position with at least 3 paws in contact with the ground for >1 min	D
Sitting	Static position with hindquarters flexed and in contact with the ground; forelimbs are extended with only paws in contact with the ground for >1 min	D
Recumbent	Static position with trunk lying in complete contact with the ground in lateral, sternal or dorsal recumbency for >1 min	D
Exploring	The dog moves slowly, sniffing and investigating the environment	D
Playing	Playful interactions with elements from the environment	D

Avoidance	Escape behavior, withdrawal, eyes or head turned away from either the handler or patient	F
Attention-seeking	Seeking attention and physical contact from handler and/or patient: nuzzling or pawing for attention, jumping up on, asking to be petted	F
Changing of posture	Frequent changes of position: standing up shortly after sitting/lying down for < 30 sec	F
Sniffing	Sniffing along object and/or the floor before responding to handler's cue	F
Persistent self-grooming	Oral behavior directed towards dog's own body (licking, chewing skin or coat) for > 1 min	F
Scratching	Purposeful movement of limbs to scratch any part of body	F
Circling	Continuous walking in short circles	F
Body shaking	Move, shake the body with energy	F

F = Frequency; D = Duration

Table 3 Differences in social synchrony duration assessed by semi-quantitative method

Syncrony behaviors	**Phase T_1(GI)**			**Total**	**STASTISTICS**
	< 1 sec %	**1-5 sec** %	**> 5 sec** %	%	**Kruskal-Wallis test** P
GAZE SYNCRONY	67.5	29.9	2.6*	100	0.001
JOINT ATTENTION	43.8	41.3	14.9*	100	0.001
TOUCH SYNCRONY	65.3	28.6*	6.1*	100	0.001

Values are expressed in terms of percentages.

T_1: during guided interactions (GI) with patients. * = P < 0.05 *vs* the other categories of duration within behavior.

Table 4 Differences in the relative frequency of spontaneous physical contact-seeking (sPCS) with patients among dogs during guided interactions

Dog	**sPCS with patients** (F)
dyad 1	2.3* ± 0.2
dyad 2	0.5 ± 0.3
dyad 3	1.0 ± 0.3
dyad 4	0.2 ± 0.1

Values are expressed as relative means (n. occurrences/min) ± S.E.
[*] = *vs* the other dogs, Kruskal-Wallis test $P < 0.05$.

Table 5 Mean concentrations of salivary cortisol and heart rate in AAA and control days

Subject	**Days**	**Cortisol (ng/ml) T_0**		**Cortisol (ng/ml) T_2**		**Wilcoxon Signed-Rank test**
		Mean	S.E.	Mean	S.E.	P
Dogs	Control	1.4	0.4	1.1	0.3	-
Dogs	AAA	1.2	0.3	0.8	0.1	-
Handlers	Control	10.0*	0.3	4.5	0.1	0.001
Handlers	AAA	9.0*	0.2	4.5	0.1	0.003
Subject	**Days**	**HR T_0**		**HR T_2**		**Mann-Whitney U test**
		Mean	S.E.	Mean	S.E.	P
Dogs	Control	60.0	3.3	65.0	3.9	-
Dogs	AAA	74.0§	2.6	80.0	3.9	0.001
Handlers	Control	79.0	2.7	78.0	1.6	-
Handlers	AAA	85.0	4.2	81.0	2.2	-

AAA: Animal Assisted Activities; * = $P < 0.05$ T_0 *vs* T_2.
HR: Heart Rate. § = $P < 0.05$ AAA *vs* Control.

Table 6 Differences in mean concentrations of salivary cortisol among dogs

Day	Time point	Dogs' Salivary Cortisol (ng/ml)								Kruskal-Wallis test
		Dog1		Dog 2		Dog 3		Dog 4		
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	P
Control	T_0	0.6	0.0	1.2*	0.0	0.6	0.1	3.3*	0.8	0.010
AAA	T_0	0.6	0.1	1.3*	0.2	0.7	0.1	2.4*	0.9	0.026
Control	T_2	0.6	0.1	0.7	0.1	0.6	0.2	2.5**	0.2	0.010
AAA	T_2	0.5	0.1	0.9	0.1	0.5	0.0	1.3**	0.5	0.045

AAA: Animal Assisted Activities; T_0: * = *vs* Dog 1 and Dog 3; T_2: ** = *vs* all other dogs.

FIGURE CAPTIONS

Fig. 1 - Spatial arrangement of AAAs. a) A moment of the group session with one of the two visiting handler-dog dyads. The experimenter (holding a white block-notes) and the two healthcare professionals (sitting on the sofa) are also visible. b) – A moment of the group session with both visiting handler-dog dyads, each working with a recumbent patient. The experimenter (holding a white block-notes) is also visible. AAA: Animal Assisted Activities.

Fig. 2 – Mean frequency (n/min) of social synchrony behaviors before (T_0), during (T_1) and after (T_2) an AAA session.

AAA: Animal Assisted Activities; GS= Gaze Synchrony; JA= Joint Attention; TS=Touch Synchrony

GS and JA: * = T_1 *vs* T_0, $P < 0.05$ Kruskal-Wallis test. T_1: $^{\#}$ = JA *vs* GS and TS, $P < 0.05$ Kruskal-Wallis test.

Fig. 3 – Mean duration of dogs' resting *versus* active behaviors before (T_0), during (T_1) and after (T_2) an AAA session, expressed as %.

AAA: Animal Assisted Activities; * = *vs* the other behaviors, $P < 0.05$, Kruskal-Wallis test.

Fig. 1

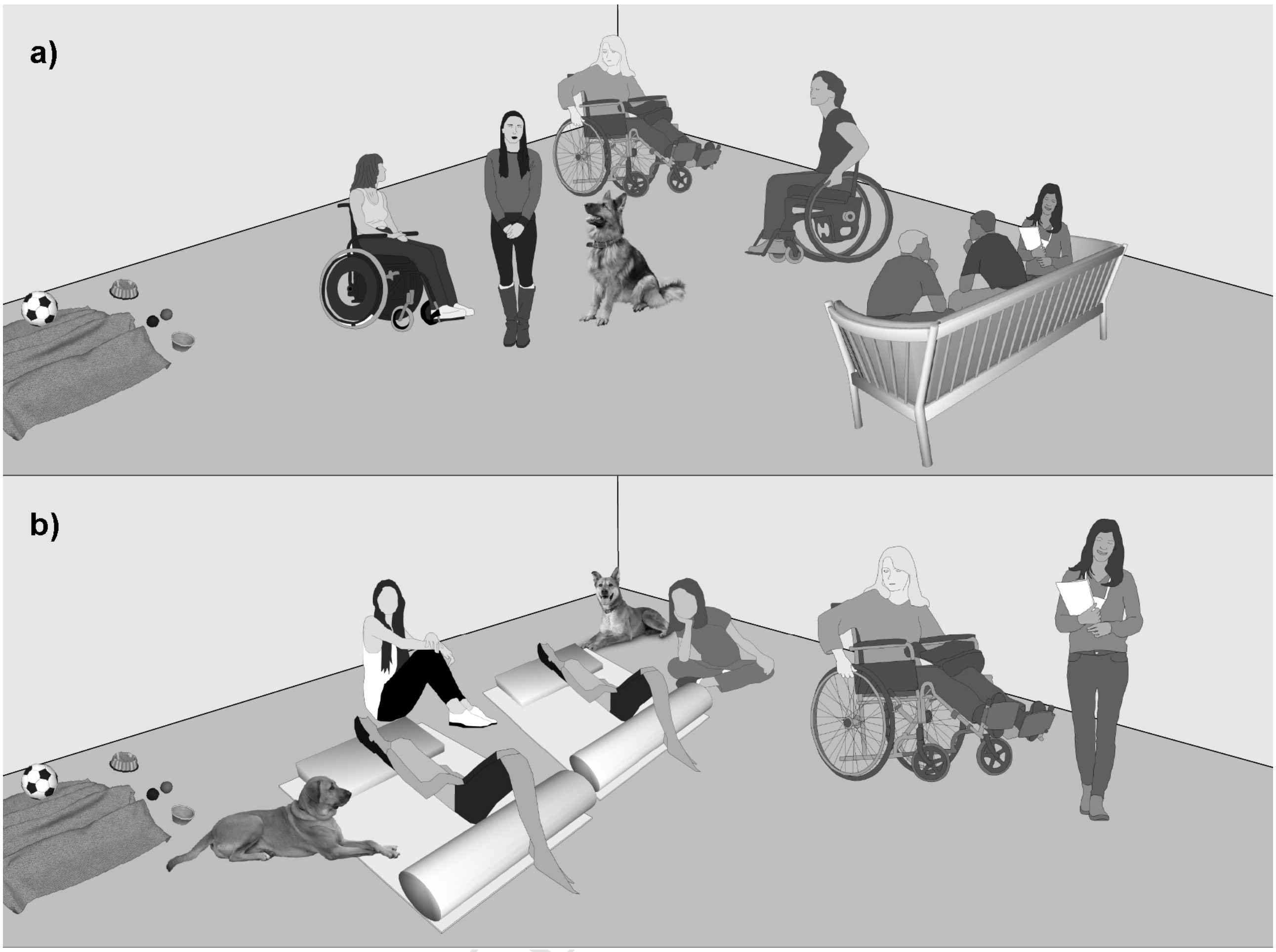
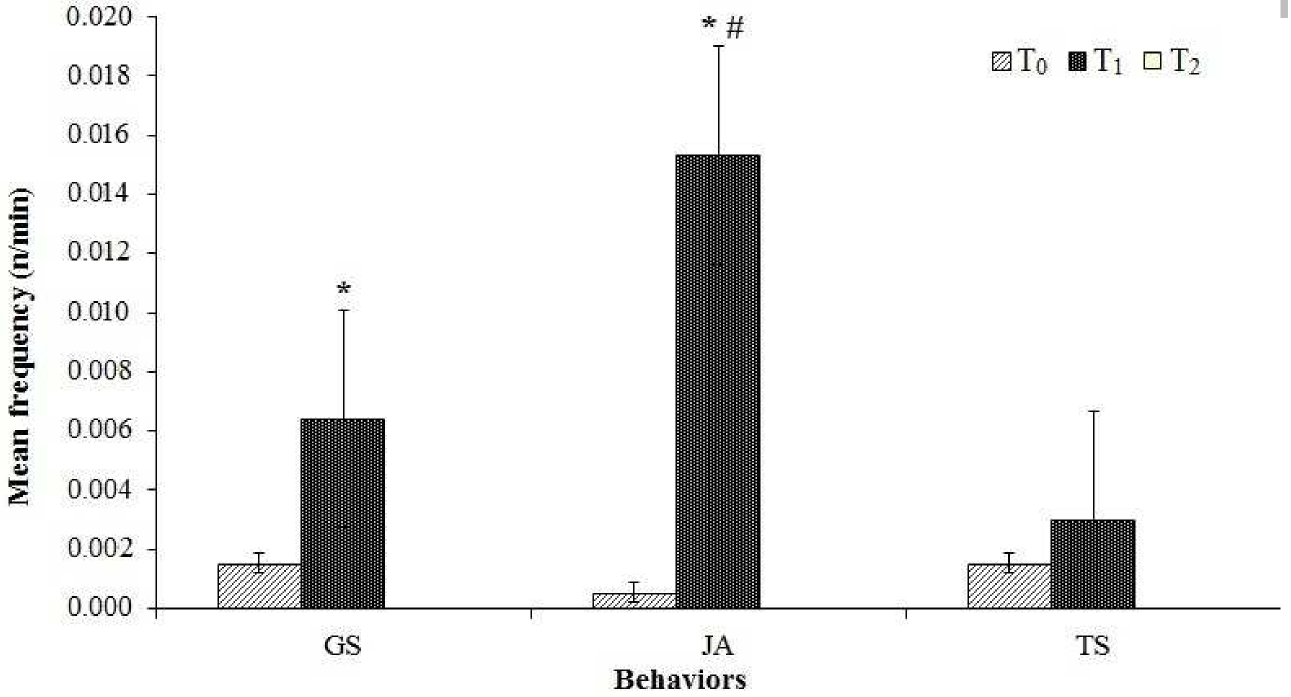


Fig. 2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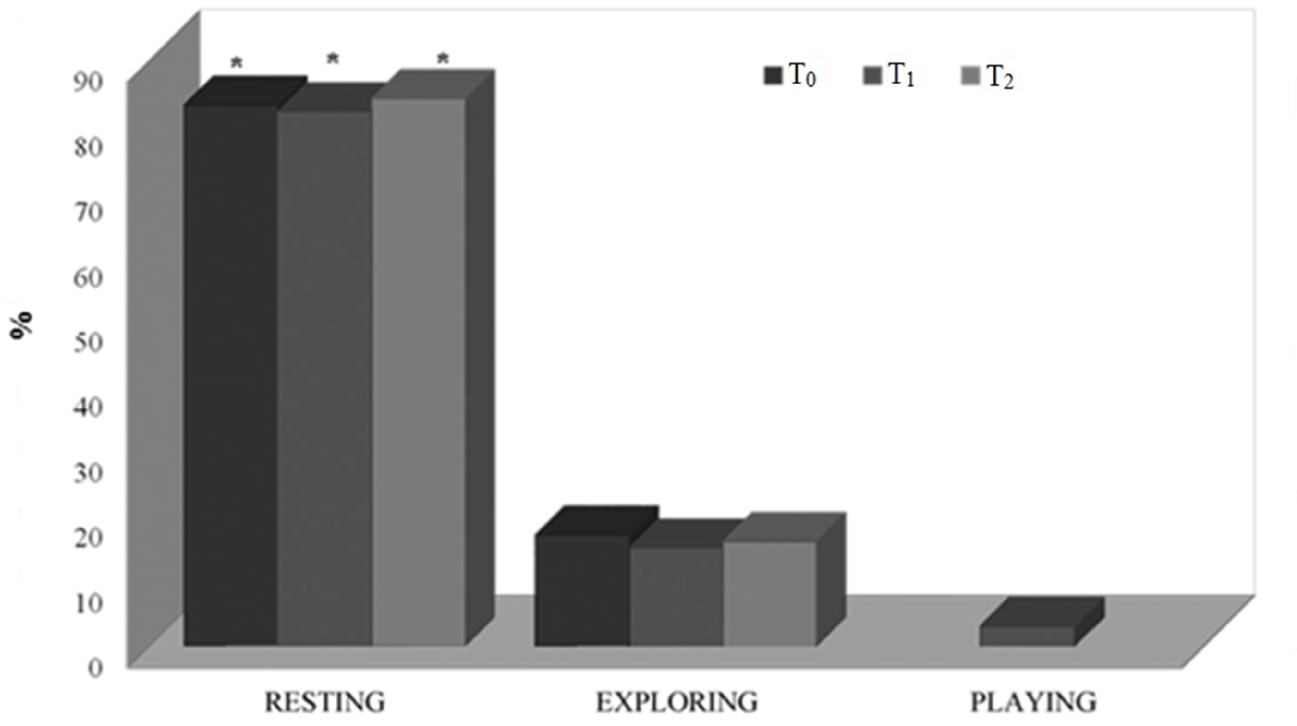
Mean frequency (n/min)
* #
*
T0 T1 T2
GS
JA
TS
Behaviors

Fig. 3



Highlights

1. Synchrony patterns between handler and dog were investigated in the context of animal assisted activities
2. All handler-dog dyads showed synchronous behaviors, particularly joint attention
3. No stress-related signs were detected in either the dogs or their human partners