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THE EVOLUTION OF HUMAN-DOG COMMUNICATION MECHANISMS DURING THE DOMESTICATION PROCESS

TESI DI DOTTORATO IN BIOLOGIA

XXXI CICLO

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CHAPTER 1

BACKGROUND

Animals have always played an important role in the lives of humans, such that, over the past 11,000 years, they have been brought under domestication in every part of the world. Domestic animals belong to mammals, birds, reptiles, amphibians, fish, insects, and even bacteria. Raised for food, secondary products, labor and companionship, domestic animals merged into human society (Alves, 2016). Although the principal motivation for the domestication of most species was their potential to furnish products consumed as food by humans (Diamond, 2002; Muller, 2002; Russell, 2002), the very first domestication was apparently not associated with this intention. It seems that the first domesticated animal was the ancestor of the nowadays wolf, giving rise to domesticated dogs (*Canis lupus familiaris*) (Muller, 2002). These animals were probably useful as guards and in helping humans during their hunting activities (Muller, 2002), roles which they still fulfill today (Koster, 2008). Later, other mammals were also domesticated, such as cattle, sheep, goats, pigs, horses, donkeys.

The degree to which a wild population of animals is willing to domestication largely depends on the degree of developmental plasticity of the species and the extent to which the captive environment, compatible with husbandry techniques, allows the expression of species-typical behavioral patterns (Balon, 1995). Although there are many reported differences between wild and domestic stocks, there are little evidences that domestication has resulted in the loss of specific behaviors or that the basic structure of the motor patterns for such behaviors has been changed (Scott and Fuller, 1965; Hale, 1969; Miller, 1977). The hypothesized loss of certain behavior under domestication can be explained, instead, by the heightening of response thresholds, changing in the quantitative rather than qualitative nature of response (e.g., fear reduction, increased stress tolerance, higher social tolerance).

1.1 How domestication is defined

Price and King (1968), with a very stringent definition, proposed that “domestication is an evolutionary process involving the genotypic adaptation of animals to the captive environment”, asserting that three different genetic processes influence populations of animals undergoing domestication: natural selection, artificial selection and relaxed selection. The first provides the basic selective mechanism for genetic change in captive populations; it is most intense during the first few generations (during the transition from field to captive environments), but it strength depends on the number of generations in captivity and how much the captive environment allows for the development and expression of species-typical biological characteristics (Spurway, 1955). The second one is the only selective mechanism unique to domestication, and it may be applied either intentionally or unconsciously by human with a selection for preferred traits. The relaxed selection is representative of the increase in both genetic and phenotypic variability for traits directly connected to behaviors essential for survival in nature, which lose much of their adaptive significance in captivity (e.g., food finding, predator avoidance).

Ratner and Boice (1975) were the first to give a more ontogenetic approach by acknowledging the contributions of both genetic change and experience separately in the development of the domestic phenotype. Price (1984) assumed that genes and developmental environment operate together to determine phenotypic outcomes.

Though, the experiential acquisitions are not transmitted to the next generation, so many researchers do not consider them in a formal definition of domestication. According to Kisling (2001), domestication was a long-term biological process that required the breeding of wild animals for many generations and was only fully accomplished when significant changes occurred in the behavior, physical attributes, and genetics of the captive species. These alterations were presumably not triggered (or at least not initially) by human communities, leading us to imagine that domestication was not premeditated or predetermined.

1.2 The domestication syndrome

Once humans started agriculture, domestication and diversification of animals accelerated, such that they probably evolved traits that were adaptive under these new conditions (Jensen, 2014). Although each species has its own history, common denominators in terms of adaptation to life with humans have caused domestic populations to diverge rapidly from their wild ancestors in appearance, physiology, and behavior.

Some behavioral characteristics make certain animal taxa (and individuals), better candidates for domestication than others (Hale, 1969; Price, 1984, 1999, 2002; Zeder, 2012). The foremost prerequisites of successful domestication must have been a reduced fear and aggressiveness toward humans, enabling reproduction in conditions that would normally elicit strong stress reactions (e.g., confinement, human proximity, and crowding) and less territoriality and aggression toward conspecific, as a consequence of living in larger groups in overcrowded conditions (Forkman et al., 2007; Campler et al., 2009; Hughes and Mcdonald, 2013). Therefore, the low reactivity toward external stimuli is a central preadaptation for animal domestication, including all domesticated mammals — carnivores (Trut, 1999; Coppinger and Coppinger, 2001), herbivores (Tennessen and Hudson, 1981), and rodents (Murphy, 1985) — as well as domestic birds (Andersson et al., 2001), fish (Waples, 1991), and even domesticated invertebrate species (Marliave et al., 1993; Price, 2002).

Belyaev (1969), a Russian geneticist and academician, believed that the phenotypic changes observed in domesticated animals were regulated by a fine balance between neurotransmitters and hormones at the level of the whole organism. The genes that control that balance occupy a high level in the hierarchical system of the genome (i.e., a pleiotropic effect for which linked traits may arise from mutations in a few key regulatory genes; Jensen, 2006), whose main role is to control the functioning of the neural and endocrine systems, which also control animal's behavior including its friendliness/hostility towards humans. So, he claimed that the key factor for domestication was the selection of a peculiar behavior: tameness. According to this concept, selecting animals for behavioral traits could have fundamentally altered the development of an organism and could have been directly related to brain neurochemistry. Because mammals from widely different taxonomic groups share similar regulatory mechanisms for hormones and neurochemistry, it was reasonable to believe that selecting them for similar behavior (tameness) should alter those mechanisms, and the other developmental pathways they control, in similar ways.

To test his hypothesis, Belyaev designed a selective-breeding program (begun about 50 years ago at the Institute of Cytology and Genetics in Novosibirsk, Siberia) characterized by a strong selection pressure for tameness, choosing as experimental model a species taxonomically close to the dog but never domesticated: *Vulpes vulpes*, the silver fox (Belyaev, 1979; Trut, 1999). Tameness is an important behavioral trait since it facilitates animal handling (Grandin, 2007) and improves animal welfare (Hemsworth and Barnett, 1987). Acquired tameness can be achieved by habituation or positive associative conditioning. In habituation, the animal's fear of humans is gradually reduced by repeated exposures in a neutral context (absence of positive/negative reinforcing by man's presence), but it may also be achieved by positive associative conditioning with humans as secondary reinforcer (as providers of food, water, shelter, grooming or companionship). In this way, the threshold for avoidance behaviors is raised. However, acquired or learned tameness is not transmitted from mother to offspring. For example, the human avoidance by the offspring of hand-reared (tame) and mother-reared (relatively untamed) ungulates is very similar when exposed to people in the absence of their mother or herd-mates (Blaxter, 1974; Lyons et al., 1988a), whereas mother-reared dairy goats exhibited less avoidance of humans when accompanied by tame herd-mates (Lyons et al., 1988b). To ensure that tameness results came from genetic selection, Belyaev and his team avoided training, submitting the foxes to a battery of tests. When a pup was one month old, an experimenter offered it food from his hand while trying to stroke and handle it. The pups were tested twice, once in a cage and once while moving freely with other pups in an enclosure, where they could choose to make contact either with the human experimenter or with another pup. The test was repeated monthly until the pups were six or seven months old. At seven or eight months, when the foxes reached sexual maturity, they were scored for tameness and assigned to a class. Foxes that were afraid of the experimenters or bit when stroked or handled, were assigned to Class III. Foxes in Class II let themselves be petted and handled, although not showing emotionally friendly response to experimenters. Foxes in Class I were friendly toward experimenters, wagging their tails and whining. Finally, the members of Class IE (after sixth generation), before they were one month old, already have started to be eager to establish human contact, whimpering to attract attention, sniffing and licking experimenters. The unselected control population continued to exhibit a wild-type behavior, including strong defensive responses toward humans. Corresponding to the hypotheses, years later Belyaev research group noted an increase of the corticosteroids in the blood plasma of the tamed foxes, which was the direct consequence of changes in the serotonin system, the leading mediator inhibiting animal's aggressive behavior (Trut, 1999). Particularly, higher levels of serotonin, and of its metabolite 5-hy-droxyindole acetic acid, have been detected in the brain of tamed foxes (Popova et al., 1991). Other endocrinological changes associated with the domestication syndrome have been recognized, such as the reduction adrenocorticotropic hormone release from the anterior pituitary of the hypothalamic-pituitary-adrenal axis associated with the stress response in mammals (Gulevich et al., 2004; Trut et al., 2009; Hare et al., 2012; Wilkins et al., 2014).

Darwin (1868) was the first recognizing that domestic animals possess a wide variety of similar morphological traits despite the lack of close evolutionary relationships between their wild progenitors (herbivores and predators, large and small). This phenotypic convergence includes change in body size and proportions, leading to the appearance of dwarf and giant breeds; change in the typical wild coat camouflage in favor of a completely lacking pigmentation in specific body areas (piebald or spotted coat). The latter characteristic has some linkage with the biochemical pathways of melanin, involved in coat coloration, and neurotransmitters, such as the dopamine, which in turn affect the behavior and the cognition (Keeler et al., 1968; Hemmer, 1990). Hair turned wavy or curly, also becoming longer or shorter. Tails changed, too, curling up in a circle or semicircle, or shortening as a result of a decrease in the number of tail vertebrae. Ears became floppy, a feature not found in any wild animals, which may be a byproduct of the neoteny (Fox, 1968; Morey, 1994, Goodwin et al., 1997; Fang et al., 2009). In many domesticated species, certain anatomical characteristics of the juvenile (e.g., shortening of the jaws and facial region) are retained into adulthood, perhaps to preserve the greater esthetic appeal of the young animal (Dechambre, 1949; Clutton-Brock, 1981; Morey, 1994). Coppinger and Smith (1983) suggest that behavioral neoteny together with a placid temperament (tameness) have been selected for in certain wild species (e.g., the ancestors of our common domestic animals) and are a prerequisite to successful domestication. The behavior of the dog provides the most compelling case of behavioral neoteny accompanying the domestication process. Comparative studies of domestic dogs and wolves (Fox, 1978; Frank and Frank, 1982; Ginsburg and Hiestand, 1992; Morey, 1994) have suggested that the behaviors of the adult dog are relatively juvenile compared to wolves. Neoteny may characterize the behavioral development of the dog because of selection for tractability, a trait frequently associated with juvenile animals. The ease with which most dogs can be trained to perform various tasks may reflect a high degree of plasticity relative to the more structured (i.e., less variable) adult-like behavior of their wild relatives. Coppinger et al. (1987) has pointed out that different dog breeds may express different degrees of behavioral neoteny. Dog breeds selected for herding livestock exhibit the eye/stalk/chase/and (sometimes) bite sequence of predatory behaviors but refrain from attack. Dog breeds selected for protecting livestock lack these predatory behaviors and will sometimes engage them (livestock) in play. An alternative to the selection hypothesis is that behavioral neoteny in domesticated animals may be environmentally induced. First, captive young animals may be conditioned to retain their juvenile behaviors. Offering positive rewards for juvenile behaviors (e.g., care-soliciting, playfulness, submissiveness to humans) may, in effect, retard the development of more independent adult activities, or alternatively, may reduce their expression. This latter effect could be achieved by raising the threshold for elicitation of adult behaviors or by sufficiently lowering the threshold for juvenile behaviors to allow for the continued expression of many juvenile characteristics throughout adulthood. Moreover, rearing animals in physical isolation from older, socially dominating conspecifics, may further discourage the development of normal adult-like patterns of agonistic behaviors (Price, 1978) and facilitate the retention of many juvenile social behaviors. Nevertheless, it is interesting to underline how the dog's sociability may be the result of the canine homologous Williams-Beuren syndrome (vonHoldt et al., 2017), a genetic disorder that in humans causes hyper-sociability (Dykens and Rosner, 1999). Indeed, a deletion in 5-Mb genomic region on chromosome 6 linked to the syndrome was observed in dogs but not in wolves (vonHoldt et al., 2017).

The neoteny was also responsible for changes in the dimensions of the skull (Wayne, 1986; Morey, 1992; Trut, 1999) and in a shortening of snout length which results in tooth crowding and a reduction in teeth number and size (Turnbull and Reed, 1974). This collection of traits has been termed as the domesticated phenotype (Price, 2002) and represents an animal equivalent of the domestication syndrome described in plants (Harlan, 1975). All these morphological characteristics have also been highlighted in domesticated foxes, so that Belyaev demonstrated that selection for tame behavior resulted in the acquisition of numerous other phenotypic traits including piebald coats, drooping ears, upturned/reduced tails, shortened snouts and under-bites, smallest legs, and shifts in developmental timing, as an effect of the domestication syndrome (Trut, 1999; Trut et al., 2009). A similar process was also observed in chickens (Agnvall et al., 2012).

It is also being observed that sustained and increasingly intensified selection for lowered reactivity resulted in profound changes in brain size in other animals (Plogmann and Kruska, 1990; Ebinger, 1995; Ebinger and Röhrs, 1995; Kruska, 1988, 1996; Rehkämper et al., 2008). As showed in lower vertebrates (Pinelli et al., 2014) changes in brain evolution can affect some areas in different manner. Accordingly, not all parts of the brain are equally affected by brain-size reduction in domesticated mammals, in which the most evident change is the reduction of the complex structures belonging to the limbic system, with domestic pigs, dogs, and sheep showing a greater than 40% reduction in size in comparison with their wild progenitors (Kruska, 1988). The limbic system, including the hippocampus, the hypothalamus, the pituitary gland, and the amygdala, regulates the endocrine functions and the autonomic nervous system activities linked to behaviors like aggression, wariness, and responses to environmentally induced stress. The reduction in the size of this portion of the brain in domesticated animals can be directly linked to an increase in the threshold reactivity for the display of behaviors such as aggression, fear, and flight (Kruska, 1988; Price, 2002). Although some researchers characterize these changes as a “decline of environmental appreciation” (Hemmer, 1990) or even more negatively as “regressive evolution” (Röhrs, 1985), the higher threshold related to stressful situations in domestic animals be highly adaptive, leading to cope better with the stimuli in the anthropogenic niches (Price, 2002).

There is considerable support for the hypothesis that domestication has prolonged and accelerated some developmental processes by selective mechanisms (Cairns, 1976). Some of the differences have obvious links to the changes in animals’ social behavior, such as the extension of the sensitive period, essential for the socialization. In dogs, for example, it is well known that the first weeks of life are crucial for creating a primary social bond with humans through the imprinting. The "window" of bonding opens when a puppy becomes able to sense and explore its surroundings, and it closes when the pup starts to be afraid of unknown stimuli (from 8 to 12 weeks, depending on the breed). According to Belyaev team (1985; Trut, 1999), non-domesticated fox pups start responding to auditory stimuli on day 16 after birth, and their eyes are completely open by day 18 or 19. On average, the domesticated fox pups respond to sounds two days earlier and open their eyes a day earlier than their non-domesticated cousins. Non-domesticated foxes first show the fear response at 6 weeks of age, whereas domesticated ones show it after 9 weeks or even later. As a result, domesticated pups have more time to become imprinted to humans.

Other major evolutionary consequences of domestication are the accelerated attainment of sexual maturity, the loss of the seasonal rhythm of reproduction and the changes in mate preferences. There are evidences that most domestic dogs become sexually mature at 6 to 9 months of age, which is considerably earlier than for wolves and other wild canid species (Scott and Fuller, 1965; Fox, 1978). Moreover, most wild animals in middle latitudes are genetically programmed to mate once a year, whereas domestic animals at the same latitudes can mate more than once a year irrespectively from the season (Christie and Bell, 1971). Otherwise wolves are forced to breed only once a year mating in the winter months (Scott and Fuller, 1965). In addition, the monogamy or specific mate preferences of wild canids has been largely eliminated in domestic dogs, shifting toward more promiscuous sexual relations (Scott and Fuller, 1965).

1.3 References

Agnvall, B., Jöngren, M., Strandberg, E., & Jensen, P. (2012). Heritability and genetic correlations of fear-related behavior in red junglefowl–possible implications for early domestication. *PloS one*, *7*(4), e35162.

Alves, R. R. N. (2016). Domestication of animals. In: Albuquerque, U.P., Alves R. (Eds.), *Introduction to Ethnobiology,* (pp. 221-225). Springer, Cham.

Andersson, M., Nordin, E., & Jensen, P. (2001). Domestication effects on foraging strategies in fowl. *Applied Animal Behavior Science*, *72*(1), 51-62.

Balon, E. K. (1995). Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture*, *129*(1-4), 3-48.

Belyaev, D. K. (1969). Domestication of animals. *Science*, *5*(1), 47-52.

Belyaev, D. K. (1979). Destabilizing selection as a factor in domestication. *Journal of Heredity*, *70*(5), 301-308.

Belyaev, D. K., Plyusnina, I. Z., & Trut, L. N. (1985). Domestication in the silver fox (*Vulpes fulvus Desm*): Changes in physiological boundaries of the sensitive period of primary socialization. *Applied Animal Behavior Science*, *13*(4), 359-370.

Blaxter, K. L. (1974). Deer farming. *Mammal Review*, *4*(3), 119-122.

Cairns, R. B. (1976). The ontogeny and phylogeny of social interactions. In: Hahn, M.E., Simmel, E.C. (Eds.), *Communicative behavior and evolution,* (pp. 115-139). Academic Press, New York.

Campler, M., Jöngren, M., & Jensen, P. (2009). Fearfulness in red junglefowl and domesticated White Leghorn chickens. *Behavioral Processes*, *81*(1), 39-43.

Christie, D. W., & Bell, E. T. (1971). Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *Journal of Small Animal Practice*, *12*(3), 159-167.

Clutton-Brock, J. (1981). Domesticated animals from early times. British Museum/W. Heinemann, London.

Coppinger, R. P., & Smith, C. K. (1983). The domestication of evolution. *Environmental Conservation*, *10*(4), 283-292.

Coppinger, R., & Coppinger, L. (2001). Dogs: A startling new understanding of canine origin, behavior and evolution. Simon and Schuster, New York, Scribner.

Coppinger, R., Glendinning, J., Torop, E., Matthay, C., Sutherland, M., & Smith, C. (1987). Degree of behavioral neoteny differentiates canid polymorphs. *Ethology*, *75*(2), 89-108.

Darwin C. (1868). The Variation of Animals and Plants Under Domestication. London: John Murray.

Dechambre, E. (1949). La theorie de la foetalisation et la formation des rages de chiens et de porcs. *Mammalia*, *13*(3), 129-137.

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication. *Nature*, *418*(6898), 700.

Dykens, E. M., & Rosner, B. A. (1999). Refining behavioral phenotypes: Personality—motivation in Williams and Prader-Willi syndromes. *American Journal on Mental Retardation*, *104*(2), 158-169.

Ebinger, P. (1995). Domestication and plasticity of brain organization in mallards (Anas platyrhynchos). *Brain, behavior and evolution*, *45*(5), 286-300.

Ebinger, P., & Röhrs, M. (1995). Volumetric analysis of brain structures, especially of the visual system in wild and domestic turkeys (*Meleagris gallopavo*). *Journal fur Hirnforschung*, *36*(2), 219-228.

Fang, M., Larson, G., Ribeiro, H. S., Li, N., & Andersson, L. (2009). Contrasting mode of evolution at a coat color locus in wild and domestic pigs. *PLoS Genetics*, *5*(1), e1000341.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, *92*(3), 340-374.

Fox, M. W. (1968). The influence of domestication upon behavior of animals. In: Fox, M.W. (Ed.), *Abnormal behavior in animals,* (pp. 64–76). W.B. Saunders, Philadelphia.

Fox, M.W. (1978). The Dog: Its Domestication and Behavior. Garland Press, New York.

Frank, H., & Frank, M. G. (1982). On the effects of domestication on canine social development and behavior. *Applied Animal Ethology*, *8*(6), 507-525.

Ginsburg, B. E., & Hiestand, L. (1992). Humanity's "best friend": The origins of our inevitable bond with dogs. In: Davis, H., Balfour, D. (Eds.), *The Inevitable Bond: Examining Scientist–Animal Interactions,* (pp. 93–108). Cambridge University Press, New York.

Goodwin, D., Bradshaw, J. W., & Wickens, S. M. (1997). Paedomorphosis affects agonistic visual signals of domestic dogs. *Animal Behavior*, *53*(2), 297-304.

Grandin, T. (2007). *Livestock handling and transport.* CAB Int., Wallingford, U.K.

Gulevich, R. G., Oskina, I. N., Shikhevich, S. G., Fedorova, E. V., & Trut, L. N. (2004). Effect of selection for behavior on pituitary–adrenal axis and proopiomelanocortin gene expression in silver foxes (*Vulpes vulpes*). *Physiology & behavior*, 82(2-3), 513-518.

Hale, E. B. (1969). Domestication and the evolution of behavior. In: Hafez, E.S.E. (Ed.), *The bevavior of domestic animals*, (pp. 22-42). Bailliere, Tindall and Cassell, London.

Hare, B., Wobber, V., & Wrangham, R. (2012). The self-domestication hypothesis: evolution of bonobo psychology is due to selection against aggression. *Animal Behavior*, *83*(3), 573-585.

Harlan, J. R. (1975). Crops and man. Foundation for modern Crop Science.

Hemmer, H. (1990). *Domestication: the decline of environmental appreciation*. Cambridge University Press.

Hemsworth, P. H., & Barnett, J. L. (1987). Human-animal interactions. In: Price, E.O. (Ed.), *The Veterinary Clinics of North America. Food Animal Practice*, *3*(2), 339-356.

Hughes, J., & Macdonald, D. W. (2013). A review of the interactions between free-roaming domestic dogs and wildlife. *Biological Conservation*, *157*, 341-351.

Jensen, P. (2006). Domestication—from behavior to genes and back again. *Applied animal behavior science*, *97*(1), 3-15.

Jensen, P. (2014). Behavior genetics and the domestication of animals. *Annual Review of Animal Biosciences*, *2*(1), 85-104.

Keeler, C., Ridgway, S., & Lipscomb, L. (1968). The genetics of adrenal size and tameness in colorphase foxes. *Journal of Heredity*, *59*, 82–84.

Kisling, V. N. (2001). Ancient collections and menageries. In: Kisling, V. N. (Ed.), *Zoo and aquarium history: ancient animal collections to zoological gardens* (pp. 1–47). CRC Press, London.

Koster, J. (2008). The impact of hunting with dogs on wildlife harvests in the Bosawas Reserve, Nicaragua. *Environmental Conservation*, *35*(3), 211–220.

Kruska, D. (1988). Mammalian domestication and its effect on brain structure and behavior. In: Jerison, H. J., & Jerison I., *Intelligence and evolutionary biology* (pp. 211–50). Springer-Verlag, New York.

Kruska, D. (1996). The effect of domestication on brain size and composition in the mink (*Mustela vison*). *Journal of Zoology*, *239*(4), 645-661.

Lyons, D. M., Price, E. O., & Moberg, G. P. (1988a). Individual differences in temperament of domestic dairy goats: constancy and change. *Animal Behavior*, *36*(5), 1323-1333.

Lyons, D. M., Price, E. O., & Moberg, G. P. (1988b). Social modulation of pituitary-adrenal responsiveness and individual differences in behavior of young domestic goats. *Physiology & behavior*, *43*(4), 451-458.

Marliave, J. B., Gergits, W. F., & Aota, S. (1993). F_{10} pandalid shrimp: sex determination; DNA and dopamine as indicators of domestication; and outcrossing for wild pigment pattern. *Zoo biology*, *12*(5), 435-451.

Miller, D. B. (1977). Social displays of Mallard Ducks (*Anas platyrhynchos*): effects of domestication. *Journal of Comparative and Physiological Psychology*, *91*(2), 221.

Morey, D. F. (1992). Size, shape and development in the evolution of the domestic dog. *Journal of Archaeological Science*, *19*(2), 181-204.

Morey, D. F. (1994). The early evolution of the domestic dog. *American Scientist*, *82*(4), 336-347.

Muller, W. (2002). The first steps of animal domestication. Oxbow Books, Oxford.

Murphy, M. R. (1985). History of the capture and domestication of the Syrian golden hamster (*Mesocricetus auratus Waterhouse*). In: Siegel, H., *The hamster: Reproduction and behavior,* (pp. 3-

20). Springer, Boston, MA.

Pinelli, C., Rastogi, R. K., Scandurra, A., Jadhao, A. G., Aria, M., & D'Aniello, B. (2014). A comparative cluster analysis of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase histochemistry in the brains of amphibians. *Journal of Comparative Neurology*, *522*(13), 2980-3003.

Plogmann, D., & Kruska, D. (1990). Volumetric comparison of auditory structures in the brains of European wild boars (*Sus scrofa*) and domestic pigs *(Sus scrofa* f. dom.). *Brain, Behavior and Evolution*, *35*(3), 146-155.

Popova, N. K., Voitenko, N. N., Kulikov, A. V., & Avgustinovich, D. F. (1991). Evidence for the involvement of central serotonin in mechanism of domestication of silver foxes. *Pharmacology Biochemistry and Behavior*, *40*(4), 751-756.

Price, E. O. (1978). Genotype versus experience effects on aggression in wild and domestic Norway rats. *Behavior*, *64*(3), 340-353.

Price, E. O. (1984). Behavioral aspects of animal domestication. *The quarterly review of biology*, *59*(1), 1-32.

Price, E. O. (1999). Behavioral development in animals undergoing domestication. *Applied Animal Behavior Science*, *65*(3), 245-271.

Price, E. O. (2002). Animal domestication and behavior. CABI Publishing, Wallingford, UK.

Price, E. O., & King, J. A. (1968). Domestication and adaptation. In: Hafez, E. S. E. (Ed.), *Adaptation of Domestic Animals*, (pp. 34-45). Lea and Febiger, Philadelphia.

Ratner, S. C., & Boice, R. (1975). Effects of domestication on behavior. In: Hafez, E. S. E. (Ed.), *The behavior of domestic animals*, (vol. 3 pp. 3-19). Williams and Wilkins, Baltimore.

Rehkämper, G., Frahm, H. D., & Cnotka, J. (2008). Mosaic evolution and adaptive brain component alteration under domestication seen on the background of evolutionary theory. *Brain, behavior and evolution*, *71*(2), 115-126.

Röhrs, M. (1985). Cephalization, neocorticalization and the effects of domestication on brains of mammals. *Fortschritte der Zoologie*, *30*, 545-547.

Russell, N. (2002). The wild side of animal domestication. *Society & Animals*, *10*(3), 285-302.

Scott, J. P., & Fuller, J. L. (1965). *Genetics and the Social Behavior of the Dog*. University of Chicago Press, Chicago.

Spurway, H. (1955). The causes of domestication: an attempt to integrate some ideas of Konrad Lorenz with evolution theory. *Journal of Genetics*, *53*(2), 325-362.

Tennessen, T., & Hudson, R. J. (1981). Traits relevant to the domestication of herbivores. *Applied Animal Ethology*, *7*(1), 87-102.

Trut, L. N. (1999). Early Canid Domestication: The Farm-Fox Experiment: Foxes bred for tamability in a 40-year experiment exhibit remarkable transformations that suggest an interplay between behavioral genetics and development. *American Scientist*, *87*(2), 160-169.

Trut, L., Oskina, I., & Kharlamova, A. (2009). Animal evolution during domestication: the domesticated fox as a model. *Bioessays*, *31*(3), 349-360.

Turnbull, P. F., & Reed, C. A. (1974). The fauna from the terminal Pleistocene of Palegawra Cave, a Zarzian occupation site in northeastern Iraq. *Fieldiana. Anthropology*, *63*(3), 81-146.

vonHoldt, B., Shuldiner, E., Koch, I. J., Kartzinel, R. Y., Hogan, A., Brubaker, L., Wanser, S., ... & Udell, M. A. (2017). Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Science advances*, *3*(7), e1700398.

Waples, R. S. (1991). Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(S1), 124-133.

Wayne, R. K. (1986). Cranial morphology of domestic and wild canids: the influence of development on morphological change. *Evolution*, *40*(2), 243-261.

Wilkins, A. S., Wrangham, R. W., & Fitch, W. T. (2014). The “domestication syndrome” in mammals: a unified explanation based on neural crest cell behavior and genetics. *Genetics*, *197*(3), 795-808.

Zeder, M. A. (2012). The domestication of animals. *Journal of Anthropological Research*, *68*(2), 161-190.

CHAPTER 2

THE AIMS

Darwin (1859) began its book “On the Origin of Species” arguing about domestication, highlighting that apart from size, color, and shape, the behavior of domesticated animals also differs from wild relatives.

Apart from a reduction of reactivity (i.e., lowering of human-fear and increasing of stress and social tolerance) domestication has favored animals with a high propensity to cooperate and communicate with humans. In the communication, the sender changes the behavior (and the inner state) of the receiver by means of specific behavioral display (‘signals’) aimed to obtain a personal or reciprocal advantage (Krebs and Davis, 1993). In such a context, the dog has been studied extensively over the past two decades and the dog–human communication receiving growing interest over the years (for reviews: Wynne et al., 2008; Siniscalchi et al., 2018).

Based on the differences reported between wolves and dogs in social behavior and cognition two theory tried to explain the divergences between the dogs and their ancestral progenitors (Kubinyi et al., 2007; Miklósi et al., 2003; Udell et al., 2010a). The “Domestication (or phylogenetic) hypothesis” attributes the origin of most of the dog's behaviors (evolutionary social skills) to the genetic processes (i.e., natural, artificial and relaxed selection) involved in the domestication adapting the dogs to the anthopogenetic niche (Miklósi, 2009). This theory is based on studies demonstrating that dogs outperform wolves in different communicative contexts (Frank and Frank, 1983; Frank et al., 1989; Agnetta et al., 2000; Hare et al., 2002, 2010; Miklósi et al., 2003; Virányi et al., 2008; Pongrácz et al., 2010; Frank, 2011; Miklósi and Topál, 2011; Range et al., 2014; Marshall-Pescini et al., 2015). On the other hand, the "Two-stage (or ontogenetic) hypothesis" emphasizes the role of behaviors acquired through individual experiences (developmental social skills) (Wynne et al., 2008; Udell and Wynne, 2008, 2010; Miklósi, 2009; Udell et al., 2010a,b). The authors supporting this theory focus their research on the fact that human-reared wolves, dingoes, coyotes, and foxes (as well as other non-domesticated species) can respond to human social cues (Smith and Litchfield, 2009; Barrera et al., 2012; Udell et al., 2012) and, in some cases, they outperform dogs (Udell et al., 2008, 2010b).

To date, the ontogenetic aspect, as the experiential baggage of an individual, is acquiring more and more consideration in the scientific world. One of the ways to investigate the role of ontogenesis in *Canis lupus familiaris* is studying the consequences of the human exposition in different dogs' population, investigating how the quantity and the quality human exposure modulate dog 's behavior. In particular, it is possible to verify the existence of differences in the communicative tendency toward humans related to the level of human socialization (Barrera et al., 2012; Udell, 2015; Zaine et al., 2015; Duranton and Gaunet, 2016; Fagnani et al., 2016; Brubaker et al., 2017), both when the dog is the receiver (i.e. in reading human messages) and when is the sender (i.e. in sending messages toward humans). For example, while many pet dogs excel at following a range of human pointing gestures to distant locations to find a reward, dogs living in shelters often fail to follow a human's pointing unless additional training is provided (Udell et al., 2010b). Again, when presented with an unsolvable task, dogs reared in kennels from birth spend significantly less time gazing at humans than do pets (D'Aniello and Scandurra, 2016).

The human exposure at quality level, instead, consists in establishing if the presence, absence and type of training in dogs can influence the appearance/nonappearance/changing of some behavioral patterns (D'Aniello et al., 2015; Scandurra et al., 2015). For instance, dogs with agility training gaze longer at their owners than do pets or search-and-rescue dogs during an unsolvable task (Marshall-

Pescini et al., 2009). Highly trained obedience dogs spend more time interacting with a testing apparatus and appear to be better independent problem-solvers compared to untrained pet dogs, which relate more at their owners (Osthaus et al., 2003; Marshall-Pescini et al., 2008).

The transition from the phylogenetic to the ontogenetic paradigm could radically change the scientific thinking on the domestication process, emphasizing the importance of the subject's experience acquired during its development and in adult life, rather than their evolutionary acquisition. My research project is aimed at examining the ontogenetic mechanisms that underlie dog-human relationship and communication in the most ancient domestic species.

2.1 Quality exposure level on dog-human bond

As already been noted, domestication included a developmental delay resulting in the extension and increased rate of juvenile-type behavior into adulthood (Price, 1984), such as prolonged gaze, extended greetings (Bentosela et al., 2016; Nagasawa et al., 2015), and a tendency toward hyper-social responses (Udell, 2015; vonHoldt et al., 2017). Such developmental delays are also responsible for the extension of the socialization window during which the dogs can accept the human or other species as companion, as compared to wild canids (Udell et al., 2010a). For dogs, the sensitive period for initial socialization starts around 3 weeks of age and extends to 12 to 16 weeks of age. For wolves and other wild canines, this window starts a few days after birth and ends much earlier. If a wolf has not been socialized to humans during this period, it is unlikely that a lasting bond will be formed subsequently (Udell et al., 2010a; Udell and Brubaker, 2016), whereas, with intensive human-socialization during the first month of live and further on, a puppy-wolf can lead to dog-like attachment responses (Hall et al., 2015). While an extended window for socialization increases the likelihood that dogs will form a bond with humans, it does not guarantee it. There are evidences suggesting that the dog–human relationship can be characterized as an "attachment", which closely resembles the one reported between infants and their mothers (Hart, 1995; Topál et al., 1998; Prato-Previde et al., 2003). The presence of attachment has been demonstrated in adult dogs with different life experiences (Gácsi et al., 2001; Topál et al., 2005; Fallani et al., 2006, 2007; Prato-Previde and Valsecchi, 2007; Valsecchi et al., 2010; Mariti et al., 2013; Mongillo et al., 2013; Siniscalchi et al., 2013). Of interest were the studies carried out on guide dogs that demonstrated the separations they undergo during their life (e.g., puppy-walker and trainer) do not prejudice the formation of the new bond with the blind owner (Fallani et al., 2006, 2007; Valsecchi et al., 2010). Nonetheless, guide dogs showed less proximity research than family dogs (Fallani et al., 2006) and, despite having a more controlled reaction when separated from their handler, they have been involved in increased cardiac activation (Fallani et al., 2007). A more recent work on search and rescue dogs, shows that these dogs tended to display a more secure bond with their handler (Mariti et al., 2013). Moreover, it was demonstrated that owner's attachment profile affects the owner-dog attachment bond, such that the type of the attachment that the owner has structured during his early life with his parents influence the dog's behavior unknowingly (Siniscalchi et al., 2013). These works represent a scientific evidence of the fact that the attachment bond may be influenced by the ontogenesis.

To asses this hypothesis, the water and rescue dogs were selected for their specific training which raise the cooperative relationship with humans, testing them in an adapted version of the "Strange Situation Test", originally designed to investigate the human bond between mother and child in humans (Ainsworth et al., 1978). The study has been published in Applied Animal Behavior Science and reported integrally in the chapter 3.

2.2 Quantity exposure level on dog’s comprehension of human gestures

Many studies revealed that dogs can use various forms of human communication (e.g., Hare et al., 1998; Miklósi et al., 1998; Soproni et al., 2001; Kaminski et al., 2004). Most these studies used the so-called object choice paradigm (Anderson et al., 1995), during which a human experimenter hides food under one of several containers out of the dog’s view and then indicates the target location by giving a social cue (Miklósi and Soproni, 2006). The results show that dogs are very skillful in using the pointing as a communicative gesture to find food (e.g., Miklósi et al., 1998; Agnetta et al., 2000; Soproni et al., 2001). The researchers who support the phylogenetic current suggests that selection pressures during domestication influenced dogs’ skills in this domain, supposing two main hypotheses about how selection affected this ability: the by-product hypothesis (Hare and Tomasello, 2005; Hare et al., 2005) and the adaptation-hypothesis (Miklósi et al., 2003). The first states that, as a by-product of on one trait (e.g., tameness), dogs evolved other social skills, one of which is their ability to read human given communicative cues (Hare and Tomasello, 2005). The adaptation-hypothesis, on the other hand, states that humans have actively selected dogs for their ability to use human signals (Kaminski and Nitzschner, 2013). Nevertheless, many studies have emphasized the role of ontogeny, showing how adult wolves, if socialized intensively, outperform dogs in following human pointing (e.g, Udell et al., 2008, 2010a). To clarify if this ability is a dog evolutionary competence or a consequence of its experience, several studies compared groups of family dogs with dogs from shelters (Udell et al., 2008, 2010b, 2011; Hare et al., 2010; Cunningham and Ramos, 2014) obtaining contrasting results. In these researches the previous life of the dogs before their arrival in the shelter was unknown. Some of them belonged to pure breed, which make unlikely they had not previous contacts in human families, whereby having the opportunity to learn to follow human gestures and biasing the results.

Thus, a group of dogs born and living in a kennel with a low socialization regime was selected, testing them in the object choice task paradigm and comparing the results with family dog. The study has been published in Animal Cognition and reported integrally in the chapter 4.

2.3 The importance of visual message during the typical human communication

As widely described in a very recent review on dog communication (Siniscalchi et al., 2018), several studies have investigated dogs' comprehension of human visual signals, revealing that dogs are tuned into human visual communication (Beaver, 1999; Kaminski and Nitzschner, 2013), following spontaneously human body postures, gaze direction, and pointing to find a target location (Soproni et al., 2001; Virányi et al., 2004; Miklósi and Soproni, 2006; Udell et al., 2008; Bensky et al., 2013). However, dogs are also able to receive and respond to human verbal signals (Mills, 2005; Miklósi, 2007, 2009; Grassmann et al., 2012). The human typical communication is defined as bimodal when the message is expressed using both gestures and words (Messing, 1994). Humans uses this type of communication even when they interact with other animals, like dogs. As a consequence of the domestication, there are evidences that dogs developed novel forms of the pre-existing vocalizations compared with wolves, which facilitate their communication with humans (Feddersen-Petersen, 2000; Pongrácz et al., 2010). At the same time, humans have developed the ability to derive physical information from dogs' vocalizations (i.e., dog size; Taylor et al., 2009) and its emotional valence (Pongrácz et al., 2005; Faragó et al., 2010). On the other hand, there are indications suggesting that dogs' social contact with humans represents an important regulatory factor of vocalizations expression (i.e., individual experience), as shown by the decreasing of their production in feral and stray dogs (Pongrácz et al., 2010). If the domestication process has led dogs to adapt to the human social and physical environment, causing them to communicate more effectively with humans, they should prefer the vocal communicative channel. Indeed, it has been pointed out that when dogs are required to perform a transitive action (or object-related action; Carmo and Rumiati, 2009) they respond to the vocal request better than the pointing and gazing gestures (Grassmann et al., 2012). Unfortunately, the two dogs tested by Grassmann et al. (2012) underwent a specific word-based fetching game training where the owners were not accustomed to performing gestures. This may have caused dog's preference to follow the verbal command.

To evaluate the outcomes of Grassmann et al. (2012) it has been tested firstly how much the dogs, accustomed to responding equally to gestural and verbal requests, weighing the information given by posture and voice when the owner asks them to perform intransitive actions, not related to an object (Carmo and Rumiati, 2009) (i.e., execution of an obedience command) in a contrasting paradigm (i.e., the information received by gestures were contradictory with the vocal command). Secondly, it has been verified if the dogs gave different responses in relation to the familiarity of the command-giver. Finally, the dogs have been tested in a different context, in which their owner asked them to retrieve an object (i.e., transitive action) from a certain distance calling it by name while pointing and looking at another. The studies have been published in Animal Cognition and Applied Animal Behavior Science and reported integrally from chapter 5 to 7.

2.4 The unexplored olfactory communication

Dogs use a wide spectrum of signals when communicating with humans (and other dogs), that include tactile, visual, acoustic and olfactory indications (Siniscalchi et al., 2018). They engage in the same behavioral scheme used during conspecific interactions, even though some signals can acquire different meaning when directed toward humans (e.g., eye contact, Topál et al., 2014). Usually researches dealing with the study of dog-human communication focus on visual and acoustic systems, whereas the olfactory communication, which is probably also the most important for dogs (dogs' olfactory sensitivity is 10,000–100,000 times higher than humans; Hecht and Horowitz, 2015; Wells, 2017), is poorly studied (Siniscalchi et al., 2016). The reason for the lack of research examining the communicative potential of the olfactory sensory system in dogs might be due to the undervalued role of olfaction in human–human communication and to human minor sensibility to odors (Marshall and Moulton, 1981; Bradshaw and Rooney, 2016). However, some studies have shown that in humans, chemosignals can carry information helping to discriminate the genetic nature of the sender (Jacob et al., 2002), the gender (Penn et al., 2007) or emotional states (e.g., Mujica-Parodi et al., 2009; Prehn et al., 2006; Zhou and Chen, 2009; deGroot et al., 2012; Mutic et al., 2015). The transmission of olfactory information linked to the latter takes place without a conscious communicative intent (Pause, 2012; Semin and deGroot, 2013) but, despite this, induces in the recipient a behavioral, perceptive and neural reproduction of the sender's status (Semin, 2007). On the contrary, dogs can intentionally deposit their odor in the environment through urines, faces, and glandular secretions (i.e., scent marking) such that dog-dog olfactory communication can occur indirectly through scent marking or through direct interaction between two individuals (Handleman, 2012). Moreover, dogs recognize humans by their smell, preferring specific parts of human body for olfactory investigation, suggesting that different body parts produce specific odors that might convey different information (Bradshaw and Rooney, 2016; Siniscalchi, 2016).

Humans and dogs shared a long co-evolutionary history and it would be surprising that the olfactory system of dogs did not contribute to the communication between the two species. To evaluate this possibility the chemosignals contained in human sweat collected in different emotional states has been submitted to dogs, recording their behavioral and physiological changes according to the condition. The study has been published in Animal Cognition and reported integrally in the chapter 8.

2.5 An overview on dog's sex difference: is it a consequence of domestication?

As a side effect of artificial selection, the differences between male and female animals, which are maintained in nature through natural (and sexual) selection, could have changed during and after domestication. Specific sexual traits are actively selected and preserved through the sexual selection in nature. For example, differences in personality traits between male and female animals have been linked to sexual selection as an effect of intra-sexual competition and mate choice in both humans and non-human animals (Schuett et al., 2010). Living in proximity with and depending reproductively by humans could have make less necessary to maintain sex-specific traits.

In such a context, the dog may be an interesting model to investigate the human directed effects on the roles of males and females. As has been discussed, the domestication process has determined in the dog an acceleration in the achievement of sexual maturity (Scott and Fuller, 1965; Fox, 1978), the loss of the reproduction seasonal rhythm (Christie and Bell, 1971) and the onset of promiscuous sexual relations (Scott and Fuller, 1965). However, it has been little clarified how much that process has acted on individual male-female differences. To this aim the differences in male and female dogs regarding personality traits as well as cognitive and perceptual processes have been reviewed to explore whether such dissimilarities were affected by the domestication process or the sex-specific differences existing in wild animals have been maintained. The study has been published in Animals and reported integrally in the chapter 9.

2.6 References

Agnetta, B., Hare, B., & Tomasello, M. (2000). Cues to food location that domestic dogs (*Canis familiaris*) of different ages do and do not use. Animal cognition, 3(2), 107-112.

Ainsworth, M. D. S., Blehar, M. C., Waters, E., & Wall, S. (1978). Patterns of Attachment. A Psychological Study of the Strange Situation. Lawrence Erlbaum Associates, Hillsdale, New Jersey.

Anderson, J. R., Sallaberry, P., & Barbier, H. (1995). Use of experimenter-given cues during object-choice tasks by capuchin monkeys. *Animal Behavior*, *49*(1), 201-208.

Barrera, G., Jakovcevic, A., Mustaca, A., & Bentosela, M. (2012). Learning interspecific communicative responses in Pampas foxes (*Lycalopex gymnocercus*). *Behavioral Processes*, *89*, 44–51.

Beaver, B.V. (1999). Canine Behavior: A Guide for Veterinarians. W.B. Saunders Co., Philadelphia.

Bensky, M. K., Gosling, S. D., & Sinn, D. L. (2013). The world from a dog's point of view: a review and synthesis of dog cognition research. In *Advances in the Study of Behavior* (Vol. 45, pp. 209-406). Academic Press.

Bentosela, M., Wynne, C. D. L., D'Orazio, M., Elgier, A., & Udell, M. A. R. (2016). Sociability and gazing toward humans in dogs and wolves: Simple behaviors with broad implications. *Journal of the Experimental Analysis of Behavior*, *105*, 68–75.

Bradshaw, J. W., & Rooney, N. (2016). Dog social behavior and communication. In: Serpell, J. (Ed.), *The Domestic Dog,* (pp. 133-159). Cambridge University Press, Cambridge, UK.

Brubaker, L., Dasgupta, S., Bhattacharjee, D., Bhadra, A., & Udell, M. A. R. (2017). Differences in problem-solving between canid populations: Do domestication and lifetime experience affect persistence? *Animal cognition*, *20*(4), 717-723.

Carmo, J. C., & Rumiati, R. I. (2009). Imitation of transitive and intransitive actions in healthy individuals. *Brain and cognition*, *69*(3), 460-464.

Christie, D. W., & Bell, E. T. (1971). Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *Journal of Small Animal Practice*, *12*(3), 159-167.

Cunningham, C. L., & Ramos, M. F. (2014). Effect of training and familiarity on responsiveness to human cues in domestic dogs (*Canis familiaris*). *Animal cognition*, *17*(3), 805-814.

D'Aniello, B., & Scandurra, A. (2016). Ontogenetic effects on gazing behavior: a case study of kennel dogs (Labrador Retrievers) in the impossible task paradigm. *Animal cognition*, *19*(3), 565-570.

D'Aniello, B., & Scandurra, A. (2017). Impossible task paradigm. In: Vonk, J., Shackelford, T. (Eds.), *Encyclopedia of Animal Cognition and Behavior*. Springer International Publishing: Cham, Switzerland.

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

Darwin, C. (1859). On the origins of species by means of natural selection. *London: John Murray*, *247*, 1859.

de Groot, J. H., Smeets, M. A., Kaldewaij, A., Duijndam, M. J., & Semin, G. R. (2012). Chemosignals communicate human emotions. *Psychological science*, *23*(11), 1417-1424.

Duranton, C., & Gaunet, F. (2016). Effects of shelter housing on dogs' sensitivity to human social cues. *Journal of Veterinary Behavior: Clinical Applications and Research*, *14*, 20-27.

Fagnani, J., Barrera, G., Carballo, F., & Bentosela, M. (2016). Is previous experience important for inhibitory control? A comparison between shelter and pet dogs in A-not-B and cylinder tasks. *Animal cognition*, *19*(6), 1165-1172.

Fallani, G., Previde, E. P., & Valsecchi, P. (2006). Do disrupted early attachments affect the relationship between guide dogs and blind owners? *Applied Animal Behavior Science*, *100*(3-4), 241-257.

Fallani, G., Previde, E. P., & Valsecchi, P. (2007). Behavioral and physiological responses of guide dogs to a situation of emotional distress. *Physiology & behavior*, 90(4), 648-655.

Faragó, T., Pongrácz, P., Range, F., Virányi, Z., & Miklósi, Á. (2010). 'The bone is mine': affective and referential aspects of dog growls. *Animal Behavior*, *79*(4), 917-925.

Feddersen-Petersen, D. U. (2000). Vocalization of European wolves (*Canis lupus lupus* L.) and various dog breeds (*Canis lupus* f. fam.). *Archives Animal Breeding*, *43*(4), 387-398.

Fox, M.W. (1978). The Dog: Its Domestication and Behavior. Garland Press, New York.

Frank, H. (2011). Wolves, dogs, rearing and reinforcement: complex interactions underlying species differences in training and problem-solving performance. *Behavior genetics*, *41*(6), 830-839.

Frank, H., & Frank, M. G. (1983). Inhibition training in wolves and dogs. *Behavioral processes*, *8*(4), 363-377.

Frank, H., Frank, M. G., Hasselbach, L. M., & Littleton, D. M. (1989). Motivation and insight in wolf (*Canis lupus*) and Alaskan malamute (*Canis familiaris*): Visual discrimination learning. *Bulletin of the Psychonomic Society*, *27*(5), 455-458.

Gácsi, M., Topál, J., Miklósi, Á., Dóka, A., & Csányi, V. (2001). Attachment behavior of adult dogs (*Canis familiaris*) living at rescue centers: forming new bonds. *Journal of Comparative Psychology*, *115*(4), 423.

Grassmann, S., Kaminski, J., & Tomasello, M. (2012). How two word-trained dogs integrate pointing and naming. *Animal Cognition*, *15*(4), 657-665.

Hall, N. J., Lord, K., Arnold, A. M. K., Wynne, C. D. L., & Udell, M. A. R. (2015). Assessment of attachment behavior to human caregivers in wolf pups (*Canis lupus lupus*). *Behavioral processes*, *110*, 15-21.

Hare, B., & Tomasello, M. (2005). Human-like social skills in dogs? *Trends in cognitive sciences*, *9*(9), 439-444.

Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, *298*(5598), 1634-1636.

Hare, B., Call, J., & Tomasello, M. (1998). Communication of food location between human and dog (*Canis familiaris*). *Evolution of communication*, *2*(1), 137-159.

Hare, B., Plyusnina, I., Ignacio, N., Schepina, O., Stepika, A., Wrangham, R., & Trut, L. (2005). Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication. *Current Biology*, *15*(3), 226-230.

Hare, B., Rosati, A., Kaminski, J., Bräuer, J., Call, J., & Tomasello, M. (2010). The domestication hypothesis for dogs' skills with human communication: a response to Udell et al. (2008) and Wynne et al. (2008). *Animal Behavior*, *79*, e1–e6.

Hart, L. A. (1995). Dogs as human companions: a review of the relationship. In: Serpell, J. (Ed.), The Domestic Dog: Its Evolution, Behavior, and Interaction with *People* (pp. 162–178). Cambridge University Press, New York.

Hecht, J., Horowitz, A. (2015). Introduction to dog behavior. In: Weiss, E., Mohan-Gibbons, H., Zawistowski, S. (Eds.), *Animal Behavior for Shelter Veterinarians and Staff*, (pp. 5–30). Wiley-Blackwell, London, UK.

Jacob, S., McClintock, M. K., Zelano, B., & Ober, C. (2002). Paternally inherited HLA alleles are associated with women's choice of male odor. *Nature genetics*, *30*(2), 175.

Kaminski, J., & Nitzschner, M. (2013). Do dogs get the point? A review of dog–human communication ability. *Learning and Motivation*, *44*(4), 294-302.

Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for "fast mapping". *Science*, *304*(5677), 1682-1683.

Krebs, R. J., & Davis, N. B. (1993). Introduction in behavioral ecology. Oxford University Press, Oxford.

Kubinyi, E., Virányi, Z., & Miklósi, Á. (2007). Comparative social cognition: From wolf and dog to humans. *Comparative Cognition & Behavior Reviews*, *2*, 26–46.

Mariti, C., Ricci, E., Carlone, B., Moore, J. L., Sighieri, C., & Gazzano, A. (2013). Dog attachment to man: a comparison between pet and working dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, *8*(3), 135-145.

Marshall-Pescini, S., Passalacqua, C., Barnard, S., Valsecchi, P., & Prato-Previde, E. (2009). Agility and search and rescue training differently affects pet dogs' behavior in socio-cognitive tasks. *Behavioral processes*, *81*(3), 416-422.

Marshall-Pescini, S., Valsecchi, P., Petak, I., Accorsi, P. A., & Previde, E. P. (2008). Does training make you smarter? The effects of training on dogs' performance (*Canis familiaris*) in a problem solving task. *Behavioral Processes*, *78*(3), 449-454.

Marshall-Pescini, S., Virányi, Z., & Range, F. (2015). The effect of domestication on inhibitory control: wolves and dogs compared. *PloS one*, *10*(2), e0118469.

Marshall, D. A., & Moulton, D. G. (1981). Olfactory sensitivity to α-ionone in humans and dogs. *Chemical senses*, *6*(1), 53-61.

Messing, L. (1994). Bimodal Communication, Signing Skill & Tenseness. *Sign Language Studies*, *84*(1), 209-220.

Miklósi, Á. (2007). Dog behavior, evolution and cognition. Oxford University Press, Oxford.

Miklósi, A. (2009). Evolutionary approach to communication between humans and dogs. *Veterinary research communications*, *33*(1), 53-59.

Miklósi, Á., & Soproni, K. (2006). A comparative analysis of animals' understanding of the human pointing gesture. *Animal cognition*, *9*(2), 81-93.

Miklósi, Á., & Topál, J. (2011). On the hunt for the gene of perspective taking: pitfalls in methodology. *Learning & behavior*, *39*(4), 310-313.

Miklósi, Á., Kubinyi, E., Topál, J., Gácsi, M., Virányi, Z., & Csányi, V. (2003). A simple reason for a big difference: Wolves do not look back at humans, but dogs do. *Current Biology*, *13*, 763–766.

Miklósi, Á., Polgárdi, R., Topál, J., & Csányi, V. (1998). Use of experimenter-given cues in dogs. *Animal cognition*, *1*(2), 113-121.

Mills, D. S. (2005). What's in a word? A review of the attributes of a command affecting the performance of pet dogs. *Anthrozoös*, *18*(3), 208-221.

Mongillo, P., Pitteri, E., Carnier, P., Gabai, G., Adamelli, S., & Marinelli, L. (2013). Does the attachment system towards owners change in aged dogs? *Physiology & behavior*, *120*, 64-69.

Mutic, S., Parma, V., Brünner, Y. F., & Freiherr, J. (2015). You smell dangerous: communicating fight responses through human chemosignals of aggression. *Chemical senses*, *41*(1), 35-43.

Nagasawa, M., Mitsui, S., En, S., Ohtani, N., Ohta, M., Sakuma, Y., ... & Kikusui, T. (2015). Oxytocin-gaze positive loop and the coevolution of human-dog bonds. *Science*, *348*(6232), 333-336.

Osthaus, B., Lea, S. E. G., & Slater, A. M. (2003). Training influences problem-solving abilities in dogs (*Canis lupus familiaris*). In *Proceedings of the Annual Meeting of British Society of Animal Science, York* (Vol. 103, p. 103).

Pause, B. M. (2012). Processing of body odor signals by the human brain. *Chemosensory perception*, *5*(1), 55-63.

Penn, D. J., Oberzaucher, E., Grammer, K., Fischer, G., Soini, H. A., Wiesler, D., ... & Brereton, R. G. (2007). Individual and gender fingerprints in human body odour. *Journal of the Royal society interface*, *4*(13), 331-340.

Pongrácz, P., Molnár, C., & Miklósi, Á. (2010). Barking in family dogs: an ethological approach. *The Veterinary Journal*, *183*(2), 141-147.

Pongrácz, P., Molnár, C., Miklósi, A., & Csányi, V. (2005). Human listeners are able to classify dog (*Canis familiaris*) barks recorded in different situations. *Journal of comparative psychology*, *119*(2), 136.

Prato-Previde, E., & Valsecchi, P. (2007). Effect of abandonment on attachment behavior of adult pet dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, *2*(3), 87-88.

Prato-Previde, E., Custance, D. M., Spiezio, C., & Sabatini, F. (2003). Is the dog-human relationship an attachment bond? An observational study using Ainsworth's strange situation. *Behavior*, *140*(2), 225-254.

Prehn, A., Ohrt, A., Sojka, B., Ferstl, R., & Pause, B. M. (2006). Chemosensory anxiety signals augment the startle reflex in humans. *Neuroscience letters*, *394*(2), 127-130.

Price, E. O. (1984). Behavioral aspects of animal domestication. *The quarterly review of biology*, *59*(1), 1-32.

Range, F., Jenikejew, J., Schröder, I., & Virányi, Z. (2014). Difference in quantity discrimination in dogs and wolves. *Frontiers in psychology*, *5*, 1299.

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D'Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behavior. *Animal cognition*, *18*(4), 937-944.

Schuett, W., Tregenza, T., & Dall, S. R. (2010). Sexual selection and animal personality. *Biological Reviews*, *85*(2), 217-246.

Scott, J. P., & Fuller, J. L. (1965). *Genetics and the Social Behavior of the Dog*. University of Chicago Press, Chicago.

Semin, G. R. (2007). Grounding communication: Synchrony. In: Kruglanski, A. W., Higgins, E. T. (Eds.), *Social psychology: handbook of basic principles* (pp. 630–649). Guilford Press, New York.

Semin, G. R., & De Groot, J. H. (2013). The chemical bases of human sociality. *Trends in Cognitive Sciences*, *17*(9), 427-429.

Siniscalchi, M., d'Ingeo, S., Minunno, M., & Quaranta, A. (2018). Communication in dogs. Animals, 8(8).

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2016). The dog nose "KNOWS" fear: asymmetric nostril use during sniffing at canine and human emotional stimuli. *Behavioral brain research*, *304*, 34-41.

Siniscalchi, M., Stipo, C., & Quaranta, A. (2013) "Like owner, like dog": correlation between the owner's attachment profile and the owner-dog bond. *PLoS ONE, 8,* e78455.

Smith, B. P., & Litchfield, C. A. (2009). Dingoes (*Canis dingo*) can use human social cues to locate hidden food. *Animal Cognition*, *13*, 367–376.

Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2001). Comprehension of human communicative signs in pet dogs (*Canis familiaris*). *Journal of comparative psychology*, *115*(2), 122.

Taylor, A. M., Reby, D., & McComb, K. (2009). Context-related variation in the vocal growling behavior of the domestic dog (*Canis familiaris*). *Ethology*, *115*(10), 905-915.

Topál, J., Gácsi, M., Miklósi, Á., Virányi, Z., Kubinyi, E., & Csányi, V. (2005). Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies. *Animal behavior*, *70*(6), 1367-1375.

Topál, J., Kis, A., & Oláh, K. (2014). Dogs’ sensitivity to human ostensive cues: a unique adaptation? In: Kaminski, J., Marshall-Pescini, S. (Eds.), *The Social Dog,* (pp. 319-346). Academic Press: Cambridge, MA, USA.

Topál, J., Miklósi, Á., Csányi, V., & Dóka, A. (1998). Attachment behavior in dogs (*Canis familiaris*): a new application of Ainsworth's (1969) Strange Situation Test. *Journal of comparative psychology*, *112*(3), 219.

Udell, M. A. R. (2015). When dogs look back: Inhibition of independent problem-solving behavior in domestic dogs (*Canis lupus familiaris*) compared with wolves (*Canis lupus*). *Biology Letters*, *11*(9), Article 20150489.

Udell, M. A. R., & Brubaker, L. (2016). Are Dogs Social Generalists? Canine Social Cognition, Attachment, and the Dog-Human Bond. *Current Directions in Psychological Science*, *25*(5), 327-333.

Udell, M. A. R., & Wynne, C. D. L. (2008). A review of domestic dogs' (*Canis familiaris*) human-like behaviors: or why behavior analysts should stop worrying and love their dogs. *Journal of the experimental analysis of behavior*, *89*(2), 247-261.

Udell, M. A. R., & Wynne, C. D. L. (2010). Ontogeny and phylogeny: both are essential to human-sensitive behavior in the genus *Canis*. *Animal Behavior*, *79*(2), e9-e14.

Udell, M. A. R., Dorey, N. R., & Wynne, C. D. L. (2008). Wolves outperform dogs in following human social cues. *Animal Behavior*, *76*(6), 1767-1773.

Udell, M. A. R., Dorey, N. R., & Wynne, C. D. L. (2010a). What did domestication do to dogs? A new account of dogs sensitivity to human actions. *Biological Reviews*, *85*, 327–345.

Udell, M. A. R., Dorey, N. R., & Wynne, C. D. L. (2010b). The performance of stray dogs (*Canis familiaris*) living in a shelter on human-guided object-choice tasks. *Animal Behavior*, *79*(3), 717-725.

Udell, M. A. R., Dorey, N. R., & Wynne, C. D. L. (2011). Can your dog read your mind? Understanding the causes of canine perspective taking. *Learning & Behavior*, *39*(4), 289-302.

Udell, M. A. R., Spencer, J., Dorey, N. R., & Wynne, C. D. L. (2012). Human-socialized wolves follow diverse human gestures... and they may not be alone. *International Journal of Comparative Psychology*, *25*(2), 97–117.

Valsecchi, P., Previde, E. P., Accorsi, P. A., & Fallani, G. (2010). Development of the attachment bond in guide dogs. *Applied animal behavior science*, *123*(1-2), 43-50.

Virányi, Z., Gácsi, M., Kubinyi, E., Topál, J., Belényi, B., Ujfalussy, D., & Miklósi, Á. (2008). Comprehension of human pointing gestures in young human-reared wolves (*Canis lupus*) and dogs

(*Canis familiaris*). *Animal cognition*, 11(3), 373.

Virányi, Z., Topál, J., Gácsi, M., Miklósi, Á., & Csányi, V. (2004). Dogs respond appropriately to cues of humans' attentional focus. *Behavioral processes*, *66*(2), 161-172.

vonHoldt, B., Shuldiner, E., Koch, I. J., Kartzinel, R. Y., Hogan, A., Brubaker, L., Wanser, S., ... & Udell, M. A. (2017). Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Science advances*, *3*(7), e1700398.

Wells, D.L. (2017). Behavior of Dogs. In: Jensen, P. (Ed.), *The Ethology of Domestic Animals: An Introductory Text*, (vol. 3 pp. 228–238). CABI: Oxford, UK.

Wynne, C. D. L., Udell M. A. R. & Lord K. A. (2008). Ontogeny's impacts on human-dog communication. *Animal Behavior*, *76*, e1-e4.

Zaine, I., Domeniconi, C., & Wynne, C. D. L. (2015). The ontogeny of human point following in dogs: When younger dogs outperform older. *Behavioral processes*, *119*, 76-85.

Zhou, W., & Chen, D. (2009). Fear-related chemosignals modulate recognition of fear in ambiguous facial expressions. *Psychological science*, *20*(2), 177-183.

CHAPTER 3

BEHAVIORAL EFFECTS OF TRAINING ON WATER RESCUE DOGS IN THE STRANGE SITUATION TEST

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Abstract

Water rescue training produces dog-human dyads specialized in rescuing people in the water by promoting a strong cooperative relationship between dogs and their handlers. The present study aims to assess whether this training also affects the human-dog attachment bond using an adapted version of the "Strange Situation Test" (SST), consisting of 7 episodes of 3 minutes each. Thirteen mutually exclusive and two non-mutually exclusive behaviors were considered. Sixty-five dogs (Labrador and Golden retrievers) were tested: 29 dogs had a Water Rescue Certificate® (Trained group), 22 dogs had yet to begin the training program (Naïve group), and 14 dogs were untrained (Old group) as an age control that was equivalent to the Trained group.

All dogs showed a pattern of attachment behaviors with their owners, as they played with and greeted the owner more than the stranger and remained oriented towards the door more in the presence of the stranger during the separation episodes. Furthermore, all groups engaged in play with the stranger more when their owner was present rather than absent (episode 2 vs. 3). The Trained group was less explorative than the Naïve and Old groups. Trained dogs engaged in different behaviors not for playing purposes holding a ball in their mouth more than the other groups. Social play behaviors were expressed equally by the Trained and the Naïve groups and less in the Old group. Overall, the water rescue training affected the dogs' behavior in the SST; however these behavioral differences were (probably) not related to differences in attachment quality.

Keywords: attachment bond, dog-human relationship, strange situation test, training, water rescue dogs

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3.1 Introduction

Many species of mammals and birds form a variety of social bonds with their conspecifics to maximize their survival and reproduction (Wilkinson, 1985; Connor et al., 2001; de Villiers et al., 2003; Wasilewski, 2003; Emery et al., 2007; Cameron et al., 2009; Mitani, 2009). One type of social bond that typically develops between parents and offspring is attachment. Attachment is a long-term bond through which offspring take advantage by their parents. It is expressed behaviorally through a preference for the attachment figure(s) over other individuals and evokes distress when involuntary separations occur. Attachment is also expressed through behaviors aimed at obtaining and maintaining proximity to the caregiver (Bowlby, 1958, 1969; Rajecki et al., 1978; Ainsworth and Bell, 1970; Ainsworth, 1989; Bretherton, 1992). Although affiliative bonds and attachment usually occur between individuals of the same species, humans form strong affectional bonds with other animals (e.g., horses, cats, dogs), extending parental-like behavior towards them (Rowan and Beck, 1994; Hart, 1995; Archer, 1997; Prato-Previde et al., 2006). Using adapted versions of the "Strange Situation Test" (SST), originally devised to investigate the mother-infant attachment bond in humans (Ainsworth and Bell, 1970; Ainsworth et al., 1978), many authors have shown that dogs form an affectional bond with their owners that fulfil all attachment criteria: proximity maintenance and comfort seeking towards the owner, indication of the "secure base" effect in the presence of the owner and distress and protest behavior upon short-term separation from the owner (Topál et al., 1998; Prato-Previde et al., 2003; Palestrini et al., 2005; Palmer and Custance, 2008; Rehn et al., 2013). Attachment has been reported in adult dogs with varying life experiences (Gácsi et al., 2001; Fallani et al., 2006, 2007; Prato-Previde and Valsecchi, 2007; Valsecchi et al., 2010; Mariti et al., 2013; Mongillo et al., 2013) and has also been documented in 4-month-old puppies (Topál et al., 2005).

Three studies have investigated attachment in guide dogs that, due to their specific training, results in three subsequent bonds with humans: the first with their puppy-walker (during the first year of life), the second with their trainer (during the training period) and the third with the visually impaired owner to which they are assigned. These studies showed that the previous separations did not prejudice the formation of a secure affectional bond with the visually impaired owner and that training can modulate behavioral responses in the SST (Fallani et al., 2006, 2007; Valsecchi et al., 2010). In particular, guide dogs showed a lower degree of proximity seeking behaviors than pet dogs did (Fallani et al., 2006), and although guide dogs exhibited more controlled behavioral reactions when they were separated from their impaired visual owners, they had stronger cardiac activation (Fallani et al., 2007). However, apart from some other behavioral differences, the attachment bond in guide dogs seemed very similar to that of pet dogs. In a recent paper, Mariti et al. (2013) reported a trend toward a more secure attachment bond in search and rescue dogs (no significant differences were found), leaving open the possibility that the attachment could be different in working dogs. Thus, to investigate this topic, we studied water rescue dogs, which are particularly appropriate to verify whether the attachment bond is affected in working dogs because the main purpose of training is to promote strong cooperation and synchronization between the dog and handler that can persist in stressful and challenging situations. To strengthen the bond in the human-dog dyad, many of the exercises that the dogs complete are based on a separation in which the handler runs away while the dog is restrained by a trainer and then is released to regain proximity to the handler, which is rewarded by playing behavior or food. The method of restraining a dog while the owner runs off is commonly used in puppy/beginner training courses also for pet dogs. If this really affects the bond is yet unclear.

Water rescue training involves the formation of dog-human dyads specialized in rescuing drowning people. These dyads cooperate with the coast guard and port authorities when patrolling bathing areas in summer. Water rescue dogs belong to medium-large size breeds (over 30 kg) originally selected to work in aquatic environments, such as Newfoundlands, Labrador retrievers and Golden retrievers. All dogs live with their owners/handlers as pets, but the handler and dog act as a water rescue team when necessary. For dogs to be eligible for training, no specific criteria are required apart from the absence of behavioral pathologies (e.g., aggressiveness) or fear of the water that would make them unsuitable for water rescues. Thus, no other initial screening for dogs' social skills is carried out. The dyad members each have distinctive tasks in a water rescue: the handler is responsible for calming and grasping the drowning person and then supporting and preparing him or her for the journey back; the dog tows both people to safety (to shore or to an emergency boat).

The first stage of training (lasting approximately 12 months) includes advanced obedience training, often involving positive, game-based reinforcement, to consolidate the dog-owner relationship. The second stage (lasting 10 months) involves work in the water that is generally self-rewarding to the dog (intrinsic reinforcement). During this stage, dog and handler carry out common tasks, further strengthening their relationship. Upon completion of training and a successful final examination, dog-human dyads are qualified as water rescue teams (obtaining a Water Rescue Certificate®). It is to be noted that failure to obtain the certificate mainly results from a low athletic performance (based on the standard required by the school) and/or a low motivation of the handler in carrying out a highly demanding and long-lasting training.

Given that the water rescue training is aimed to strengthen the dog-owner cooperative relationship, the goal of the present study is to assess, using the SST, whether this training also affects the human/dog attachment bond.

3.2 Materials and methods

3.2.1 Subjects

The subjects were 65 adult dogs (35 males and 30 females; 51 Labrador retrievers and 14 Golden retrievers) recruited from the Italian School of Water Rescue Dogs (Scuola Italiana Cani Salvataggio - SICS) and through personal contacts and advertisements in public places, veterinary surgeons and through the Internet. All dogs lived in a human household with at least two people, and all owners reported that their dogs were accustomed to staying home alone without problems. Twenty-nine dogs (23 Labrador retrievers and 6 Golden retrievers, 13 males and 16 females, mean age ± SD = 4.3 ± 2.0 years) had the SICS Water Rescue Certificate® (Trained group) and had been working as rescue dogs for at least one year at the time of testing; 22 dogs (17 Labrador retrievers and 5 Golden retrievers, 13 males and 9 females, mean age ± SD = 1.3 ± 0.4 years) had yet to begin the training program (Naïve group). As the Trained group was older than the Naïve group because of the long period of training that they had undergone, we also introduced 14 untrained dogs (Old group) to act as an age control, equivalent to the Trained group (11 Labrador retrievers and 3 Golden retrievers, 9 males and 5 females, mean age ± SD = 4.9 ± 2.6 years).

3.2.2 Experimental design

Dogs were tested in a standardized, unfamiliar environment using a protocol adapted from the SST (Ainsworth and Bell, 1970) used to test the attachment bond in pet and working dogs (Fallani et al., 2006, 2007; Prato-Previde et al., 2003; Valsecchi et al., 2010). The tests were conducted at the University of Naples "Federico II" (Naples) and at the training centre of the Italian School of Water Rescue Dogs (Velletri, Rome) in rooms of 12 and 16 m2, respectively, both unfamiliar and novel to the dogs. The rooms contained two chairs (for the stranger and owner), a table, dog toys (two tennis balls and two plastic bottles), a water bowl and two Sony Handycam video cameras (HDR-CX115 and HDR-PJ260VE). The rooms had the same geometry, and the door was on the same side in both rooms (Fig. 3.1). Prior to testing, dog-human pairs were escorted to a different room, where the procedure was described to ensure that the owners were prepared to act in line with the procedure of the experiment. However, the specific goal of the study was not disclosed at this stage. The participants were then moved to the experimental room, and the video cameras were activated. Testing lasted 21 min and consisted of seven 3-min consecutive episodes (Table 3.1) in which the same unfamiliar male stranger was introduced and the dogs were subjected to two short episodes of separation from their owners. Immediately after each test, the experimental room, water bowl and toys were cleaned with a non-toxic, weakly scented disinfectant.

The stranger and the owner did not induce or stimulate behaviors except for the social play. Saying the dog's name was allowed only when the people were close to the dog to calm it.

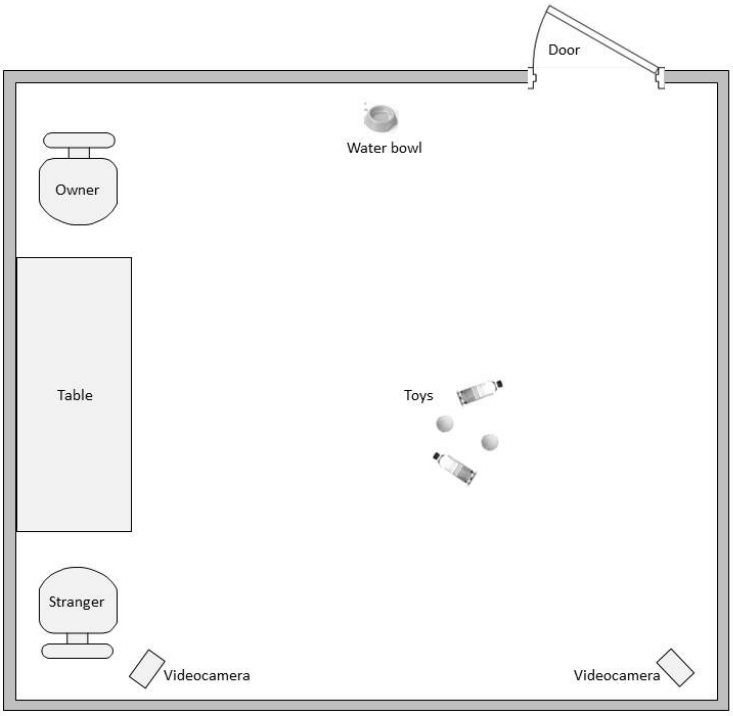

Fig 3.1 Representation of the experimental rooms equipped with two video cameras, a water bowl, dog toys, a desk and two chairs (for the stranger and owner).

Table 3.1 Description of the Strange Situation procedure

Episode	**Description**
Episode 1: owner and dog	The owner sat quietly and the dog was free to explore the room
Episode 2: owner, dog and stranger	The stranger entered the room, sat quietly for 1 minute, conversed with the owner for the second minute, approached the dog and attempted to stimulate play during the last minute. At the end of this episode the owner left the room unobtrusively
Episode 3: stranger and dog (1st separation episode)	The stranger continued to play with the dog if it was willing; if it was inactive or distressed, the stranger attempted to distract it with play or by providing verbal and tactile comfort
Episode 4: owner and dog (1st reunion episode)	The owner entered the room and greeted and/or comforted his/her dog as usual after returning from work or shopping. The stranger quietly exited the room. The owner had been told that he/she was free to play with the dog throughout the episode. At the end of this episode the owner left the room
Episode 5: dog alone (2nd separation episode)	The dog remained alone for three minutes

Episode 6: stranger and dog	The stranger entered the room and followed the same protocol as in episode 3
Episode 7: owner and dog (2nd reunion episode)	The owner entered the room greeted and followed the same protocol as in episode 4. The stranger left the room unobtrusively. At the end of this episode the experimenter arrived and officially terminated the procedure

3.2.3 Data collection and analysis

Based on the scientific support from literature (e.g. Mongillo et al., 2013; Palestrini et al., 2005; Prato-Previde et al., 2003; Rehn et al., 2013; Valsecchi et al., 2010) the dog behavior was coded from the video by a trained observer using a 5-second point sampling method by Solomon Coder beta® 14.05.19 (ELTE TTK, Hungary). For each dog, we obtained a sample of 252 points (12 points sample/min x 3 min x 7 episodes); for statistical analysis, the absolute number of occurrences of each behavior was used as the dependent variable. An ethogram composed of 13 mutually exclusive behaviors was compiled. In addition, two non-mutually exclusive behaviors were included: “vocalizing” and “ball in mouth”. This latter behavior was included in the ethogram after observing water rescue dogs consistently retaining the ball in their mouth for a long time while engaging in non-play behaviors. Vocal behavior was recorded as bouts. These behaviors were selected after a preliminary analysis of the videotapes (i.e., ball in the mouth) and on the basis of previous studies based on the SST with dogs (Topál et al., 1998; Prato-Previde et al., 2003; Palestrini et al., 2005; Fallani et al., 2006, 2007; Palmer and Custance, 2008; Valsecchi et al., 2010) (see Table 3.2 for a detailed description of the behaviors). A second, independent coder analysed a random sample of 16 videos (25% of the sample), and inter-observer reliability of the frequencies of behaviors was calculated as percentage agreement (the lowest value was 90%). Because a Shapiro-Wilk test revealed that most of the data were not normally distributed, the statistical analyses were carried out using non-parametric tests of each behavior. Intergroup differences were analysed by a Kruskal-Wallis test for independent samples, followed by a post hoc Mann-Whitney U test with Bonferroni correction, in cases of significant differences. To assess the dogs’ attitudes towards their owners and the stranger within each treatment group, we used the Wilcoxon signed rank test for intragroup comparisons of the dog behaviors. The occurrence of the behaviors towards the person was compared between episodes in which only the stranger (3+6) or owner (4+7) were present. Greetings were examined only in those episodes in which the person (stranger or owner) entered the room (episodes 2+6 vs. 4+7). All statistical tests were implemented in SPSS 21.

Table 3.2 Behaviors recorded in the Strange Situation procedure. All behaviors listed are considered mutually exclusive except for the ball in the mouth and vocalizing.

Behavior	**Definition**
Social play	Any vigorous or galloping movement behavior performed when interacting with the owner or stranger, including running, jumping and chasing toys
Oriented to door	Staring fixedly at the door, either when close to it or from a distance
Passive behavior	Sitting, standing or lying down without any obvious orientation toward the environment or person
Oriented to person	Staring fixedly at the person without any type of interaction with him/her
Exploration	Activity directed toward physical aspects of the environment including sniffing, visual inspection and gentle oral examination such as licking
Individual play	Any vigorous or galloping movement behavior directed toward a toy when clearly not interacting with a person, including chewing, biting, shaking from side to side, scratching or batting with the paw, chasing rolling balls and tossing using the mouth
Physical contact	Being in physical contact with the owner or stranger regardless of visual orientation, excluding during greeting and social play
Locomotion	Walking, pacing or running around without exploring the environment or playing
Drink	Drinking from the water bowl
Greeting behavior	All greeting behaviors toward the entering owner or stranger, such as approaching, tail wagging, jumping, and physical contact. Greetings were allowed in a range of 10 and 15 seconds (max 2 sampling points). To this purpose an advice given from the door to the person after 10 seconds signaled the end of the greeting (if still running) and the beginning of the play invitation

Approach person	Actively approaching while clearly visually oriented toward the owner or stranger
Following	Following the owner or the stranger around the room
Scratch the door	All active behaviors resulting in physical contact with the door, including scratching the door with the paws, jumping on the door, and pulling on the door handle with the forelegs or mouth
Ball in the mouth	Keeping the ball in the mouth while performing other behaviors (not mutually exclusive)
Vocalising	Barking and whining recorded as bouts (not mutually exclusive)

3.3 Results

A descriptive analysis based on the percent of the sample points of all behaviors considered is reported in Fig. 3.2. Significant differences will be detailed below according to three levels of analysis: (a) Overall intergroup analysis: comparison of each behavior (throughout the whole test) between groups of dogs; (b) Episodes intergroup analysis: differences between groups in episodes where either the owner or the stranger were present in the room; and (c) Intragroup analysis: changes toward episodes to compare the behaviors in the presence of owner and stranger in each group. For ease of reading, only the behaviors showing a significant difference in at least one of three levels will be detailed.

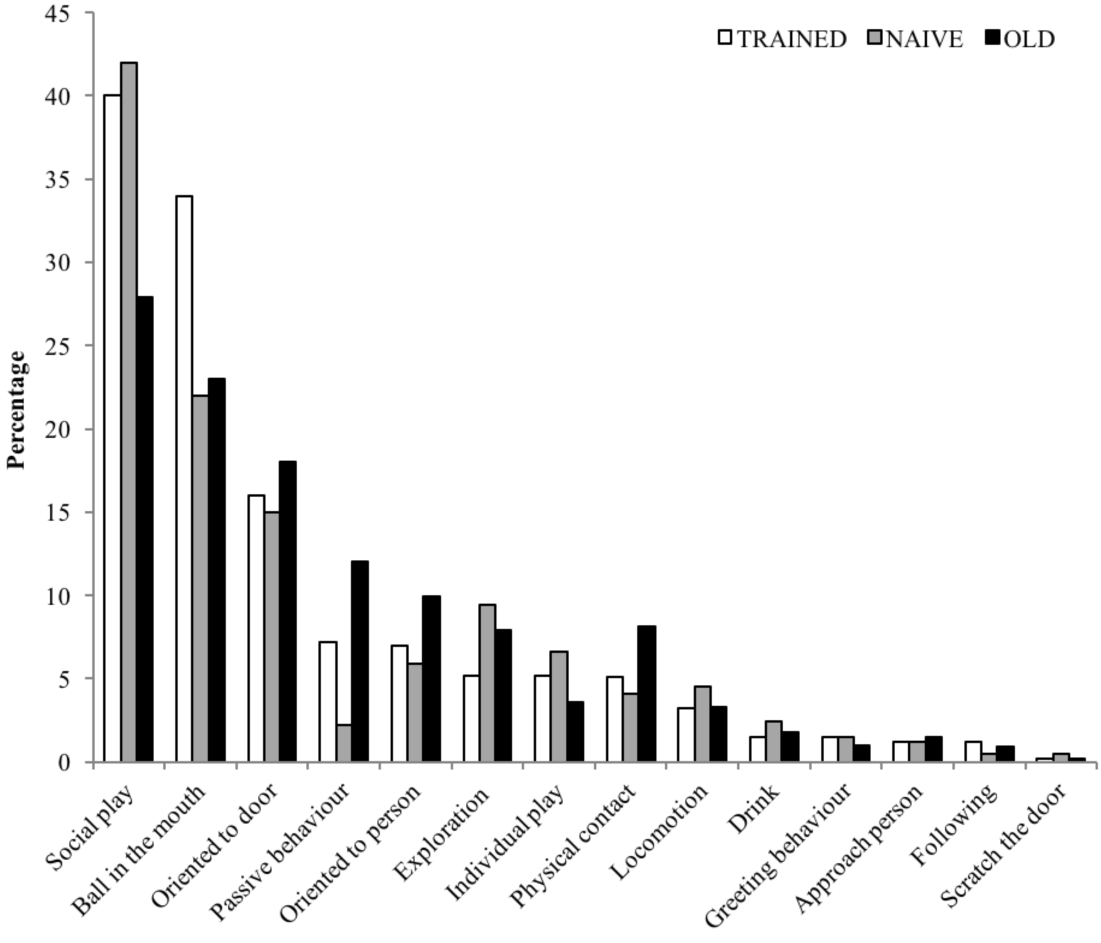

Fig 3.2 Descriptive non statistical percentage of sampling points for each behavior in the entire experimental procedure. The most frequent behaviors are represented on the left. All behaviors are mutually exclusive, except for "ball in the mouth". In the legend, Trained indicates dogs with the SICS Water Rescue Certificate®; Naïve indicates dogs at the beginning of their training program; Old indicates untrained dogs.

3.*3.1 Social play behavior*

Social play was the most expressed behavior and was significantly different between the groups ($H = 7.66$, $P = 0.02$). A post hoc Mann-Whitney U test showed no differences between the Trained and Naïve groups, which engaged in social play behavior in 40% and 42% of all sample points, respectively. The Old group, with only 28% of sample points reflecting social play, was generally less playful than both the Trained ($U = 110$, $P = 0.04$) and Naïve ($U = 75$, $P = 0.03$) groups.

These differences appeared also with the stranger (episodes 3+6; $H = 9.51$, $P = 0.009$) and the owner (episodes 4+7; $H = 6.29$, $P = 0.04$) alone, with the post hoc test showing that the Trained group was significantly more playful with their owner than the Old (*U* = 82.5, *P* = 0.006) group (Fig. 3.3).

An intragroup analysis revealed that all groups played with their owners in episodes 4+7 significantly more often than they played with the stranger in episodes 3+6 (Trained: $z = 4.4$; Naïve: $z = 3.18$; Old: $z = 3.3$, $P < 0.001$ in all cases) (Fig. 3.3). However, while the play behavior with the owner did not change between the first and the second reunion episodes (episode 4 vs. 7), it was lower after the stranger's second entrance (episode 3 vs. 6) and was above chance in the Trained ($z = 3.05$, $P = 0.002$) and the Naïve ($z = 3.13$, $P < 0.001$) groups but not in the Old group.

A further analysis comparing the last minute of episode 2 (i.e. owner present) and the first minute of episode 3 (i.e. owner absent) revealed a non-significant trend for dogs to play less with the stranger once their owner had exited the room in the Trained ($z = 1.02$, $P = 0.09$) and Naïve ($z = 1.85$; $P = 0.07$) groups and a significant difference in the Old ($z = 2.81$; $P = 0.005$) group (Fig. 3.4).

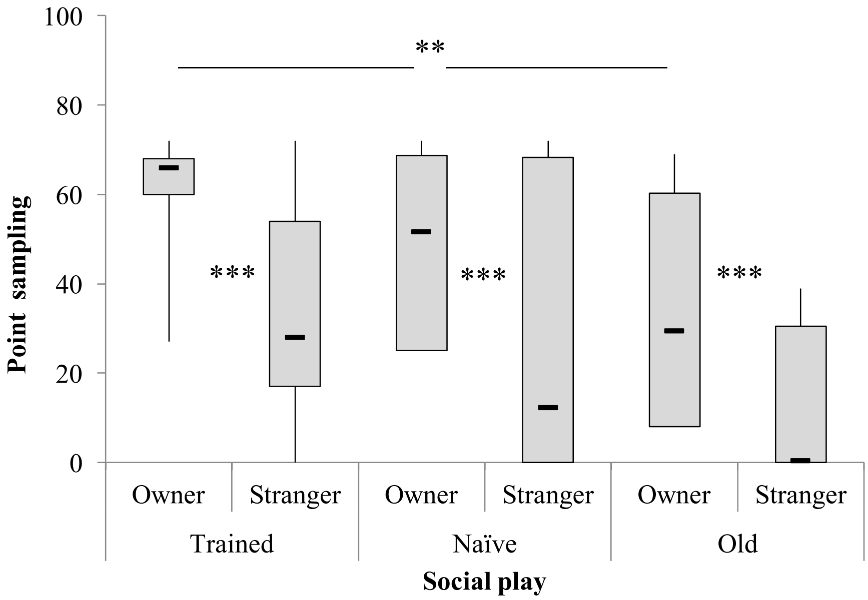

Fig 3.3 Social play behavior with the owner (sum of point sampling in episodes 4+7) and the stranger (sum of point sampling in episodes 3+6). The three groups differed significantly in the social play behavior with the stranger ($H = 9.51$, $P = 0.009$) and the owner ($H = 6.29$, $P = 0.04$), with a post hoc test showing that the Trained dogs were more playful with their owners than the Old ($U = 82.5$, $P = 0.006$) dogs. An intragroup analysis revealed that the dogs in all groups played with their owners significantly more often than with the strangers (Trained: $z = 4.4$; Naïve: $z = 3.18$; Old: $z = 3.3$, $P < 0.001$ in all cases). Asterisks over the boxes indicate differences between groups; asterisks between the boxes indicate differences between the owner and stranger in the same group. Bold horizontal lines: medians; grey boxes: quartiles; thin vertical lines: minimum and maximum values. *P <0.05; **P <0.01; ***P <0.001.

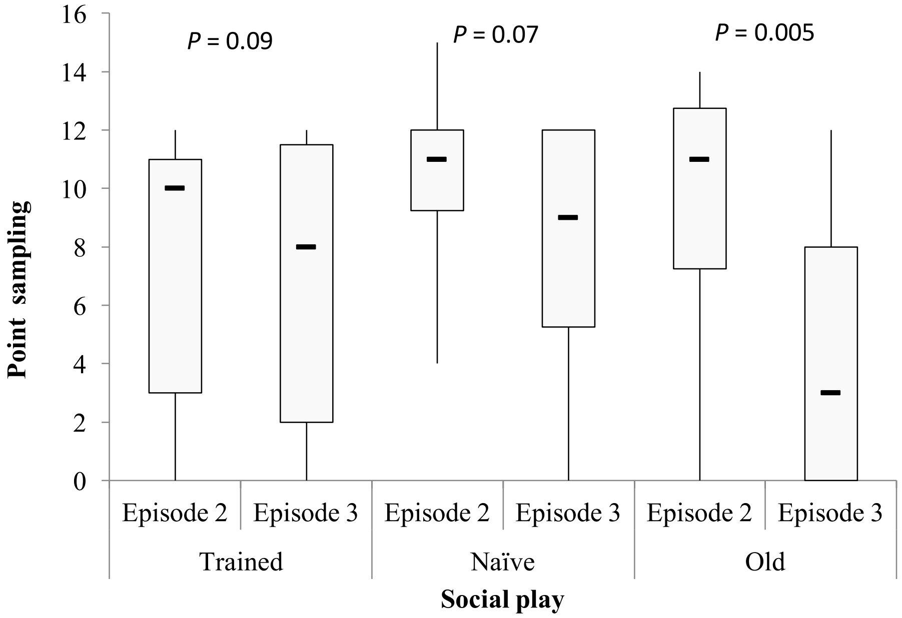

Fig 3.4 Social play behavior with the stranger in the last minutes of episode 2 (i.e. owner present) and the first of episode 3 (i.e. owner absent). Non-significant trend for dogs to play less with the stranger once their owner had exited the room was observed in the Trained and Naïve groups and a significant difference was obtained in the Old group.

3.3.2 Ball in the mouth

The second most frequent behavior was the behavior of holding the ball in their mouth, which was not mutually exclusive, with the Trained group showing this behavior in 34% of sample points, followed by the Old (23%) and Naïve (22%) groups (H = 5.46, P = 0.04), without significant differences in the post hoc test.

No intergroup or intragroup differences were recorded in the presence of the stranger (episodes 3+6) or the owner (episodes 4+7) alone, neither comparing owner present vs. stranger present. In all cases, this behavior was largely associated with the social play behavior. However, a re-examination of episode 5, when the ball in the mouth behavior was almost never associated with play but rather with the behaviors of locomotion and orientation toward the door, also revealed a difference between the groups (H = 8.59, P = 0.014), with the Trained group (55%) showing a this behavior more than both the Naïve (32%; U = 214, P = 0.04) and the Old (21%; U = 115, P = 0.04) groups, while the latter two groups were not significantly different.

3.3.3 Orientation towards the door

An orientation towards the door was largely expressed in episode 5, when the dogs were alone, although it was also evident, albeit to a lesser extent, throughout the whole test whenever the owner was not in the room. No intergroup differences were registered regarding this behavior either in relation with the stranger or the owner alone.

However, the analysis between episodes 3+6 and 4+7 revealed that all groups consistently remained oriented towards the door in the presence of the stranger, more so than when the owner was present (Trained: $z = 4.37$, $P < 0.001$; Naïve: $z = 3.36$, $P < 0.001$; Old: $z = 2.89$, $P = 0.002$).

3.3.4 Passive behavior

Passive behavior was expressed in large quantities in the first two episodes and in episode 5 by the Trained and Old groups, while the Naïve group was generally less passive throughout the whole test. Overall significant differences were recorded ($H = 15.14$, $P < 0.001$), with the highest passivity registered in the Old group (12% of sample points), followed by the Trained (7%) and Naïve (2%) groups. A post hoc test showed no significant differences between the Old and Trained groups, and both groups' passivity percentages were significantly higher than that in the Naïve dogs ($U = 49$, $P = 0.002$ and $U = 162.5$, $P = 0.008$, respectively).

For a clear-cut case of secure base effects, the passivity was analysed in the episode in which only the owner was present (e.g. 1+2+4+7). Also in this case significant intergroup differences appeared ($H = 13.9$, $P < 0.001$), with Naïve less passive than Trained ($U = 163.5$, $P = 0.007$) and Old groups ($U = 56{,}5$ $P = 0.004$) and no significant differences between the Old and Trained groups.

No intergroup differences were recorded in the presence of either the stranger or the owner alone, nor were intragroup differences observed between the presence of the owner in episodes 4+7 and the presence of the stranger in episodes 3+6.

3.3.5 Orientation to the person

Significant differences were found in the dogs' orientation to the person ($H = 6.09$, $P = 0.048$), which was more frequent in the Old group (10% of sample points) than in the Trained (7%) and Naïve dogs (6%), but no differences were recorded in the post hoc test.

No intergroup differences were recorded in relation to the stranger or the owner, nor were intragroup preferences obtained for the owner vs. the stranger or vice versa.

3.3.6. Exploration

Differences in exploration during the whole test were above chance ($H = 7.85$, $P = 0.02$). A post hoc test showed significant differences between the Naïve (9% of sample points) and Trained (5%) groups ($U = 171$, $P = 0.02$), but the Old (8%) group was not different from either Naïve or Trained groups. However, the exploration was recorded in large percentages only during the first episode, showing the same statistical trend ($H = 8.88$, $P = 0.02$), with the Naïve appearing more explorative than Trained ($U = 167.5$, $P = 0.01$) after post hoc tests.

In episodes 3+6 (in the presence of the stranger) and in 4+7 (in the presence of the owner), exploration was only occasionally observed and in only a few dogs. However, no differences were recorded between these two episode pairs in an intergroup or an intragroup comparison.

3.3.7 Individual play

Individual play was prevalently expressed in the first two episodes. Overall, it was greater in the Naïve (7% of sample points) than in the Trained (5%) and Old (4%) groups, but it was registered only as a tendency ($H = 5.7$, $P = 0.06$). Otherwise, significant differences were recorded comparing only the episodes in which the owner was present ($H = 6.8$, $P = 0.03$), with Naïve more playful than Old ($U = 76.5$, $P = 0.04$).

As with exploration, because of very low values, no differences were recorded between the groups in the presence of the stranger (episodes 3+6) or the owner (episodes 4+7) or in the intragroup comparison (episodes 3+6 vs. 4+7).

3.3.8 Greeting behavior

Greeting behaviors were observed in the episode 2, 6 (e.g. entrance of the stranger) and 4, 7 (e.g. entrance of the owner) and were comparable among groups, both as a general pattern, as well as for the stranger or the owner alone. However, an intragroup analysis revealed that all groups greeted their owner significantly more often than the stranger (Trained: $z = 3.91$, $P < 0.001$; Naïve: $z = 2.99$, $P = 0.002$; Old: $z = 2.16$, $P = 0.03$).

3.4 Discussion

The aim of this study was to examine the expression of the parameters related to the attachment bond in dogs trained for water rescue and untrained beginners in controlled situations using an adapted version of the Strange Situation Test. The water rescue dogs have been studied with a social referencing test (Merola et al., 2013) and the impossible task paradigm (D'Aniello et al., 2015); to our knowledge, this is the first research on SST.

Overall, in the present study, all dogs showed a pattern of attachment with their owners in some affiliative key behavior: the dogs preferentially played with and greeted the owner more than the stranger and remained oriented towards the door more in the presence of the stranger during the episodes of separation from the owner. More important, all groups engaged in playing with the stranger more often when their owner was present rather than absent (episode 2 vs. 3), which indicates a secure-base effect. However, in our sample, we did not record differences among the groups in other affiliative behaviors such as orientation to the person, approaching the person, physical contact or following the person, so these factors were not able to underline a statistical preference toward the attachment figure. In our dogs, social play occupied the largest percentage of the sample points in episodes 3+6 (only stranger present) and 4+7 (only owner present). Labrador retrievers, which represented most of our sample (78.5%), are very high in playfulness and are the highest ranked breed in curiosity/fearlessness (Svartberg, 2006). Thus, it is possible that the affiliated non-play behaviors were expressed in very low values, causing the difference in the behaviors to not reach statistical significance in our dogs. It may be that the Labrador retrievers need a stronger stressor in order to activate the attachment system.

These explanations may also be valid for the individual play behavior that was not differently expressed in the presence of the owner and the stranger and was also not expressed significantly more by any of the three groups in the study. However, in presence of the owner the Naïve appeared significantly more playful than the Old group, which probably point out on an age effect on the individual play, since both groups were equivalent as life style.

In the current study, it emerged that the Trained dogs showed the lowest levels of exploration, which, when expressed in the presence of the attachment figure, has been associated with a secure-base effect in humans (Ainsworth, 1989) and chimpanzees (Ainsworth and Bell, 1970; Ainsworth et al., 1978; Bard, 1991), as well as in dogs (Topál et al., 1998; Palmer and Custance, 2008). This might suggest that Trained dogs feel less securely attached.

The exploratory behavior seems to be unaffected by age because aged dogs explored in a similar way to adult dogs (Mongillo et al., 2013). Alternatively, the fact that Water Rescue dogs were less explorative could be the result of the training in which they are trained to remain seated for long spells of time on command and are accustomed to being attentive and inactive for long periods in the vicinity of their owners during their service in new environments, such as a beach where they are supposed to not cause any disturbance to the people. A similar reason could apply also to the decrease in the exploratory behavior over time in guide dogs (Valsecchi et al., 2010), who are also required to remain passive in several situations: when off duty, they may have to lie quietly near their blind owner for long periods while the owner eats, works, etc. (Fallani et al., 2006). On the contrary, search and rescue dogs appeared to be as explorative as pet dogs (Mariti et al., 2013).

A high level of passivity has been considered to be an indicator of a secure-base effect (Palmer and Custance, 2008). Other authors have considered passiveness as an active suppression of behavioral signs rather than a relaxed reaction to social challenges (see Topál et al., 1998; Mongillo et al., 2013). Passive behavior was expressed in large amounts by the Old group and, although to a lesser extent, by the Trained group as well. Both were significantly more passive than the Naïve dogs. Thus, the passivity pattern, different from exploration, seems to be most influenced by age, with younger dogs appearing less passive (see Mongillo et al., 2013). Similar results have also been reported for guide dogs appearing more passive than younger apprentice and sub-adult dogs (Fallani et al., 2006; Valsecchi et al., 2010). Thus, passivity, in addition to exploration behavior, is inconclusive in differentiating the attachment bond in our groups.

The Naïve group engaged in social play more than the Old group, which does not fit with prior literature showing no significant differences in aged and adult dogs (Mongillo et al., 2013). However, in their paper, the authors compared dogs that were four and nine years old, whereas our Naïve dogs were very young (approximately one year), so the results are not directly comparable. Our Naïve and Old groups were without formal training because the former was at the beginning of their course to become water rescue dogs. Thus, the effect of age on social play is probable, as previously obtained in another study on the same breeds using the SST (Fallani et al., 2006; Valsecchi et al., 2010). Interestingly, the Trained group played as much as the Naïve group did and more than the Old group. It could be that the owners are better prepared to stimulate the Trained dogs with social play behavior because their owners performed such interactions better after instruction by a trainer; however, the same trend was also obtained with respect to the stranger, who was always the same. Perhaps this result may be the effect of the extensive use of social play in water rescue training, counteracting the decline of this behavior with age and allowing older dogs to interact with people in a youthful manner.

An interesting and unexpected result was that Trained dogs engaged in many different non-play behaviors such as holding a ball in their mouth. This behavior has not been reported in previous SST studies and may be a side effect of water rescue training: play is elicited with the offer of a toy (ball, rope) to encourage focus on and physical contact with the person. Although play serves as an effective reward for the training of many tasks (Rooney et al., 2004; Bradshaw et al., 2015), it is insufficient for explaining the emergence of this behavior in the SST. Trained dogs tended to interact with their physical/social environments with the ball in their mouth throughout the test. It is possible that the Trained dogs learned to keep a toy in their mouth to enhance their communication with people. However, given that it was also associated with non-play behaviors, particularly in the isolation episode 5, it is more probable that it is a learned strategy for coping with stressful situations. Maybe this behavior has been observed in other guide dog studies using retrievers, but that it might have been included in the "play" variable (Fallani et al, 2007; Valsecchi et al, 2010). Further investigations of the ontogenesis and function of this behavior are required.

Overall, the water rescue dogs did not show a different attachment bond with their owners than untrained dogs. Similarly, other studies on training effects have not provided support that training specifically causes different attachment strategies: search and rescue dogs showed some tendency toward a different attachment but without any statistical difference (Mariti et al., 2013), and guide dogs were very similar to pets (Fallani et al., 2006). Thus, it seems that the training that working dogs undergo does not improve the human/dog attachment bond, which independently develops just as wellby spending their daily lives together. However, training experiences could result in specific behavioral traits consistent with the different work requested of the dogs.

3.5 References

Ainsworth, M. D. S. (1989). Attachments beyond infancy. *American psychologist*, *44*(4), 709.

Ainsworth, M. D. S., & Bell, S. M. (1970). Attachment, exploration, and separation: Illustrated by the behavior of one-year-olds in a strange situation. *Child development*, 49-67.

Ainsworth, M. D. S., Blehar, M. C., Waters, E., & Wall, S. N. (2015). Patterns of attachment: A psychological study of the strange situation. Psychology Press. Lawrence Erlbaum Associates, Hillsdale, New Jersey.

Archer, J. (1997). Why do people love their pets? *Evolution and Human behavior*, *18*(4), 237-259.

Bard, K. A. (1991). Distribution of attachment classifications in nursery chimpanzees. *American Journal of Primatology*, *24*, 88.

Bowlby, J. (1958). The nature of the child’s tie to his mother. *International Journal of Psycho-Analysis, 39*, 350-373.

Bowlby, J. (1969). Attachment and loss: vol. 1. Attachment. Basic Books, New York.

Bradshaw, J. W., Pullen, A. J., & Rooney, N. J. (2015). Why do adult dogs ‘play’? *Behavioral processes*, *110*, 82-87.

Bretherton, I. (1992). The origins of attachment theory: John Bowlby and Mary Ainsworth. *Developmental psychology*, *28*(5), 759.

Cameron, E. Z., Setsaas, T. H., & Linklater, W. L. (2009). Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences*, *106*(33), 13850-13853.

Connor, R. C., Heithaus, M. R., & Barre, L. M. (2001). Complex social structure, alliance stability and mating access in a bottlenose dolphin ‘super-alliance’. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1464), 263-267.

D’Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

De Villiers, M. S., Richardson, P. R., & Van Jaarsveld, A. S. (2003). Patterns of coalition formation and spatial association in a social carnivore, the African wild dog (*Lycaon pictus*). *Journal of Zoology*, *260*(4), 377-389.

Emery, N. J., Seed, A. M., Von Bayern, A. M., & Clayton, N. S. (2007). Cognitive adaptations of social bonding in birds. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *362*(1480), 489-505.

Fallani, G., Previde, E. P., & Valsecchi, P. (2006). Do disrupted early attachments affect the relationship between guide dogs and blind owners? *Applied Animal Behavior Science*, *100*(3-4), 241-257.

Fallani, G., Previde, E. P., & Valsecchi, P. (2007). Behavioral and physiological responses of guide dogs to a situation of emotional distress. *Physiology & behavior*, *90*(4), 648-655.

Gácsi, M., Topál, J., Miklósi, Á., Dóka, A., & Csányi, V. (2001). Attachment behavior of adult dogs (*Canis familiaris*) living at rescue centers: forming new bonds. *Journal of Comparative Psychology*, *115*(4), 423.

Hart, L. A. (1995). Dogs as human companions: a review of the relationship. In: Serpell, J. (Ed.), The domestic dog: its evolution, behavior, and interaction with people (pp. 162-178). Cambridge University Press.

Mariti, C., Ricci, E., Carlone, B., Moore, J. L., Sighieri, C., & Gazzano, A. (2013). Dog attachment to man: a comparison between pet and working dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, *8*(3), 135-145.

Merola, I., Marshall-Pescini, S., D’Aniello, B., & Prato-Previde, E. (2013). Social referencing: water rescue trained dogs are less affected than pet dogs by the stranger's message. *Applied animal behavior science*, *147*(1-2), 132-138.

Mitani, J. C. (2009). Male chimpanzees form enduring and equitable social bonds. *Animal Behavior*, *77*(3), 633-640.

Mongillo, P., Pitteri, E., Carnier, P., Gabai, G., Adamelli, S., & Marinelli, L. (2013). Does the attachment system towards owners change in aged dogs? *Physiology & behavior*, *120*, 64-69.

Palestrini, C., Previde, E. P., Spiezio, C., & Verga, M. (2005). Heart rate and behavioral responses of dogs in the Ainsworth's Strange Situation: a pilot study. *Applied Animal Behavior Science*, *94*(1-2), 75-88.

Palmer, R., & Custance, D. (2008). A counterbalanced version of Ainsworth's Strange Situation Procedure reveals secure-base effects in dog–human relationships. *Applied animal behavior science*, *109*(2-4), 306-319.

Previde, E. P., & Valsecchi, P. (2007). 11: Effect of abandonment on attachment behavior of adult pet dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, *2*(3), 87-88.

Prato-Previde, E., Custance, D. M., Spiezio, C., Sabatini, F., 2003. Is the dog-human relationship an attachment bond? An observational study using Ainsworth's Strange Situation. Behavior. 140, 225-254.

Prato-Previde, E., Custance, D. M., Spiezio, C., & Sabatini, F. (2003). Is the dog-human relationship an attachment bond? An observational study using Ainsworth's strange situation. *Behavior*, *140*(2), 225-254.

Rajecki, D. W., Lamb, M. E., & Obmascher, P. (1978). Toward a general theory of infantile attachment: A comparative review of aspects of the social bond. *Behavioral and Brain Sciences*, *1*(3), 417-436.

Rehn, T., McGowan, R. T., & Keeling, L. J. (2013). Evaluating the strange situation procedure (SSP) to assess the bond between dogs and humans. *PloS one*, *8*(2), e56938.

Rooney, N. J., Bradshaw, J. W., & Almey, H. (2004). Attributes of specialist search dogs—a questionnaire survey of UK dog handlers and trainers. *Journal of Forensic Science*, *49*(2), 1-7.

Rowan, A. N., Beck, A. M., (1994). The health benefits of human-animal interactions. *Anthrozoös, 7,* 85-89.

Svartberg, K. (2006). Breed-typical behavior in dogs—historical remnants or recent constructs? *Applied Animal Behavior Science*, *96*(3-4), 293-313.

Topál, J., Gácsi, M., Miklósi, Á., Virányi, Z., Kubinyi, E., & Csányi, V. (2005). Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies. *Animal behavior*, *70*(6), 1367-1375.

Topál, J., Miklósi, Á., Csányi, V., & Dóka, A. (1998). Attachment behavior in dogs (*Canis familiaris*): a new application of Ainsworth's (1969) Strange Situation Test. *Journal of comparative psychology*, *112*(3), 219.

Valsecchi, P., Previde, E. P., Accorsi, P. A., & Fallani, G. (2010). Development of the attachment bond in guide dogs. *Applied animal behavior science*, *123*(1-2), 43-50.

Wasilewski, A. (2003). "Friendship" in ungulates? – Sociopositive relationships between non-related herd members of the same species. Fachbereich Biologie, Philipps-Universität Marburg.

Wilkinson, G. S. (1985). The social organization of the common vampire bat. *Behavioral Ecology and Sociobiology*, *17*(2), 123-134.

CHAPTER 4

WHAT'S THE POINT? GOLDEN AND LABRADOR RETRIEVERS LIVING IN KENNELS DO NOT UNDERSTAND HUMAN POINTING GESTURES

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Abstract

In many studies that have investigated whether dogs' capacities to understand human pointing gestures are aspects of evolutionary or developmental social competences, family-owned dogs have been compared to shelter dogs. However, for most of these studies, the origins of shelter dogs were unknown. A portion of shelter dogs may have lived with families before entering shelters, and from these past experiences, they may have learned to understand human gestures. Furthermore, differences in methodology and analysis approaches used in such studies are notable (e.g., different pointing protocols, statistical analysis approaches and experimental areas (i.e., indoor/outdoor)). Such differences in methodologies and analysis techniques used make it difficult to compare results obtained from different studies and may account for the divergent results obtained. We thus attempted to control for several parameters by carrying out a test on dynamic proximal and distal pointing. We studied eleven kennel dogs of known origin that were born and raised in a kennel with limited human interaction. This group was compared to a group of eleven dogs comparable in terms of breed, sex and age living with human families since they were puppies. Our results demonstrate that pet dogs outperform kennel dogs in their comprehension of proximal and distal pointing, regardless of whether an absence of choices made signified a wrong choice or whether such conditions were excluded from statistical analysis, meaning that dogs living in kennels do not understand pointing gestures. Even when genetic effects of the domestication process on human-dog relationships cannot be considered negligible, our data suggest that dogs need to learn human pointing gestures and they underscore the importance of ontogenetic processes.

Keywords: cue following, pointing, dog, ontogeny, domestication, kennel dogs

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4.1 Introduction

The “Domestication Hypothesis” proposes that the natural and artificial selection of the wild progenitor of dogs caused genetic changes that allowed these animals to understand human signals (Agnetta et al., 2000; Miklósi et al., 2003; Hare and Tomasello, 2005; Hare et al., 2002, 2010; Kaminski and Nitzschner, 2013). This theory is largely based on studies demonstrating that dogs outperform wolves raised by humans in certain cue-following tasks (Hare et al., 2002; Virányi et al., 2008; Gácsi et al., 2009).

Alternatively, the “Two-Stage Hypothesis” suggests that the ability to follow human cues is acquired over a dog’s lifespan after humans are accepted as companions in early ontogeny and when opportunities to learn from human are provided overtime (Udell and Wynne, 2008, 2010; Wynne et al., 2008; Udell et al., 2010a). In fact, it was found that when wolves are raised with intensive socialization with humans, they outperform dogs in following human social cues (Udell et al., 2008) and they are also able to understand a broad range of human gestures (Udell et al., 2012).

These theories are not necessarily antagonists to each other. Indeed, the synergistic hypothesis suggests that the dog-wolf difference in the sensitivity for human gestural cues emerges both at the evolutionary and at the developmental level (Gácsi et al., 2009; Miklósi and Topál, 2011).

To date, more than fifty papers have addressed human pointing gesture comprehension among dogs. In many cases, the specific goal was to determine whether dogs’ capacities to understand human pointing gestures are an evolutionary or developmental social competency. To this end, family-owned dogs have been compared to shelter dogs and wolves.

It is assumed that shelter dogs less socialized by humans and given fewer opportunities to learn from human rather than family-owned dogs should serve as a good model through which to explore ontogenetic acquisition. Hare et al. (2002) compared dog puppies living with human families with those hosted in shelters and found that both groups of dogs are able to understand human pointing gestures even at the youngest age (9–13 weeks) at above-chance levels. Accordingly, it was concluded that degrees of human socialization do not affect dogs’ capacities to read and interpret human pointing gestures correctly, thus minimizing ontogenetic effects. On the other hand, opposing study results report that puppies from homes better understand pointing gestures (Zaine et al. 2015). For adult dogs, Udell et al. (2008) showed that while shelter dogs understand momentary distal pointing at chance levels, pet dogs perform above chance levels; none of the shelter dogs followed pointing gestures successfully, and as a group, shelter dogs were significantly less successful at understanding human pointing gestures than pet dogs. This outcome was confirmed in follow-up studies by the same authors, underscoring that most shelter dogs learn to understand momentary distal pointing when subjected to additional training (Udell et al., 2010a, 2011). These findings, in sharp contrast with those of Hare et al. (2002), appear to conclude the opposite trend in that for dogs, domestication is not a predictor of sensitivity to human cues, highlighting the role of ontogenetic processes. Hare et al. (2010) repeated Udell and Wynne's experiments by studying a larger sample of shelter dogs to determine how skilful they were at using human pointing gestures. Twenty-three dogs hosted in a shelter were studied, of which 12 were suspected of having lived with families before their arrival at the shelter and with the other 11 dogs suspected of being feral with limited exposure to humans. The results showed that while both groups used pointing at above-chance levels, there was no significant difference in the performance of the two groups. More recently, Cunningham and Ramos (2014) used a different technical approach whereby dogs were not made to move to a cued location but instead to follow pointing gestures through a gaze shift toward a specific location and where food rewards were not given. They compared shelter dogs with trained and highly trained dogs and did not find any differences between the groups, once again emphasizing the importance of genetic acquisition for dog performance in heterospecific communicative skills. Unlike studies on puppies, the main problem associated with studies on adult shelter dogs is that stray and abandoned dogs can be of unknown origin as noted in the following quote: "*However, some caution is required as the histories of the shelter dogs were not known. It may be that these animals had lived for prolonged periods in a human home with sufficient ontogenetic experience to produce cue-following behavior in line with levels demonstrated by pet dogs*" (Cunningham and Ramos, 2014). Some shelter dogs examined in the above studies included purebred dogs that were not likely born as strays without human support. Such subjects, in experiencing life with a family for a period of time, may have had opportunities to learn pointing gestures to some extent. Their contributions may improve the number of correct responses given in shelter dog groups, lessening their differences with family-owned dogs and making it difficult to obtain significant differences between pet and shelter dogs. Lazarowski and Dorman (2015) attempted to settle this matter by comparing human pointing comprehension levels between dogs residing exclusively in kennel environments (i.e., never experiencing a life with a family) and pet dogs living in human homes. The authors found that the latter significantly outperformed the kennel dogs, thus reasserting once again the importance of ontogenetic effects. However, in this research study, the pet dogs studied were much older (mean = 4.75 years) than the kennel dogs examined (i.e., 13–17 months of age). Human pointing comprehension may increase as a function of age, as demonstrated through studies on puppies (Dorey et al., 2010), infant chimpanzees (Okamoto-Barth et al., 2008) and toddlers (Butterworth, 2002).

In addition to these problems, other variations make it difficult to obtain consistent study results, such as differences in experimental areas where tests are conducted (e.g., outdoor and indoor), different pointing protocols used (e.g., type of pointing gestures and the number of trials), differing emotional states among subjects (e.g., shelter dogs have higher levels of stress-related hormones (Hennessy et al., 1998), potentially affecting behavioral outcomes) and differing statistical analysis approaches used (e.g., coding an absence of choice as an incorrect choice or only considering choices as correct or incorrect through statistical comparisons). In the present study, we tried to control for these parameters by conducting a simple test based on dynamic proximal and distal pointing gestures. We studied adult dogs of known origin, which are referred to as kennel dogs following Lazarowski and Dorman (2015). Unlike shelter dogs, kennel dogs do not include dogs of unknown origin. All of the studied dogs were born and had lived their entire lives in a kennel with limited human interaction and were compared to dogs comparable by breed, sex and age that had lived in family homes since they had been puppies. We investigated whether dogs' capacities to understand human pointing gestures are affected by the lacking of extensive human influence. Due to several contradictions present in the literature, we cannot make specific predictions; however, from Udell et al.'s (2010b) results, we expect both groups of dogs to perform better at following easily understandable dynamic proximal pointing gestures compared to dynamic distal pointing.

4.2 Materials and Methods

4.2.1 Subjects

Twenty-nine dogs were included in the study: thirteen kennel dogs and sixteen pet dogs. All kennel dogs came from the FOOF dog museum (Caserta, Italy; www.foof.it). This establishment breeds dogs and sells purebred puppies. Unsold puppies of many breeds are held for exposition purposes. All of the dogs studied had never experienced mistreatment and were reared in very comfortable conditions. They live in kennels equipped with a floor cooling system in the summer and with a heating system in the winter with covered and open areas. Each kennel holds two or three dogs. The dogs have access to a recreational area where they can run and play freely in small groups for approximately 20 minutes per day. Social interactions between humans and dogs are very limited. Visitors are only allowed to observe the dogs, and interactions are forbidden. The dogs are fed and cleaned by the same caretaker once a day for 10–15 min. Periodically, the dogs are treated by a veterinarian. While we initially tried to select different breeds of dogs from the kennel, it was not possible to test the dogs in several cases because they feared the experimenters. We thus re-oriented our focus to Labrador and Golden Retrievers for two reasons: first, they rank very high in sociability, curiosity, fearlessness (Svartberg, 2006), and boldness, which measures the willingness to play and be approached and which has negative loadings for avoidance behavior and behavioral indicators of fear (Starling et al., 2013), thus eliminating strong pre-selection requirements; second, they are among the most common breeds, making it easier to find a control group of the same breeds matched for sex and age.

Pet dogs were recruited from personal contacts, Internet advertisements, parks and veterinary surgery clinics; all of these dogs had lived in houses with the same family since they had been puppies.

Two Golden Retrievers (both intact females) in the kennel group and 4 Golden Retrievers (all intact males) and 1 intact male Labrador Retriever in the pet group were not interested in food during the pre-test and were fearful of the experimenters, so they were not studied. Thus, 11 dogs in the kennel group (mean age 2.8±0.98) and 11 in the pet group (mean age 2.9±1.99) were studied (Table 4.1). Three Labrador Retrievers of the kennel group had already been examined in a previous study on gazing behaviors based on the impossible task paradigm (D'Aniello and Scandurra 2016). Besides the dogs excluded from the test, stress signals were occasionally observed in few dogs of both groups and were not tested statistically. All selected dogs (of both groups) approached the experimenters in a friendly manner, without showing fear or avoiding behaviors.

Table 4.1 Selected pet and kennel dogs by name (when known), sex, sex status, age and breed. *Dogs without names.

	Name	Sex	Sex Status	Age (years)	Breed	Group
1	Luna	F	Neutered	3.3	Labrador Retriever	Pet
2	Aaron	M	Neutered	5.5	Labrador Retriever	Pet
3	Fluke	M	Intact	6.0	Labrador Retriever	Pet
4	Oliver	M	Intact	1.2	Golden Retriever	Pet
5	Maya	F	Intact	1.2	Labrador Retriever	Pet
6	Joey	F	Intact	1.1	Labrador Retriever	Pet
7	Aron	M	Intact	1.6	Labrador Retriever	Pet
8	Argo	M	Intact	2.5	Labrador Retriever	Pet
9	Lamù	F	Neutered	6.0	Labrador Retriever	Pet
10	Bill	M	Intact	2.0	Labrador Retriever	Pet
11	Kora	F	Intact	1.6	Labrador Retriever	Pet
12	Due	M	Intact	4.0	Labrador Retriever	Kennel
13	Lupa	F	Intact	3.0	Labrador Retriever	Kennel
14	Perla	F	Intact	4.0	Labrador Retriever	Kennel
15	Diva	F	Intact	4.0	Labrador Retriever	Kennel
16	Loh	M	Intact	1.5	Labrador Retriever	Kennel
17	Dog 1*	M	Intact	2.5	Golden Retriever	Kennel
18	Charlie	M	Intact	2.5	Golden Retriever	Kennel
19	Dog 2*	M	Intact	2.0	Labrador Retriever	Kennel
20	Dog 3*	F	Intact	1.2	Labrador Retriever	Kennel
21	Kate	F	Intact	3.0	Labrador Retriever	Kennel
22	Puma	F	Intact	3.0	Labrador Retriever	Kennel

4.2.2 Experimental setting

The tests were conducted indoors in two different locations: at the University of Naples "Federico II" in Naples (room 14 m^2) and at the FOOF dog museum (Caserta) in a delimited indoor space of approximately 15 m^2. The dogs had never been to either place (except for 3 Labrador Retriever of the kennel group). The kennel staff and owners were asked not to feed the dogs over the 4 h preceding the test. For the motivation test trials, we placed single bowls inside another bowl positioned roughly 40 cm in front of the signaller (see below) and spaced roughly 2 metres apart (Fig. 4.1). Before the experimental phase, the dogs were allowed to move freely to explore and familiarize themselves with the room and with the research group for roughly 5 min. Three researchers were involved in the testing room: one (E1) for dog management and for holding the dogs, one who made pointing gestures (E2) and another (E3) who took notes and randomly assigned trials to the cue-giver. The same male E2 made pointing gestures throughout the entire experiment while E3 was a female. Two different female students served as E1. Once each test was completed, the bowls and room were cleaned with a lightly perfumed non-toxic disinfectant.

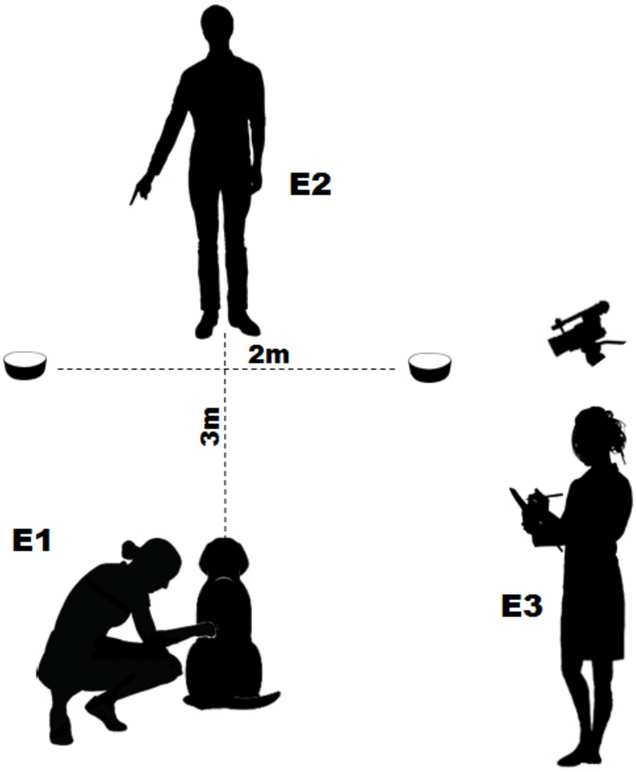

Fig 4.1 Experimental setting of the distal pointing condition. One female experimenter managed and held the dogs (E1); one male experimenter served as the cue-giver (E2); the third experimenter was a female who took notes and randomly assigned trials to the cue-giver (E3).

4.2.3 Procedure

The dogs' interest in the food given was measured by giving a limited amount of food to each dog before the test and by assessing whether each dog was willing to follow the E2 (pre-test motivation).

The procedure was performed as follows. E1 brought each dog to the starting position in front of E2, who stood 3 m from the midline between the bowls. E2 got each subject's attention and then placed a morsel of food in a pair of stacked bowls. The subject was released by E1 and was allowed to eat food, then was brought to the starting position by E1. This phase consisted of 8 trials. After 4 repetitions had been completed, the two stacked bowls were reversed so that the food odour was evenly distributed. The purpose of this phase was to assess each subject's level of motivation and to familiarize each subject with the experimental objects (pre-trial motivation) so as not to affect the test results.

The testing phase involved replicated trials in which E2 randomly made two types of signals and a control as suggested by E3: 8 dynamic proximal pointing (i.e., E2 knelt with his finger placed 10–15 cm from the closest edge of the targeted container) and 8 dynamic distal pointing (i.e., E2 stood with his finger positioned 50–60 cm from the closest edge of the targeted container). All trials were provided randomly. The same point was not proposed more than two times consecutively.

In similar experiments it was observed that a Clever Hans effect is unlikely to happen in pointing tasks (Hegedüs et al., 2013). However, we add 8 control trials (i.e., E2 remained in a neutral position (kneeling or standing) with his arms extended along his body looking forward) to study the effect of the missing of signals in a comparative way between the two groups. Although, different pointing protocol could have different outcomes (Pongrácz et al., 2013), it was chosen a dynamic pointing as it turned out to be the one which induced the fewest no-choices in dogs (Zaine et al., 2015). Both, proximal and distal, were sustained pointing, during which the E2's arm and hand were extended into a traditional point in the direction of the target container remaining in place and motionless until the end of the trial. The dogs were released immediately after showing the pointing. The trial ended when the dogs choose one of the targets or, in the case of no-choices, after 10 seconds. When a subject approached to the correct bowl, the E2 dropped a piece of food into the chosen container. When incorrect choices were made, no food was given.

To ensure the dogs' motivation and willingness to respond during the test, the bowls were stacked and positioned in the centre in front of the cue-giver every 2 trials and a morsel of food was place inside the bowl placed in front of the dogs for a total of 16 trials (inter-trial motivation). We also conducted this test to prevent the occurrence of unwanted learning effects during testing.

When necessary, E2 refocused the dogs by making vocal sounds or by calling the dogs by name, providing cues only when each dog was focused on him and establishing eye contact before signalling. When studying dogs, it is very important to maintain their attention in tests based on object choice tasks involving human pointing gestures (Carballo et al., 2016). While signalling, E2 looked away from the dogs.

4.2.4 Scoring and data analysis

We considered three possible answers: *correct*, when within 10 seconds a dog moved toward the target with his or her nose very close to (less than 10 cm) or in the bowl; *wrong*, when within 10 seconds a dog moved toward the wrong bowl with his or her nose in or very close to the bowl; and *no choice*, when within 10 seconds a dog was unable to comply with the previous requirements (i.e., never approaching the bowl, remaining static, or walking toward the experimenters).

From these three possible answers, we adopted two different assumptions: A1: *treating no-choices as errors* and A2: *excluding no-choices from the analysis*.

We first conducted an analysis at the individual dog level to identify which dogs performed above chance levels using the Wilcoxon sign rank test approach. Then, to compare response distributions between Pet and Kennel groups for each assumption, we performed a 2x2 contingency table exact Fisher's test considering the number of dogs performing above chance levels in each condition.

After verifying that the data were not normally distributed through a Kolmogorov-Smirnov test, a Wilcoxon Signed Rank test for medians was conducted to verify whether the samples as groups had median percentages of correct choices greater than chance levels for both assumptions of each condition (i.e., control, proximal and distal).

A Mann-Whitney U test was conducted to compute any difference in correct choices made under each condition between the two groups. Friedman’s ANOVA was used to compare correct choices made under the three conditions between the two groups followed by a post hoc Wilcoxon Signed Rank test with Bonferroni correction.

4.3 Results

None of the dogs failed any of the motivational tests (i.e., pre-test, pre-trial and inter-trial tests).

Results of the Wilcoxon sign rank test for individual dogs based on different assumptions are reported in Table 4.2.

Table 4.2 Binomial test results for the pet and kennel dogs for the control, proximal and distal conditions. According to Assumption 1, treating no-choices as errors, and under Assumption 2, excluding no-choices from the analysis. *Dogs without names.

			Assumption 1				**Assumption 2**			
Dog	**Group**	**Condition**	**Total Trials**	**Right Choice**	**Observe Value (%)**	**P-value**	**Total Trials**	**Right Choice**	**Observe Value (%)**	**P-value**
AARON	Pet	Control	8	3	38	0.359	3	3	100	0.074
AARON 2	Pet	Control	8	0	0	0.998	1	0	0	1.000
ARGO	Pet	Control	8	1	13	0.943	3	1	33	0.607
BILL	Pet	Control	8	1	13	0.943	1	1	100	0.500
FLUKE	Pet	Control	8	0	0	0.998	1	0	0	1.000
JOEY	Pet	Control	8	1	13	0.943	1	1	100	0.500
KORA	Pet	Control	8	0	0	0.998	0	0	-	-
LAMU'	Pet	Control	8	2	25	0.696	2	2	100	0.173
LUNA	Pet	Control	8	3	38	0.359	3	3	100	0.074
MAYA	Pet	Control	8	0	0	0.998	3	0	0	0.978
OLIVER	Pet	Control	8	4	50	0.141	6	4	67	0.065
AARON	Pet	Proximal	8	7	88	0.006	7	7	100	0.005
AARON 2	Pet	Proximal	8	8	100	0.003	8	8	100	0.003
ARGO	Pet	Proximal	8	8	100	0.003	8	8	100	0.003
BILL	Pet	Proximal	8	7	88	0.006	8	7	88	0.006
FLUKE	Pet	Proximal	8	3	38	0.359	4	3	75	0.093
JOEY	Pet	Proximal	8	8	100	0.003	8	8	100	0.003

KORA	Pet	Proximal	8	8	100	0.003	8	8	100	0.003
LAMU'	Pet	Proximal	8	7	88	0.006	7	7	100	0.005
LUNA	Pet	Proximal	8	8	100	0.003	8	8	100	0.003
MAYA	Pet	Proximal	8	6	75	0.017	6	6	100	0.010
OLIVER	Pet	Proximal	8	6	75	0.017	8	6	75	0.017
AARON	Pet	Distal	8	5	63	0.048	7	5	71	0.032
AARON 2	Pet	Distal	8	7	88	0.006	8	7	88	0.006
ARGO	Pet	Distal	8	8	100	0.003	8	8	100	0.003
BILL	Pet	Distal	8	5	63	0.048	5	5	100	0.018
FLUKE	Pet	Distal	8	2	25	0.696	2	2	100	0.173
JOEY	Pet	Distal	8	7	88	0.006	7	7	100	0.005
KORA	Pet	Distal	8	7	88	0.006	7	7	100	0.005
LAMU'	Pet	Distal	8	5	63	0.048	5	5	100	0.018
LUNA	Pet	Distal	8	8	100	0.003	8	8	100	0.003
MAYA	Pet	Distal	8	5	63	0.048	6	5	83	0.023
OLIVER	Pet	Distal	8	6	75	0.017	6	6	100	0.010
CHARLIE	Kennel	Control	8	1	13	0.943	3	1	33	0.607
DIVA	Kennel	Control	8	1	13	0.943	1	1	100	0.500
DOG 1*	Kennel	Control	8	1	13	0.943	1	1	100	0.500
DOG 2*	Kennel	Control	8	0	0	0.998	0	0	-	-
DOG 3*	Kennel	Control	8	3	38	0.359	3	3	100	0.074
DUE	Kennel	Control	8	1	13	0.943	1	1	100	0.500
KATE	Kennel	Control	8	0	0	0.998	0	0	-	-
LOH	Kennel	Control	8	1	13	0.943	1	1	100	0.500
LUPA	Kennel	Control	8	1	13	0.943	2	1	50	0.500
PERLA	Kennel	Control	8	0	0	0.998	1	0	0	1.000
PUMA	Kennel	Control	8	0	0	0.998	0	0	-	-
CHARLIE	Kennel	Proximal	8	1	13	0.943	2	1	50	0.500
DIVA	Kennel	Proximal	8	3	38	0.359	3	3	100	0.074
DOG 1*	Kennel	Proximal	8	0	0	0.998	0	0	-	-
DOG 2*	Kennel	Proximal	8	0	0	0.998	0	0	-	-
DOG 3*	Kennel	Proximal	8	3	38	0.359	4	3	75	0.093
DUE	Kennel	Proximal	8	1	13	0.943	2	1	50	0.500
KATE	Kennel	Proximal	8	2	25	0.696	3	2	67	0.207
LOH	Kennel	Proximal	8	2	25	0.696	3	2	67	0.207
LUPA	Kennel	Proximal	8	3	38	0.359	3	3	100	0.074
PERLA	Kennel	Proximal	8	1	13	0.943	3	1	33	0.607
PUMA	Kennel	Proximal	8	6	75	0.017	6	6	100	0.010
CHARLIE	Kennel	Distal	8	1	13	0.943	2	1	50	0.500
DIVA	Kennel	Distal	8	2	25	0.696	6	2	33	0.500
DOG 1*	Kennel	Distal	8	0	0	0.998	0	0	-	-
DOG 2*	Kennel	Distal	8	0	0	0.998	1	0	0	1.000
DOG 3*	Kennel	Distal	8	0	0	0.998	1	0	0	1.000
DUE	Kennel	Distal	8	2	25	0.696	4	2	50	0.289
KATE	Kennel	Distal	8	0	0	0.998	1	0	0	1.000

LOH	Kennel	Distal	8	0	0	0.998	3	0	0	0.978
LUPA	Kennel	Distal	8	3	38	0.359	7	3	43	0.272
PERLA	Kennel	Distal	8	0	0	0.998	3	0	0	0.978
PUMA	Kennel	Distal	8	2	25	0.696	3	2	67	0.207

Figure 4.2 shows that, under assumption A1, all dogs of both groups made at least one choice and were all treated statistically for up 8 trials for proximal and distal pointing conditions. No dogs in both groups performed above chance levels in the control condition (Fisher's test $P = 1.000$). Ten dogs of the pet group vs. 1 dog of kennel group for the proximal condition (Fisher's test $P < 0.001$) and 10 dogs of the pet group vs. 0 dogs of the kennel group for the distal condition (Fisher's test $P < 0.001$) performed above chance levels.

In the assumption A2, some dogs were eliminated from the statistical analysis because they made *no-choice* in all trials. Furthermore, as *no-choice* were excluded from the analysis, the number of trials available was often less than 8. In this case, as shown in Figure 4.2, 0 pet dog (out of 10) vs. 0 kennel dogs (out of 8) performed above chance levels in the control condition (Fisher's test $P = 1.000$). Ten pet dogs (out of 11) vs. 1 kennel dog (out of 9) in the proximal condition (Fisher's test $P < 0.001$) and 10 pet dogs (out of 11) vs. 0 kennel dogs (out of 10) in the distal condition (Fisher's test $P < 0.001$) performed above chance levels.

The Wilcoxon Signed Rank test results show that in A1 the pet group performed above chance levels under the proximal ($z = 2.971$, $P = 0.003$) and distal conditions ($z = 2.465$, $P = 0.014$) while in all other cases, performance levels were below chance levels (pet: control $z = 2.831$, $P = 0.005$; kennel: control and distal $z = 3.002$, $P = 0.003$, proximal $z = 2.506$, $P = 0.012$).

In A2, without considering *no-choices*, control conditions for both groups and the distal pointing condition for the kennel group revealed chance levels, whereas proximal conditions in both groups exceeded chance levels (pet: $z = 3.064$, $P = 0.002$; kennel: $z = 2.217$, $P = 0.027$) in addition to distal conditions for the pet group ($z = 3.064$, $P = 0.002$).

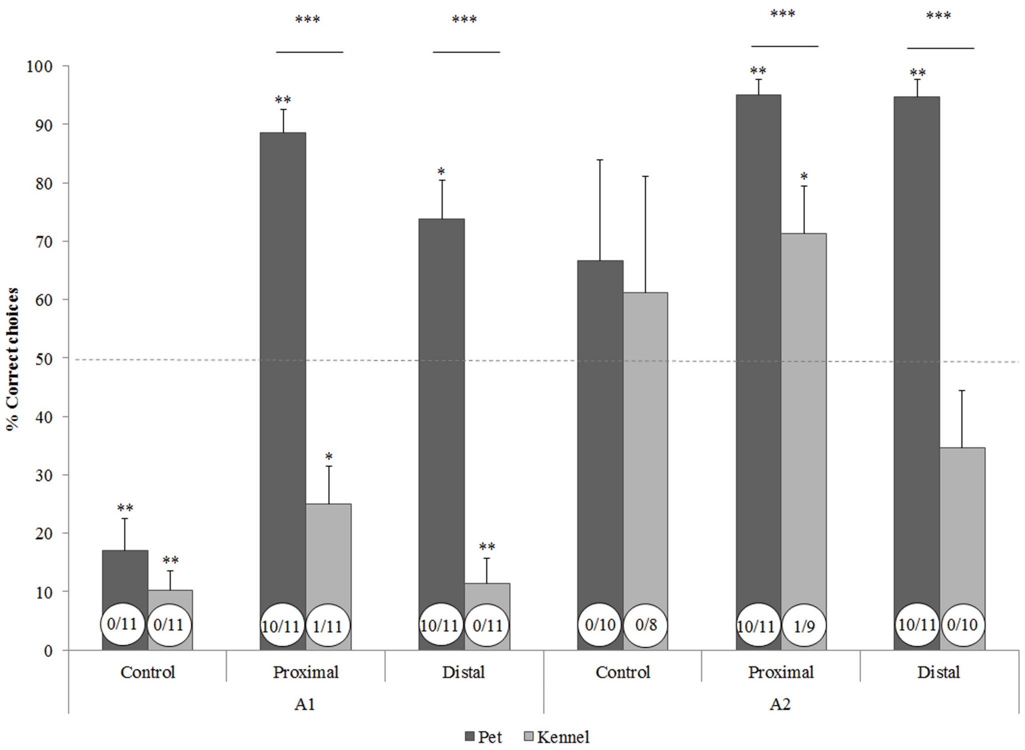

Fig 4.2 Differences in the pointing gesture comprehension performance of pet and kennel dogs under Assumption 1 (A1) and Assumption 2 (A2) of the control, proximal and distal conditions with the chance level set at 0.50. Bars show the mean percentages of correct choices made. White circles denote the number of dogs performing above chance levels from the total (see Table 4.2). Asterisks over lines denote significant differences between the number of dogs in each group exceeding chance levels according to the Fisher test; asterisks over bars denote group performance above and below chance levels. $*P < 0.05$; $**P < 0.01$; $*** P \leq 0.001$.

As shown in Figure 4.3, intergroup comparison between correct choices frequency revealed a significant difference in the occurrence of signal understanding between the groups, showing that the pet dogs followed proximal and distal pointing cues significantly better than the kennel dogs (Mann-Whitney U test: $N_{Pet} = 11$, $N_{Kennel} = 11$; proximal: $U = 2.0$, $P < 0.001$; distal: $U = 2.5$, $P < 0.001$). No difference was found for the control condition ($N_{Pet} = 11$, $N_{Kennel} = 11$; control: $U = 50.0$, $P = 0.519$).

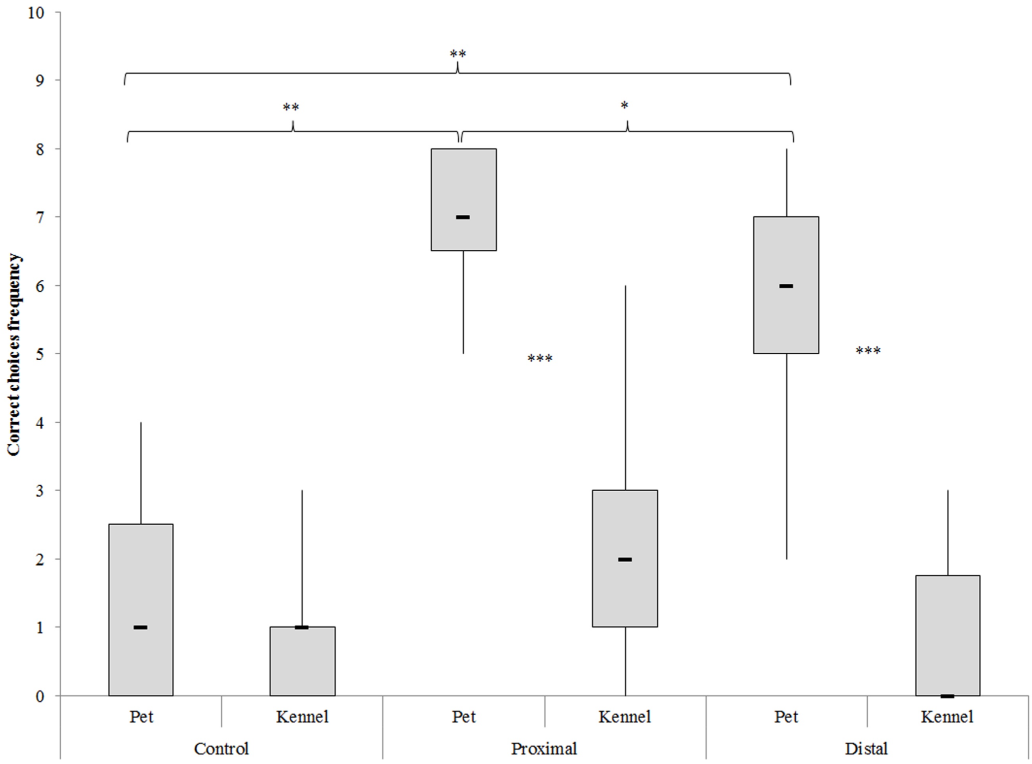

Fig 4.3 Intergroup and within-group comparisons. The box plots show the frequency of correct choices made under control, proximal and distal conditions among the pet and kennel dogs. Asterisks over the boxes denote significant differences in the same group as shown by the Wilcoxon post hoc test; asterisks between boxes denote differences between the two groups in proximal and distal conditions as shown by the Mann-Whitney U test. Bold horizontal lines: medians; grey boxes: quartiles; thin vertical lines: minimum and maximum values. *$P < 0.05$; **$P < 0.01$; *** $P \leq 0.001$.

Our Friedman's ANOVA test results for within-group comparisons revealed significant differences between the three conditions for the pet group ($\chi^2 = 20.83$, $df = 2$, $P < 0.001$). The Wilcoxon Signed Rank post hoc test showed that pet dogs understand dynamic proximal pointing better than dynamic distal pointing and control condition (control vs. proximal: $z = -2.944$, $P = 0.003$; control vs. distal $z = -2.947$, $P = 0.003$; proximal vs. distal: $z = -2.565$, $P = 0.010$). No significant differences were found for the kennel group between the three conditions ($\chi^2 = 5.07$, $df = 2$, $P = 0.079$).

4.6 Discussion

In this study, we compared the degrees to which two groups of dogs with differing levels of human socialization understand human pointing cues. None of the dogs failed any of the motivational tests (i.e., pre-test, pre-trial and inter-trial), and stress signals were only occasionally observed, ensuring that dogs of both groups were equally motivated and willing to approach the task throughout the entire test period. On the other hand, we found that Labrador Retrievers, the most common breed in our samples, cooperated well throughout the test regardless of their living conditions or specific training (Scandurra et al., 2015; D'Aniello et al., 2015) and while hosted in a kennel with limited human socialization (D'Aniello and Scandurra, 2016).

We received a very high percentage of absent choices, and especially for the kennel group. Absent choices have been interpreted several ways in the literature and have been the subject of debate (e.g., a dog's lack of interest in a given task, a misinterpretation of signals or a lack of interest in food rewards) (see Udell et al., 2008; Wynne et al., 2008; Hare et al., 2010; Udell el al., 2010b). We chose not to repeat the trial when dogs made no choices to avoid extending the study period, exhausting the dogs or creating a learning effect, but we performed two different analyses (i.e., considering absent choices as wrong choices and excluding absent choices). All of the results show that the pet dogs largely outperformed the kennel dogs in object selection tasks regardless of whether a no-choice was considered as wrong choice (i.e., assumption A1) or absence of no-choices was excluded from the statistical analysis (i.e., assumption A2). In particular, in A1, the pet dog group performed above chance levels in the proximal and distal conditions and below chance levels in control condition, whereas the kennel dog group performed below chance levels in all conditions. This outcome could be due to the prevalence of no-choices outcomes for our sample in the absence of signals (i.e., control conditions) and for the kennel group in the presence of signals (i.e., proximal and distal pointing), indicating that the kennel dogs as a group did not understand the pointing cues and that with an absence of cues, both groups appeared to be disoriented. In A2, the control condition for both groups and the distal pointing condition for the kennel group generated chance levels, whereas proximal and distal conditions exceeded chance levels for the pet group, similar to the proximal condition for the kennel group. The latter result is an interesting outcome observed in another study showing that some shelter dogs can understand simpler human pointing cues (Udell et al., 2010b). Although we cannot rule out effects of domestication on human pointing cue comprehension (Hare et al., 2002), it is unlikely that this process works only for some dogs and not for others; it is more probable that some dogs are able to learn proximal pointing gestures in a low socialization regime. We did not examine “Kaspar Hauser” dogs, and dog caretakers may use pointing gestures to refer to food bowls during feeding. On the other hand, it is known that dogs are able to understand human pointing cues very easily as puppies (Riedel et al., 2008; Virányi et al., 2008) even with a low level of human socialization (Hare et al., 2002; Zaine et al., 2015), prompting some researchers to suggest that dog uses of human communicative cues do not require extensive exposure to humans (Hare et al., 2002; Wynne et al., 2008).

Previous studies have shown that even when no-choices are considered, dogs living in shelters with few opportunities for human socialization perform pointing tasks at chance levels (Udell et al., 2008, 2010b). This discrepancy with our finding that kennel dogs perform below chance levels according to assumption A1 could be due to the fact that some shelter dogs studied in the cited papers were able to understand human pointing cues due to have been in substantial contact with humans before arriving at the given shelters. A similar explanation could be applied for dogs performing at above chance levels in similar studies (Hare et al., 2010). Our results also differ from those of Lazarowski and Dorman (2015), who studied kennel dogs that had never lived with human families. However, unlike the dogs examined in our study, kennel dogs examined in Lazarowski and Dorman’s (2015) study were purpose-bred research dogs that had undergone human socialization, potentially explaining higher levels of human pointing comprehension achieved from their group of subjects relative to the dogs examined in the present study.

Our statistical test on individual dogs showed that significantly more pet dogs than kennel dogs performed above chance levels; furthermore, the pet dogs as a group understood proximal and distal pointing cues significantly better than the kennel dogs according to our intergroup comparison of correct choice frequency levels. Altogether, such results suggest a clear difference between pet and kennel dogs, with the former being more skilful at understanding human pointing cues for both conditions and assumptions. Thus, our data, in accordance with other studies (Udell and Wynne, 2008; Udell et al., 2008, 2010b; Lazarowski and Dorman, 2015), support the notion that socialization is essential to suitable responses to human pointing gestures.

Within-group comparisons of correct choice frequency show that pet dogs understand proximal pointing cues better than dynamic pointing cues and the control condition, whereas no significant differences were found in the kennel group for the two pointing cue and control conditions. This latter result shows that as predicted, proximal pointing is generally easier for dogs to understand (Reid, 2009; Udell et al., 2010a; Lazarowski and Dorman, 2015). However, it should take into account also the possibility of a floor effect too, since the responses were generally very low.

In conclusion, in this paper we show that pet dogs largely outperform kennel dogs at understanding proximal and distal pointing cues in regards to making correct choices, meaning that regardless of whether dogs interpret pointing as a directional of referential signal (see Tauzin, 2015; Scheider et al., 2013), most dogs with a low degrees of human socialization do not understand pointing gestures. Thus, our results are in agreement with the synergetic model on the emergence of interspecific social skills in dogs (Gácsi et al., 2009; Miklósi and Topál, 2011), which predicts that dogs require social experiences with humans to master social skills. Therefore, while effects of domestication on dogs' social relationships with humans are not negligible, we stress the importance of ontogenetic processes.

4.7 References

Agnetta, B., Hare, B., & Tomasello, M. (2000). Cues to food location that domestic dogs (*Canis familiaris*) of different ages do and do not use. *Animal cognition*, *3*(2), 107-112.

Butterworth, G., Franco, F., McKenzie, B., Graupner, L., & Todd, B. (2002). Dynamic aspects of visual event perception and the production of pointing by human infants. *British Journal of Developmental Psychology*, *20*(1), 1-24.

Carballo, F., Freidin, E., Casanave, E., & Bentosela, M. (2016). Following human pointing: Where do dogs (*Canis familiaris*) look at to find food? *Behavioral processes*, *128*, 37-40.

Cunningham, C. L., & Ramos, M. F. (2014). Effect of training and familiarity on responsiveness to human cues in domestic dogs (*Canis familiaris*). *Animal cognition*, *17*(3), 805-814.

D'Aniello, B., & Scandurra, A. (2016). Ontogenetic effects on gazing behavior: a case study of kennel dogs (Labrador Retrievers) in the impossible task paradigm. *Animal cognition*, *19*(3), 565-570.

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

Dorey, N. R., Udell, M. A., & Wynne, C. D. (2010). When do domestic dogs, *Canis familiaris*, start to understand human pointing? The role of ontogeny in the development of interspecies communication. *Animal Behavior*, *79*(1), 37-41.

Gácsi, M., Gyoöri, B., Virányi, Z., Kubinyi, E., Range, F., Belényi, B., & Miklósi, Á. (2009). Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills. *PLoS One*, *4*(8), e6584.

Hare, B., & Tomasello, M. (2005). Human-like social skills in dogs? *Trends in cognitive sciences*, *9*(9), 439-444.

Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, *298*(5598), 1634-1636.

Hare, B., Rosati, A., Kaminski, J., Bräuer, J., Call, J., & Tomasello, M. (2010). The domestication hypothesis for dogs' skills with human communication: a response to Udell et al. (2008) and Wynne et al. (2008). *Animal Behavior*.

Hegedüs, D., Bálint, A., Miklósi, Á., & Pongrácz, P. (2013). Owners fail to influence the choices of dogs in a two-choice, visual pointing task. *Behavior*, *150*(3-4), 427-443.

Hennessy, M. B., Williams, M. T., Miller, D. D., Douglas, C. W., & Voith, V. L. (1998). Influence of male and female petters on plasma cortisol and behavior: can human interaction reduce the stress of dogs in a public animal shelter? *Applied Animal Behavior Science*, *61*(1), 63-77.

Kaminski, J., & Nitzschner, M. (2013). Do dogs get the point? A review of dog–human communication ability. *Learning and Motivation*, *44*(4), 294-302.

Lazarowski, L., & Dorman, D. C. (2015). A comparison of pet and purpose-bred research dog (*Canis familiaris*) performance on human-guided object-choice tasks. *Behavioral processes*, *110*, 60-67.

Miklósi, Á., & Topál, J. (2011). On the hunt for the gene of perspective taking: pitfalls in methodology. *Learning & behavior*, *39*(4), 310-313.

Miklósi, Á., Kubinyi, E., Topál, J., Gácsi, M., Virányi, Z., & Csányi, V. (2003). A simple reason for a big difference: wolves do not look back at humans, but dogs do. *Current biology*, *13*(9), 763-766.

Okamoto-Barth, S., Tomonaga, M., Tanaka, M., & Matsuzawa, T. (2008). Development of using experimenter-given cues in infant chimpanzees: longitudinal changes in behavior and cognitive development. *Developmental science*, *11*(1), 98-108.

Reid, P. J. (2009). Adapting to the human world: dogs' responsiveness to our social cues. *Behavioral processes*, *80*(3), 325-333.

Pongrácz, P., Gácsi, M., Hegedüs, D., Péter, A., & Miklósi, Á. (2013). Test sensitivity is important for detecting variability in pointing comprehension in canines. *Animal cognition*, *16*(5), 721-735.

Riedel, J., Schumann, K., Kaminski, J., Call, J., & Tomasello, M. (2008). The early ontogeny of human–dog communication. *Animal Behavior*, *75*(3), 1003-1014.

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D'Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behavior. *Animal cognition*, *18*(4), 937-944.

Scheider, L., Kaminski, J., Call, J., & Tomasello, M. (2013). Do domestic dogs interpret pointing as a command? *Animal Cognition*, *16*(3), 361-372.

Starling, M. J., Branson, N., Thomson, P. C., & McGreevy, P. D. (2013). Age, sex and reproductive status affect boldness in dogs. *The Veterinary Journal*, *197*(3), 868-872.

Svartberg, K. (2006). Breed-typical behavior in dogs—historical remnants or recent constructs? *Applied Animal Behavior Science*, *96*(3-4), 293-313.

Tauzin, T., Csík, A., Kis, A., & Topál, J. (2015). What or where? The meaning of referential human pointing for dogs (*Canis familiaris*). *Journal of Comparative Psychology*, *129*(4), 334.

Udell, M. A., & Wynne, C. D. (2008). A review of domestic dogs' (*Canis familiaris*) human-like behaviors: or why behavior analysts should stop worrying and love their dogs. *Journal of the experimental analysis of behavior*, *89*(2), 247-261.

Udell, M. A., & Wynne, C. D. (2010). Ontogeny and phylogeny: both are essential to human-sensitive behavior in the genus *Canis*. *Animal Behavior*, *79*(2), e9-e14.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2008). Wolves outperform dogs in following human social cues. *Animal Behavior*, *76*(6), 1767-1773.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2010a). What did domestication do to dogs? A new account of dogs' sensitivity to human actions. *Biological reviews*, *85*(2), 327-345.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2010b). The performance of stray dogs (*Canis familiaris*) living in a shelter on human-guided object-choice tasks. *Animal Behavior*, *79*(3), 717-725.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2011). Can your dog read your mind? Understanding the causes of canine perspective taking. *Learning & Behavior*, *39*(4), 289-302.

Udell, M. A., Spencer, J. M., Dorey, N. R., & Wynne, C. D. (2012). Human-socialized wolves follow diverse human gestures… and they may not be alone. *International Journal of Comparative Psychology*, *25*(2).

Virányi, Z., Gácsi, M., Kubinyi, E., Topál, J., Belényi, B., Ujfalussy, D., & Miklósi, Á. (2008). Comprehension of human pointing gestures in young human-reared wolves (*Canis lupus*) and dogs (*Canis familiaris*). *Animal cognition*, *11*(3), 373.

Wynne, C. D., Udell, M. A., Lord, K. A. (2008). Ontogeny's impacts on human–dog communication. *Animal Behavior, 76(4),* e1-e4.

Zaine, I., Domeniconi, C., & Wynne, C. D. (2015). The ontogeny of human point following in dogs: When younger dogs outperform older. *Behavioral processes*, *119*, 76-85.

CHAPTER 5

THE IMPORTANCE OF GESTURAL COMMUNICATION: A STUDY OF HUMAN-DOG COMMUNICATION USING INCONGRUENT INFORMATION

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Abstract

We assessed how water rescue dogs, that are equally accustomed to respond to gestural and verbal requests, weighted gestural versus verbal information when asked by their owner to perform an action. Dogs were asked to perform 4 different actions (SIT, LIE DOWN, STAY, COME) providing them congruent (Phases: 1- gestural and 2-verbal) or incongruent information (Phase 3: gestural and verbal commands referred to two different actions). In Phases 1 and 2 we recorded the frequency of correct responses as 0 or 1, whereas in Phase 3 we computed a 'preference index' (percentage of gestural commands followed over the total commands responded). Results showed that dogs followed gestures significantly better than words when these two types of information were used separately. Females were more likely to respond to gestural than verbal commands and males responded to verbal commands significantly better than females. In the incongruent condition, when gestures and words simultaneously indicated two different actions, the dogs overall preferred to execute the action required by the gesture rather than that required verbally, except when the verbal command COME was paired with the gestural command STAY with the owner moving away from the dog. Our data suggest that in dogs accustomed to respond both to gestural and verbal requests, gestures are more salient than words. However, dogs' responses appeared to be dependent also on the contextual situation: dogs' motivation to maintain proximity with the owner that was moving away could have the lead them to make the more 'convenient' choice between the two incongruent instructions.

Keywords: human-dog communication, incongruent information, gestural cue, verbal cue, water rescue dog

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5.1 Introduction

Dogs and humans communicate effectively and in a complex way during everyday social interactions as well as in specific working and collaborative contexts (Miklósi, 2009).

There is clear evidence that dogs are very skilled at interpreting different human visual communicative signals, such as pointing, gazing and body postures, and use them appropriately in different contexts and tasks (Bensky et al., 2013; Kaminski and Nitzschner, 2013 for reviews).

Dogs are also responsive to both words and non verbal vocal sounds (Mills 2005, for a review); in fact, they can learn to recognize and utilize several hundred words (Kaminski et al., 2004; Pilley and Reid, 2011) and their behavioral response and performance appear to be influenced by vocal qualities common to human speech (Fukuzawa et al., 2005) and by specific characteristics of human vocalizations (Scheider et al., 2011; Gibson et al., 2014). Moreover, dogs are sensitive to contextual cues: for example, human's visual cues such as posture, gestures, eye direction/visibility or even physical distance affect the likelihood they would respond to verbal information (Virányi et al., 2004; Fukuzawa et al., 2005).

Most often, humans when communicating with dogs spontaneously combine verbal and gestural cues; however, little is known about the relative salience of the vocal and gestural communicative channel for dogs. Recently, Grassmann et al. (2012) tested two word-trained dogs in an object fetching situation in which an unfamiliar person asked the dog to fetch an object by name, by pointing to it (proximal pointing) and in conflict conditions were the pointing gesture and the spoken word simultaneously indicated two different objects. It emerged that, when pointing gesture and spoken word conflicted, the dogs preferentially fetched the object indicated by name, thus suggesting that their training based on fetching objects by name might have induced a preference for following the verbal information in the fetching task. These finding suggested that dogs integrated the proximal pointing with the name to perform the fetching game, but they did not allow to clearly disentangle the role of the two communicative channels. For this purpose we decided to use simple verbal commands and non-referential gestures to test dogs not specifically word-trained. In particular, we tested water rescue dogs, trained to reliably perform different actions using both gestural and verbal commands (see D'Aniello et al. 2015 for further details on water rescue dogs training and activities), requiring them to perform four well-known actions using verbal commands only, gestural commands only and in a conflicting situation in which words and gestures were delivered simultaneously but referred to different actions.

We assumed that water rescue dogs would have performed reliably when receiving either gestural or verbal information only, since they do not have an induced preference for following verbal information over gestural one. As to our knowledge no other studies have tested dogs providing them incongruent information for a given action, no clear prediction on dogs’ behavior was possible. Dogs could make no choice, choose at chance level between the contrasting verbal/gestural commands: nevertheless, if dogs have a special attitude for attending to human gestures in our conflicting situation they should rely more on gestures than words to choose the action to perform.

5.2 Materials and methods

5.2.1 Participants

29 dog-owner dyads participated in the study. All dyads were recruited at the Italian School of Water Rescue Dogs (Scuola Italiana Cani Salvataggio – SICS) and were already qualified as water rescue teams (obtaining a SICS Water Rescue Certificate®). These dogs are trained to perform very reliably different commands (sit, lie down, stay, come, fetch, turn) on the ground, even from a distance and behind a wire mesh barrier, before being trained for their specific tasks.

Of the 29 dyads tested, 25 met the requirements (see below) and were used for data analysis: 10 Golden retrievers (3 males and 7 females) and 15 Labrador retrievers (9 males and 6 females); mean age ± SD: 62.16 ± 5.11 months.

5.2.2 Experimental procedure

The experimental protocol included a pre-test, in which the dogs were given 4 basic commands SIT, LIE DOWN, STAY, COME which are routinely performed and equally learned by these dogs.

Commands were given using both words and gestures as normally occur during training. Only dogs that executed all the 4 commands correctly were admitted to the test, which consisted of the 3 consecutive phases:

Phase 1 (gestural commands). The 4 basic commands were delivered by the owner using only gestures, without verbal information.

Phase 2 (verbal commands). The same as phase 1 except that the 4 commands were delivered to dogs using voice only. The owner was asked to take a neutral position with arms extended along the body and the head directed forward without looking at the dog.

Phase 3 (contrasting commands). The owner repeated the 4 commands providing incongruent information: 1) the verbal command LIE DOWN was associated with the gesture indicating SIT; 2) the verbal command SIT was associated with the gesture indicating LIE DOWN; 3) the verbal command COME was associated with the gesture indicating STAY; 4) the verbal command STAY was associated with the gesture indicating COME. Verbal and gestural commands were given simultaneously to the dog.

In all test phases dogs had to respond to the command within 5 sec. Precise instructions were given to the owners to control for any involuntary cue and all videos were checked before analysis to discard those dyads in which the owner did not strictly adhere to the instructions. Four dogs whose owner made mistakes in giving command in the incongruent condition were excluded from the analysis.

Testing was carried out indoor, to minimize sources of distraction for dogs, in a bare room measuring about 15 x 13 m. On the floor of the room two "X" (X1 and X2) were marked with black tape at a distance of 5 m and were connected by two arrows, in order to clearly indicate the path that the owner had to move along. At the start of the test, the owner was asked to stand at position X1 and, keeping the dog on his/her left side, to give the following commands: SIT, LIE DOWN and STAY. Then the owner walked to position X2, turned facing the dog and gave it the command COME. Dogs were given 5 sec to perform each command and an interval of 10 sec was set between a command and the subsequent. Before the beginning of the test, dogs were allowed to move freely to become familiar with the place. After each testing phase, the dog was allowed to move around the room for 2 min. At the end of the test, before a new dog-owner dyad entered, the room was washed with a non-toxic disinfectant. All tests were video recorded using a Sony Handycam HDR-PJ260VE.

5.2.3 Data scoring

In Phases 1 and 2 the frequency of correct responses to the commands was scored: a dog received a score of 1 when responding correctly and 0 for incorrect responses. Thus, for each dog the total score ranged from 0 to 4.

In Phase 3, when the verbal and gestural commands were conflicting, we evaluated whether the dog followed the verbal or the gestural command.

To evaluate whether overall dogs preferentially followed gestural commands to verbal ones, when both were simultaneously and incongruently given (Phase 3), we computed a "preference index" by calculating for each dog the percentage of gestural commands followed, over the total commands responded to. Therefore, if a dog responded to 3 out of the 4 commands imparted, and followed the gestural command 2 out of 3 times its preference index was 0.66.

The inter-observer reliability was conducted on 25% of the dogs tested and no disagreement on response emerged.

5.2.4 Data analysis

The possible effect of commands' order on the dogs performance was assessed using two different sequences of commands. Five dogs received the sequence: SIT, LIE DOWN, STAY, COME (sequence 1); a second sample of five dogs received a different sequence of commands: LIE DOWN, SIT, COME, STAY (sequence 2). No differences between the two samples of dogs were found in the proportion of correct responses of Phase 1: sequence 1 (median 4), sequence 2 (median 4); $P = 0.423$; $U = 10$; and Phase 2: sequence 1 (median 3), sequence 2 (median 3); $P = 0.577$; $U = 9.5$.

To assess whether dogs were more likely to respond correctly to verbal vs. gestural commands (Phase 1 and Phase 2), we carried out a Generalized Estimated Equation (GEE) with the total score (Poisson distribution) as the dependent variable and command type (verbal vs. gestural), sex and breed

(Golden retriever vs. Labrador retriever) as the within group independent variables. As no effect of breed emerged this variable was removed from the model. Post-hoc tests were carried out using the Bonferroni correction.

We used Mann-Whitney U test to assess possible sex difference in the ‘preference index’. Furthermore, to assess whether dogs’ preference index was different from chance, a one-sample Wilcoxon test was used. All analyses were carried out using SPSS 21 with alpha set at 0.05.

5.3 Results and discussion

Our results revealed a main effect of command type (*Wald* = 22.55, *df* = 1, $P < 0.001$) and sex (*Wald* = 8.3, *df* = 1, $P = 0.004$) and a significant command type x sex interaction (*Wald* = 9.97, *df* = 1, $P = 0.002$) on dogs’ performance over the 4 trials. Females responded more properly to gestural than verbal commands (mean gestural = 3.92, mean verbal = 2.85, $P < 0.001$), while males showed no preference (mean gestural = 4, mean verbal =3.75, $P = 0.27$); moreover, males responded to verbal commands significantly better than females ($P = 0.005$).

In Phase 1 (gestural commands) all dogs, except one female (that did not respond to the command LIE DOWN), performed correctly the requested actions, whereas in Phase 2 (verbal commands) 3 dogs (all females) failed to respond properly to the command SIT, 12 dogs (9 females and 3 males) failed to respond to the command LIE DOWN, and 3 dogs (all females) failed the command STAY.

This finding is interesting and indicates that in our testing situation dogs interpreted gestures significantly better than words when these two types of information were used separately. Since all the dogs correctly performed the 4 commands when they were given with words and gestures associated, words could be to some extent redundant when associated with gestures, which are more salient as cues for dogs. However, as the order in which the two types of command in Phases 1 and 2 was the same for all dogs the interpretation of the different levels of performance to the two types of command remains problematic.

The fact that females made less errors with gestural than verbal commands, whereas males performed in a comparable way with both types of commands is in line with other studies indicating that female dogs are more concentrated on visual cues than males (Müller et al., 2011; Åkerberg et al., 2012). Nevertheless, as males performed very well on the verbal commands, we cannot exclude that the emerged sex difference could be a statistical artifact due to a ceiling effect.

The Mann Whitney U test revealed no difference in the “preference index” of male and female dogs ($U = 48$, $z = -1.69$, $P = 0.11$), thus data were pooled to carry out the Wilcoxon test. The percentage of trials in which dogs chose to follow the gestural (rather than the verbal) command in incongruent trials was significantly above chance (mean preference index = 70.33, $T = 26$, $z = -3.321$, $P < 0.001$). Dogs showed an overall preference for gesture over word, but this preference was less marked when the verbal command COME was paired with the gestural command STAY and the owner moved away from the dog. In this situation 8 dogs (32%) followed the gesture, whereas 14 dogs (56%) acted according to the vocal command (Fig. 5.1).

This finding is interesting and deserves further investigation: it suggests that when gesture and word conflicted the dogs preferentially followed the gesture, but when their owner was moving away from the dog towards position X2, his/her movement and the motivation for maintaining proximity to him/her overshadowed the preference for the gesture. In the conflicting situation in which the incongruent commands were SIT and LIE DOWN the dogs’ choice could be determined by a preference at the code level (gesture vs. word), as the spatial relationship with the owner was unvaried; when the gestural command COME conflicted with the vocal command STAY, the gesture was in accordance with an action that allowed the dog to regain proximity to the owner.

Current results differ from those by Grassmann et al. (2012), showing that dogs in an object fetching game in which pointing and words were in conflict preferentially fetched the required objects by name. These discrepancies might depend on different factors. On one side, the two dogs tested by Grassmann et al. (2012) had received a specific word-based training, which may have led them to preferentially follow the verbal commands. Furthermore, their owners were not accustomed to perform pointing or other gestures in the fetching game training; this raises the possibility that the dogs did not understand that the pointing gesture meant fetching an object, but rather interpreted it as directional signal (Grassmann et al., 2012; but see Kaminski, 2008; Topál et al., 2009; Scheider et al., 2011; Wobber and Kaminski, 2011). An additional experimental condition, i.e. just pointing at the object to fetch without saying the label, would have allowed to specifically evaluate the impact of pointing per se. Finally, despite the dogs in Grassmann et al. (2012) study were specifically word-trained, the gestural indication was not completely ignored: when pointing was in conflict with label both dogs approached the pointed-to object first but then fetched the labeled one, thus showing that they also attended to the gesture.

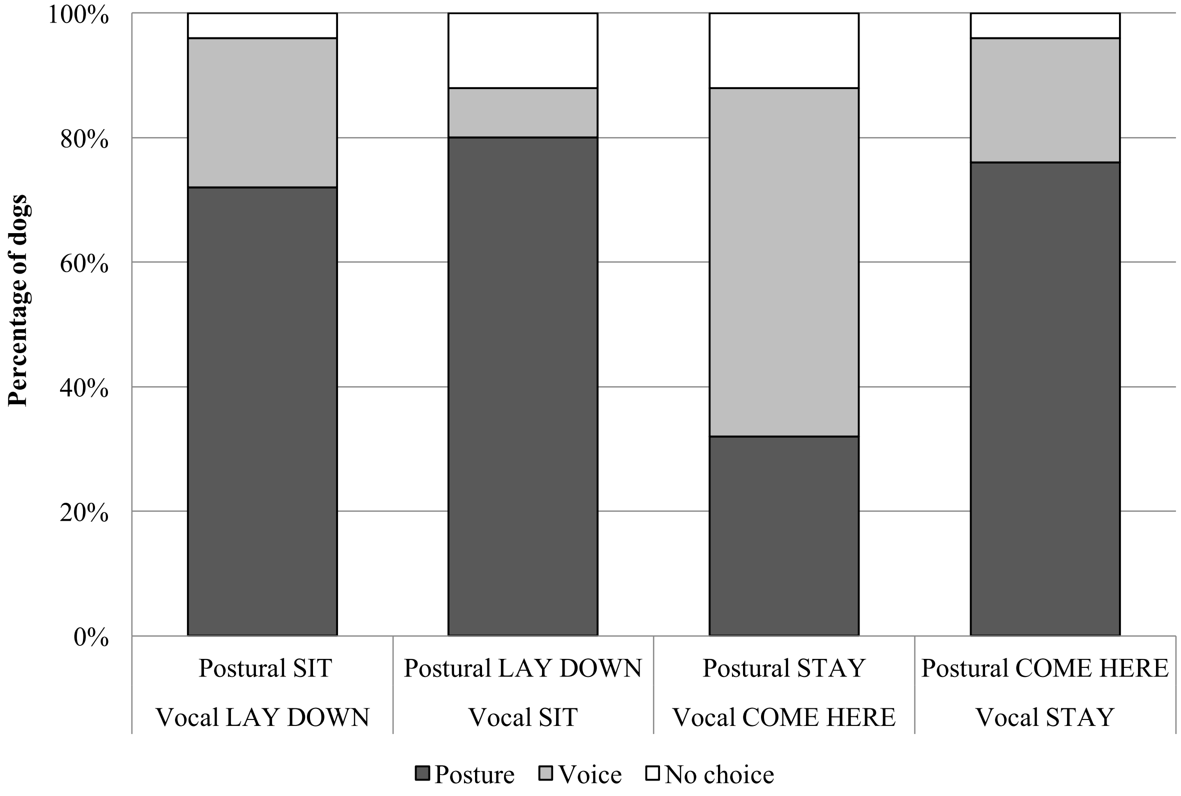

Fig 5.1 Results in Phase 3 (incongruent condition). The numbers in the boxes indicate the dogs' choices. Dark gray boxes: response to the gestural commands; light gray boxes: response to verbal commands; white boxes: no response

In conclusion our data suggest that, when dogs are equally accustomed to respond to visual and verbal commands, gestural cues are dominant; this supports the evidence that body language plays a main role, being the most important communication channel for dogs (Beaver, 1999). However, dogs' responses are also dependent on contextual information (e.g. Fukuzawa et al., 2005; Scheider et al.,

2011) and in the present study the preference for the verbal command COME over the gestural command STAY could have been influenced by the movement of the owner towards the X2 position. In particular, the dogs' motivation to maintain proximity with the owner could have the led the majority of them to make the more 'convenient' choice between the two incongruent instructions.

These preliminary findings should be confirmed and expanded using the same protocol with more combinations of contrasting commands, varying the order of the sequence, the voice tone and considering also other breeds and other training regimes, to clarify whether and how strongly gestural communication maintains the role that we highlighted in Labrador retriever and Golden retriever trained for water rescue.

5.4 References

Åkerberg, H., Wilsson, E., Sallander, M., Hedhammar, Å., Lagerstedt, A. S., Larhammar, D., & Meyerson, B. (2012). Test for personality characteristics in dogs used in research. *Journal of Veterinary Behavior: Clinical Applications and Research*, *7*(6), 327-338.

Beaver, B.V. (1999). Canine Behavior: A Guide for Veterinarians. W.B. Saunders Co., Philadelphia.

Bensky, M. K., Gosling, S. D., & Sinn, D. L. (2013). The world from a dog's point of view: a review and synthesis of dog cognition research. In *Advances in the Study of Behavior* (Vol. 45, pp. 209-406). Academic Press.

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

Fukuzawa, M., Mills, D. S., & Cooper, J. J. (2005). More than just a word: non-semantic command variables affect obedience in the domestic dog (*Canis familiaris*). *Applied Animal Behavior Science*, *91*(1-2), 129-141.

Grassmann, S., Kaminski, J., & Tomasello, M. (2012). How two word-trained dogs integrate pointing and naming. *Animal Cognition*, *15*(4), 657-665.

Gibson, J. M., Scavelli, S. A., Udell, C. J., & Udell, M. A. R. (2014). Domestic dogs (*Canis lupus familiaris*) are sensitive to the "human" qualities of vocal commands. *Anim. Behav. Cogn.*, *1*(3), 281.

Kaminski, J. (2008). Dogs (*Canis familiaris)* are adapted to receive human communication. In: Berthoz, A., & Christen, Y. (Eds.), *Neurobiology of "Umwelt": how living beings perceive the world, research and perspectives in neurosciences*. Springer, Heidelberg

Kaminski, J., & Nitzschner, M. (2013). Do dogs get the point? A review of dog–human communication ability. *Learning and Motivation*, *44*(4), 294-302.

Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for" fast mapping". *Science*, *304*(5677), 1682-1683.

Miklósi, A. (2009). Evolutionary approach to communication between humans and dogs. *Veterinary research communications*, *33*(1), 53-59.

Mills, D. S. (2005). What's in a word? A review of the attributes of a command affecting the performance of pet dogs. *Anthrozoös*, *18*(3), 208-221.

Müller, C. A., Mayer, C., Dörrenberg, S., Huber, L., & Range, F. (2011). Female but not male dogs respond to a size constancy violation. *Biology letters*, rsbl20110287.

Pilley, J. W., & Reid, A. K. (2011). Border collie comprehends object names as verbal referents. *Behavioral processes*, *86*(2), 184-195.

Scheider, L., Grassmann, S., Kaminski, J., & Tomasello, M. (2011). Domestic dogs use contextual information and tone of voice when following a human pointing gesture. *PLoS One*, *6*(7), e21676.

Topál, J., Gergely, G., Erdőhegyi, Á., Csibra, G., & Miklósi, Á. (2009). Differential sensitivity to human communication in dogs, wolves, and human infants. *Science*, *325*(5945), 1269-1272.

Virányi, Z., Topál, J., Gácsi, M., Miklósi, Á., & Csányi, V. (2004). Dogs respond appropriately to cues of humans' attentional focus. *Behavioral processes*, *66*(2), 161-172.

Wobber, V., Kaminski, J. (2011). What do dogs understand about human communicative signals? A novel synthesis. In: deGiovine, V. (Ed.), *Dogs: biology, behavior, and health disorders (*pp. 93-110). Nova Press, New York.

CHAPTER 6

EFFECTIVENESS OF VERBAL AND GESTURAL SIGNALS AND FAMILIARITY WITH SIGNAL-SENDERS ON THE PERFORMANCE OF WORKING DOGS

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Abstract

We assessed how highly trained dogs respond to gestural versus verbal signals when their handlers or an unfamiliar person asked them to perform an obedience task. Dogs were requested to perform four different actions ("Sit", "Down", "Stay" and "Come") upon receiving congruent (only gestural or only verbal) or incongruent signals (gestural and verbal signals contradict each other). The dogs' performance measures were the frequency of correct responses and their response latency. Generalized Estimation Equation models were used to determine whether the type of signal, the coherence of the signals and familiarity with the signaler influenced dogs' responses. Our results show that the probability of dogs expressing the requested behavior was lower when the stranger gave verbal signals, than in any of the other conditions. In the incongruent condition, the probability that dogs expressed the behavior indicated by the verbal signal was lower for signals provided by the stranger than for signals provided by the owner. The reverse was observed for gestural signals. In general, longer latencies to perform the "Come", "Down" and "Sit" behaviors were observed in response to the stranger's verbal signals than when the stranger gave gestural or incongruent signals. Additionally, the response latency to the stranger's verbal stimuli took longer than verbal stimuli were provided by the owner in the case of "Come" ($P = 0.002$) and "Sit" ($P < 0.001$) actions. Our data support the argument that for highly trained dogs, gestural signals are less dependent upon signal-giver familiarity, whereas verbal signals are less effective when they are given by an unfamiliar person.

Keywords: dog, gestural cue, human-dog communication, contrasting paradigm, familiarity, vocal cue

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6.1 Introduction

Accurate and smooth information flow is an important part of successful and smooth communication. A possible source of communication failure is when a signal or message delivered in different modalities conflict. The focus of the current research is how the congruence and incongruence of a signal given in two modalities (verbal versus gestural) influences the responses of highly trained dogs when they are delivered by an unfamiliar person or their handlers.

A meta-analysis of human studies (Hostetter, 2011) comparing messages presented only verbally (speech) with messages that are presented both verbally and with gestures, found that gestures have a significant effect and improved communication efficacy. Gestures accompanying speech also increase memory for verbal content, as demonstrated by Galati and Samuel (2011). Information from speech and gestures seem to be processed in parallel in the human brain (Özyürek et al., 2007; Willems et al., 2007). In the case of humans, action-related language activates the motor system and it has been shown that the neural processing of gestures is similar to neural processing in comprehending words (see Willems and Hagoort, 2007 for a review), revealing a strong interdependence between gestures and speech. There is evidence that young children (2 and 4 years old) tested in an object choice task rely most heavily on pointing gestures compared to words when these two sources of information conflict, indicating the importance of visual cues (Grassmann and Tomasello, 2010). Interspecies communication also often relies on bimodal signals. Indeed, humans combine gestural and verbal cues simultaneously when they interact with dogs.

Human gestures are very important for dogs and many studies have underlined the significance of specific human visual signals in affecting their attention and behavior. Gazing, head-nodding, head turning and glancing at a target (Hare et al., 1998, 2002; Agnetta et al., 2000; Soproni et al., 2001; Hare and Tomasello, 2005), as well as human pointing gestures (Miklósi et al., 1998; Hare and Tomasello, 1999; Soproni et al., 2001, 2002; Miklósi and Soproni, 2006; Udell et al., 2008 a,b; Lakatos et al., 2009, 2012) have been shown to direct dogs' attention and behavior.

Dogs also rely on human verbal communication, responding to both words and non-verbal vocal sounds (Mills, 2005 for a review) and are able to learn the meaning of several hundred words

(Kaminski et al., 2004; Pilley and Reid, 2011). They are also able to discriminate between live-sounds and recorded sounds and moderate the responsiveness to the command (Fukuzawa et al., 2005) suggesting their sensitivity to the vocal qualities of human speech (Gibson et al., 2014). Furthermore, dogs can also use human voice referentially, being able to locate food following the direction of the voice of a hidden person (Rossano et al., 2014).

The relative relevance of human gestural and verbal signals for dogs has received little attention so far. One study (Pongrácz et al., 2003) reported that dogs tend to respond correctly to verbal-gestural commands given by their owner whose life-size image was projected on a screen. However, when the owner commanded the dog from another room (via loudspeaker) but was not visible, then dogs barely responded. Another study (Virányi et al., 2004) revealed that well-trained dogs tended not to respond to the simple "lie down" command if their owner gave the command while facing another human person and not the dog. In comparison, dogs performed better when their owner was facing the wall, or he/she was hidden behind a screen while commanding. These results indicate that human visual attention for dogs, interpreted as a visual signal, constitutes a significant component of human-dog communication.

A study showed that there was no significant increase in error rates of dogs' obedience when human body movements were restricted and verbal signals were provided (Fukuzawa et al., 2005). Furthermore, in an object choice task in which dogs were asked to fetch an object by name while the researcher pointed and looked to another object, dogs preferentially chose the object mentioned by name rather than the object that was pointed to (Grassmann et al., 2012). These outcomes suggest that dogs rely more on verbal signals and less on gestural signals. However, the dogs tested by Grassmann et al. (2012) had received a word-based training and generally, their owners did not use gestures in training the fetching game. This could have been responsible for finding that their dogs relied mainly on verbal signals. Indeed, it has been shown that for dogs that are equally familiar to respond both to gestural and verbal signals during the training, gestures were preferred compared to verbal cues, indicating that the handler's gestural signals were more relevant than verbal ones (D'Aniello et al., 2016). An open question is how dogs would perform when the signaler is not their handler.

It is well known that dogs are able to discriminate between their owner and an unfamiliar person in a variety of situations. They show a clear preference for their caretakers given their attachment bond (see Prato-Previde and Valsecchi, 2014 for a review) and they pay more attention to their owners in different contexts (Miklósi et al., 2005; Mongillo et al., 2010). In the impossible task paradigm, water rescue dogs directed their first gaze significantly more often towards their owner (D'Aniello et al., 2015). Agility dogs, in similar experimental conditions, clearly preferred their owner, gazing at him/her for significantly longer periods (Marshall-Pescini et al., 2009). However, it seems that social familiarity alone cannot account for dogs' higher attention to their owners, since an increase in attention levels requires a close relationship, rather than mere familiarity (Horn et al., 2013). Furthermore, discrimination of a familiar person seems to be context-specific, since dogs discriminate their owner from other familiar people less well in situations requiring obedience relative to playful or fearful emotional situations (Kerepesi et al., 2015). In training contexts, dogs obey the commands of their handlers significantly more often than those of a stranger (Coutellier, 2006; Kerepesi et al., 2015). More specifically, it was found that dogs obeyed their owners' recorded voices in the presence of their owners significantly more compared to a stranger's voice in the presence of the stranger when human body movements were constrained (Coutellier, 2006). When no verbal signals are given, dogs are more likely to attend to the gestural signals of familiar others than unfamiliar ones (Cook et al., 2014).

The novel contribution of the current study is not only the relative strength of gestural and verbal signals, but also the signal source, namely the owner and a stranger. The goal was to separate the relative contribution of these two factors to the effectiveness of obedience commands. To this end, highly trained dogs, able to respond to basic obedience commands, were selected and their obedience rates to verbal and gestural signals were examined (i.e. giving gestural signals without verbal support and vice versa). Moreover, the dogs were also tested in a condition where they received signals that were incongruent between the two modalities (i.e., voice and gesture). The simultaneously delivered bi-modal signals referred to opposite actions. We chose to test water rescue dogs, which are extremely well trained to respond to obedience signals. Since they have to work in crowded beaches, such dogs are also highly socialized, which should prevent them from annoying or fearful effects of an unfamiliar signal-giver during the test (for further details of water rescue dogs training and activities see Scandurra et al., 2016). Based on the results of previous studies (Coutellier, 2006; Cook et al., 2014), we expect that the dogs' performance would generally be better when the owner provides signals than when a stranger does, regardless of the modality in which signals are provided. Moreover, using a similar protocol, we (D'Aniello et al., 2016) demonstrated that dogs obey an owner's gestures significantly more compared to when the owner gives the same signal verbally. Also, they exhibit a clear preference for gestures when conflicting gestural and verbal commands are given simultaneously. We expected that dogs would show a similar gestural preference when conflicting gestural and verbal commands are given by a stranger.

6.2 Materials and methods

6.2.1 Participants

22 dog-owner dyads participated in the study: 6 Golden retrievers (four males and two females) and 16 Labrador retrievers (eight males and eight females), mean age ± SD = 5.2 ± 1.8 years; 10 male and 12 female owners. All dyads were recruited at the Italian School of Water Rescue Dogs (Scuola Italiana Cani Salvataggio – SICS) and were already qualified as water rescue teams (having obtained a SICS Water Rescue Certificate®). The training lasts at least two years during which the owners are expected to be very competent in dog training. Water rescue dogs live in families in which one member of the family is also the handler. Before being trained for their specific tasks in water, these dogs are educated for obedience on land. During the training, all owners learn to give the obedience commands to their dogs in the same way, using the specific gestural and verbal stimuli for each single command.

6.2.2 Experimental procedure

Although water rescue dogs are highly trained, their performance quality was examined in the experimental room. Consequently, the experimental protocol included a pre-test, during which the dogs were given four commands with consistent gestural and verbal stimuli given simultaneously.

Gestural stimuli were provided as follow:

1. “Sit”: the right closed fist is moved from the chest toward the back side of dogs, while the head of the owner is turned left toward the dog;
2. “Down”: the right open hand is lowered with the palm facing the ground, while the head of the owner is turned left toward the dog;
3. “Stay”: the right open hand is showed to the dog, while the head of the owner is turned left toward the dog;
4. “Come”: away from the dog with the arms were opened sideways.

For the verbal stimuli the italian words “seduto”, “terra”, “resta” and “vieni” are uttered, respectively.

Only the dogs that executed the four commands correctly with both their owners and the stranger were included in the actual test phase. The test phase consisted of the following three episodes:

Episode 1 (gestural condition). The four commands were delivered using only gestures as described above.

Episode 2 (verbal condition). The four commands were delivered only vocally. The signaler was asked to adopt a neutral position with arms extended along the body and the head directed forward.

Episode 3 (incongruent condition). The signaler repeated the four incongruent signal commands: 1) the verbal “Down” (Down-v) was associated with the gesture “Sit” (Sit-g); 2) the verbal “Sit” (Sit-v) was associated with the gesture “Down” (Down-g); 3) the verbal “Come” (Come-v) was associated with the gesture “Stay” (Stay-g), while the handler leave the dog to take another position (see below); 4) the verbal “Stay” (Stay-v) was associated with the gesture “Come” (Come-g), while the handler was away from the dog in the another position (see below). Verbal and gestural stimuli were given synchronously and the dynamics of the gestures and the duration of the words were the same.

The three episodes were administered twice for each dog, once with the signals provided by the owner and once with signals provided by an experimenter unfamiliar to the dog. The role of stranger was performed by one of the authors (i.e. Biagio D'Aniello), who is highly specialized in dog training and was unfamiliar to the dogs. The order of the two signalers (owner and stranger) was randomized in the sample.

Testing was carried out indoors, to minimize possible distraction sources. Two "X" (X1 and X2) signs were marked on the floor with a black tape at a distance of 5 m. Before the beginning of the test, dogs were allowed to move freely to become familiar with the place and the experimenters for 5 minutes. At the start of the test, the signaler was asked to get to the X1 position and, keep the dog on his/her left side, and give the following commands: “Sit”, “Down” and “Stay”. Then the signaler walked to X2, turned around to face the dog and give it the command “Come”. If the dog followed the signaler upon the command “Stay” in the gestural and/or verbal conditions, it was positioned again in X1 and the command “Stay” was given according to the pre-test condition. Then the signaler walked to X2 to give the command “Come” according to the test conditions. After each testing episode, the dog was allowed to move freely around the room for 2 minutes. All tests were video recorded using a Sony Handycam HDR-PJ260VE.

In some cases, the owners were not able to restrain their gesture in the *verbal condition* or to provide simultaneously incongruent verbal and gestural commands in the *incongruent condition*. In case of such mistakes, a repetition of a single command was allowed only once, afterwards the test was canceled. Of 22 dog-owner dyads recruited for the study only 15 completed the test, while the remainder was excluded because the owner was unable to comply with the requirements of the procedure or due to a lack of an appropriate response by the dog in the stranger condition in the pre-test. Accordingly, the statistical analyses are based on 3 Golden retrievers (two males and one female) and 12 Labrador retrievers (six males and six females); mean age ± SD = 5.27 ± 1.39 years.

6.2.3 Data collection and analysis

The time given to the dog to execute the commands “Sit”, “Down” and “Come” was set to 5 seconds, afterwards the dog was considered unresponsive. The “Stay” command requires the dog to hold the position, while immediately afterwards the signaler leaves the dog. In this case, the dog can choose to comply with the command, remain static, or follow the signaler not obeying the command. We considered a correct response when the dog fulfilled the command for at least 5 seconds. In the incongruent condition, we further specified the response by the dog could be behavior that either followed the gesture or the verbal signal.

To determine the general efficacy of signals provided by different signalers, a Generalized Estimation Equation (GEE) model was used. The dog's response in each trial was used as a binomial dependent variable (performed/did not perform the requested behavior; in the case of the incoherent condition, response to any of the two signals was considered a correct performance). The model included the condition (verbal, gestural, incongruent) and the sender (owner, stranger) as fixed factors, as well as their first level interaction. The dogs' ID was fitted in the model to account for the repeated data collected from each dog. Pairwise contrasts were computed with sequential Bonferroni correction.

A GEE model was then used to determine the relative efficacy of verbal and gestural signals provided by the owner and the stranger in the conflicting condition. Two separate models were built using as dependent variables respectively the dog's response to the gestural signals (performed/did not perform the behavior requested by the gestural signal) and to the verbal signal (performed/did not perform the behavior requested by the verbal signal). The model included the sender (owner/stranger) as fixed factor and the dogs' ID as a subject variable, to account for the repeated data collected from each dog. Bonferroni correction was applied to results obtained from the two models, to account for the multiple comparisons.

As a further characterization of the dogs' performance, latency measures were collected, defined as the time elapsed between the starting of signals and the expression of the behavior. Latency for the 'Come' command was the time that the dog required to reach the person. The command "Stay" did not have latency measure as it required to maintain a position rather than to perform an action and was not used.

These data were analyzed with a GEE, using latency as a linear dependent variable. Since latencies were not comparable across the different commands (for differences in the inherent time requirements of the requested actions), different models were built for the behavioral response expressed by the dog in each trial. The model included the condition (verbal, gestural, incongruent) and the sender (owner, stranger) as fixed factors, as well as their first level interaction. The dogs' ID was fitted in the model to account for the repeated data collected from each dog. Pairwise contrasts were computed with sequential Bonferroni correction.

6.3 Results

For the 15 dogs that underwent the actual testing phase, the frequency of responses to the different test conditions is reported in Fig. 6.1.

The dogs' performance was nearly perfect when only the gestural signal was provided with only two non-responses. No feature was shared between such two non-responses, since they were expressed by two dogs to different commands (Down-g, Sit-g), provided by the two different senders.

When only the verbal signal was provided, the dogs' performance was slightly worse (Fig. 6.1). When the owner pronounced the command, the Down-v signal resulted in 4 non-responses and the Sit-v in one. Of the non-responses recorded with the stranger, the Down-v signal was again the most frequently one (N = 12), the remaining involving the Come-v (N = 4) and Sit-v (N = 1).

When conflicting gestural and verbal signals were provided, most of the dogs expressed the behavior indicated by the gestural signal. Only in a minority of cases did they respond to the verbal signal. Specifically, they responded to verbal signals when the stranger provided the Come-v-Stay-g (N = 3) and the Stay-v-Come-g (N = 2) signal combinations; when the signals were provided by the owner, dogs responded to the verbal signal in the following combination: Come-v-Stay-g (N = 8), Down-v-Sit-g (N = 3), Stay-v-Come-g (N = 3), and Sit-v-Down-g (N = 1). In addition, non-responses were recorded when the owner issued the following combinations of signals: Sit-v-Down-g (N = 3), Stay-v-Come-g (N = 2) and the Down-v-Sit-g (N = 1).

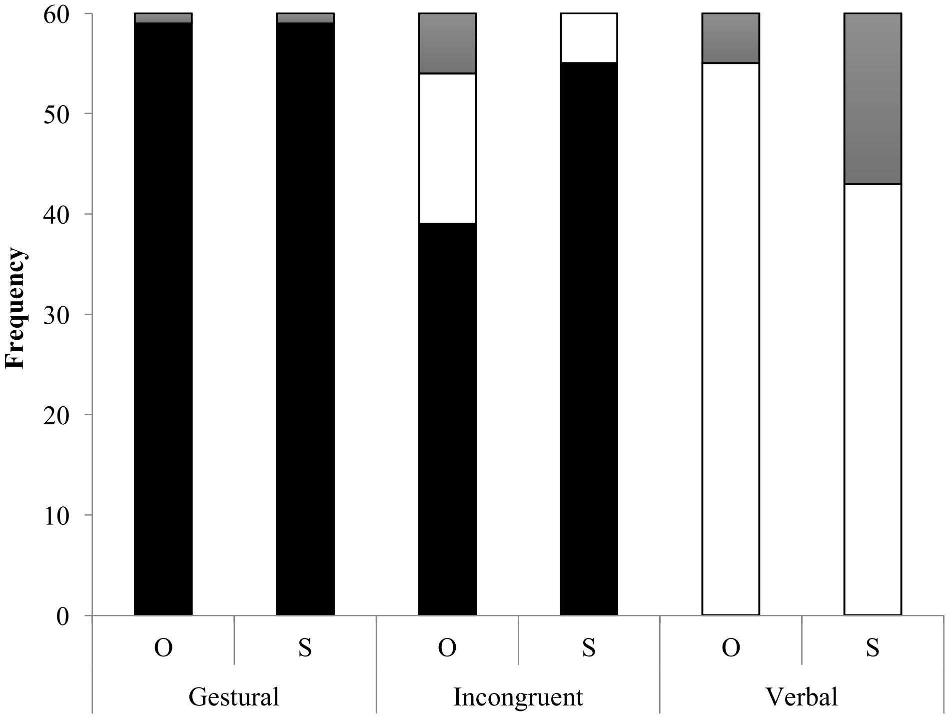

Fig 6.1 Frequency of dogs' responses when presented with gestural, verbal, or incongruent signals by their owner (O) or by a stranger (S). Black boxes: gestural commands were performed; white boxes: verbal commands were performed; grey boxes: no responses.

Results of the GEE model indicating the effect of factors on the probability that dogs expressed any of the behaviors requested are reported in Table 6.1. There was a significant effect of the sender identity*condition interaction, which was due to a lower probability that dogs expressed the requested behavior when verbal signals were provided by the stranger, than in any other sender*condition combination (Bonferroni adjusted $P < 0.001$ in all cases; $P < 0.05$ for all other comparisons).

Table 6.1 Results of the General Estimation Equation model indicating the effect of factors on the probability of dogs to express the requested behavior; in the case of incongruent signals the behavior to be expressed was the one indicated by the gestural signal. DF = Degrees of freedom.

Factor	**Wald Chi-Square**	***df***	***P***
Sender identity	0.02	1	0.88
Condition	19.18	2	0.000
Sender identity*Condition	21.57	2	0.000

In the incongruent condition, the probability of the dogs expressing the behavior indicated by the verbal signal was lower for signals given by the stranger compared to signals given by the owner. In contrast, the probability of the dogs expressing the behavior indicated by the gestural signal was higher in the case of signals given by the stranger compared to the signals given by the owner (Table 6.2).

Table 6.2 Results of the General Estimation Equation model indicating the effect of sender identity (owner vs. stranger) on the probability of dogs to express the behavior requested by gestural and by verbal signals. DF = Degrees of freedom; C.I. = confidence interval limit.

Signal type	Wald Chi-Square	*df*	Lower C.I.	Upper C.I.	*P*
Verbal	5.183	1	0.18	2.42	0.023
Gestural	155.5	1	-3.0	-0.5	0.008

Mean ± SD latencies to perform the "Come", "Down" and "Sit" behaviors can be seen in Fig. 6.2 and the results of the GEE indicating the effect of factors on the dogs' response latency are reported in Table 6.3. In general, longer latencies were observed when the stranger provided verbal signals, than when the stranger gave gestural or incongruent signals. In addition, the response latency to verbal stimuli given by the stranger was longer than when given by the owner for the "Come" ($P = 0.002$) and "Sit" ($P < 0.001$) actions.

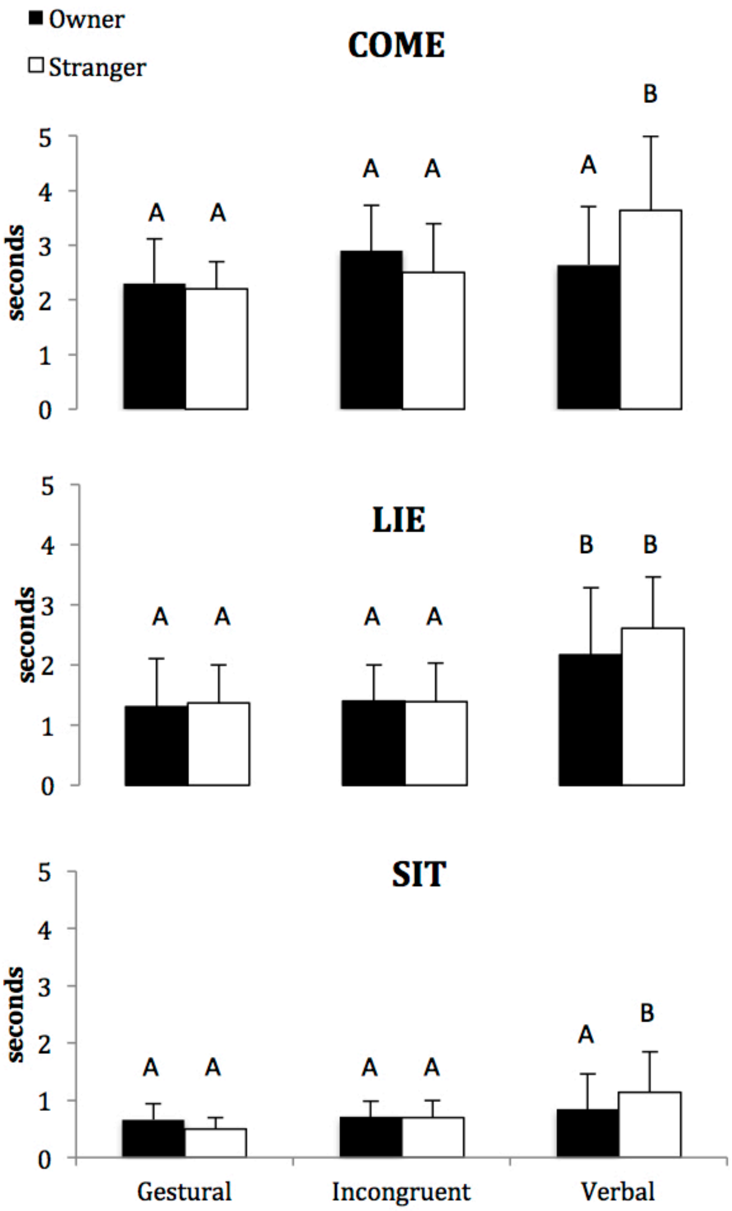

Fig 6.2 Mean ± SD of the dogs latency to express the “Come”, “Down” and “Sit” behaviors after being presented with verbal, gestural and incongruent stimuli by the owner (black boxes) and the stranger (white boxes). Different capital letters indicate significantly different mean latencies within each behavior (*P* < 0.05, pairwise comparisons with sequential Bonferroni correction after Generalized Estimation Equations model).

Table 6.3 Results of the General Estimation Equation model indicating the effect of factors on the dogs' latency to perform the "Come", "Sit" and "Down" behaviors. DF = Degrees of freedom.

Behavior	**Factor**	**Wald Chi-Square**	***df***	***P***
Come	Sender identity	2.28	1	0.131
	Condition	14.46	2	0.001
	Sender identity*Condition	12.36	2	0.002
Sit	Sender identity	0.71	1	0.400
	Condition	11.48	2	0.003
	Sender identity*Condition	18.62	2	0.000
Down	Sender identity	0.90	1	0.342
	Condition	43.72	2	0.000
	Sender identity*Condition	3.02	2	0.221

6.4 Discussion

In the current study, we selected water rescue dogs that were able to respond equally well to both owners and a stranger in the congruent condition. We assessed how the dogs assign significance to gestural compared to verbal signals when instructed to perform an action by their owners or an unfamiliar person. In the gestural condition, we found that dogs responded equally well to both their owners and a stranger. In the verbal condition, dogs performed worse with the stranger. In the incongruent condition, verbal signals of the owners were more effective, while gestural signals were preferred when the stranger was the signaller. Furthermore, dogs performed the requested behaviors less promptly if the stranger used a verbal signal compared to the corresponding gestural one. Dogs reacted similarly to their owner's and the stranger's gestural communication but manifested differential behavior in the case of verbal signals provided by the owner and the stranger.

Our working hypothesis, predicting a deterioration of dog's responses to a stranger's verbal signals was confirmed. In contrast, contrary to our expectations, owners' and stranger's gestural signals elicited very similar responses from the dogs.

The results in the verbal condition are in agreement with those of a previous study showing that, in the absence of gestures, dogs' responses are worse for the commands "Sit" and "Down" when they are provided by a stranger compared to the owner (Coutellier, 2006). Furthermore, our data in the gestural condition are in line with a study that compared responses of dogs to human pointing by owners and strangers, and found no differences between them in the case of gestural signals (Miklósi et al., 1998). However, our findings contrast strikingly with the results reported by Cook et al. (2014) showing that, in absence of a verbal indication, dogs followed gestural signals more when these were provided by an owner rather than a stranger. Particularly, dogs chose more often the container pointed by their owner rather than a stranger, even when this container never yielded a food reward. This incongruence could be due to the different experimental settings: while in Miklósi et al. (1998) the signallers provided the visual message to dogs at different times, Cook et al. (2014) used a "two pointer paradigm", in which both signallers (i.e. stranger and owner) simultaneously pointed at two different targets. Since the performance of our dogs in the gestural condition, as well as that of Miklósi et al. (1998) were very high, it is possible that a ceiling effect occurred, which could have masked possible behavioral differences between owner and stranger's gestural signals. Nevertheless, it should be pointed out that for the "Come" and "Sit" commands, the latency of our dogs in responding to the verbal stimuli was longer when the stranger issued these commands, whereas there were no performance differences to the gestural commands given by owners and strangers. All together our results suggest that for dogs it is more difficult to access the verbal signal of the stranger relative to the gestural one.

Why the verbal signals of strangers are more difficult to manage than those of owners is hard to say from our data. In an associative learning context, dogs join specific signals, such as a word or a gesture, to a given behavior. A gestural signal can be more easily imitated by a stranger who can reproduce a signal similar to the one performed by the owner. In contrast, a verbal command has properties that cannot be properly replicated. Indeed, individual's specific voice characteristics can differ in vocalization length, rate of production, pitch, tone and harmonics and they vary within the same individual with age and emotional status. All these features can affect the auditory characteristics of the verbal signals and the likelihood to replicate them. Moreover, dogs could make a connection between their owner's voice and face image (Adachi et al., 2007). Indeed, the sensitivity of dogs' audio-processing of identity-related acoustic cues was highlighted in a study (Molnár et al., 2009), where it was found that dogs can differentiate between other dogs based solely on their barks. Thus, a verbal command provided by a stranger could appear to dogs to be quite different from what they have learned from their owner, require longer processing time and have less potential to elicit the requested response in a dog. This could explain why our dogs showed lower (and slower) performances when a stranger gave a vocal command. The same explanation can be applied to the incongruent condition, with dogs showing a clear preference for gestural signals of the stranger, suggesting that in a difficult situation such as the incongruent one the dogs relied on the message that was easier to recognize.

Our results could be very useful in the applied field of working dogs, especially when the dogs are trained by a person different from that they have to work with, such as guide dogs. In this case, the dogs have to rely especially on the verbal communication, since they cannot easily receive a gestural communication from their visually impaired owner (e.g. visually impaired people cannot be aware when a dog is focused to them). Thus, trainers should focus on optimizing the generalization of verbal commands to help communication between guide dogs and future owners. To this end, dogs should be trained to work with multiple handlers, so that they can become accustomed to differences in verbal commands. However, visually impaired dog handlers should be taught verbal as well as gestural commands, that they can use after calling the dog's attention verbally.

We are not proposing an extreme view, namely that verbal signals are not in themselves a method of communication, but only a means to attract attention to the given gestural signals (see Shepherd, 2002). However, we are suggesting that gestural communication is likely to be more adaptive for certain types of working dogs. In this context, our results with water rescue dogs demonstrate that the gestures of the owner and the stranger are both equally effective in eliciting a dog’s response. In contrast, verbal signals are more difficult to generalize, thus confirming that visual communication with humans is a very flexible channel for dogs (Beaver, 1999). Comparably, human studies that use incongruent paradigms consistently showed that gesture-speech congruence leads to superior performance compared to gesture-speech incongruence, demonstrated in faster processing and fewer errors (e.g. Langton et al., 1996; Cassell et al., 1999; Langton and Bruce, 2000; Kelly et al., 2007, 2010; Chieffi et al., 2009; Galati and Samuel, 2011).

6.5 References

Adachi, I., Kuwahata, H., & Fujita, K. (2007). Dogs recall their owner's face upon hearing the owner's voice. *Animal cognition*, *10*(1), 17-21.

Agnetta, B., Hare, B., & Tomasello, M. (2000). Cues to food location that domestic dogs (*Canis familiaris*) of different ages do and do not use. *Animal cognition*, *3*(2), 107-112.

Beaver, B.V. (1999). Canine behavior: a guide for veterinarians. Philadelphia, Sanders.

Cassell, J., McNeill, D., & McCullough, K. E. (1999). Speech-gesture mismatches: Evidence for one underlying representation of linguistic and nonlinguistic information. *Pragmatics & cognition*, *7*(1), 1-34.

Chieffi, S., Secchi, C., & Gentilucci, M. (2009). Deictic word and gesture production: their interaction. *Behavioral brain research*, *203*(2), 200-206.

Cook, A., Arter, J., & Jacobs, L. F. (2014). My owner, right or wrong: the effect of familiarity on the domestic dog’s behavior in a food-choice task. *Animal cognition*, *17*(2), 461-470.

Coutellier, L. (2006). Are dogs able to recognize their handler's voice? A preliminary study. *Anthrozoös*, *19*(3), 278-284.

D’Aniello, B., Scandurra, A., Alterisio, A., Valsecchi, P., & Prato-Previde, E. (2016). The importance of gestural communication: a study of human–dog communication using incongruent information. *Animal cognition*, *19*(6), 1231-1235.

D’Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

Fukuzawa, M., Mills, D. S., & Cooper, J. J. (2005). More than just a word: non-semantic command variables affect obedience in the domestic dog (*Canis familiaris*). *Applied Animal Behavior Science*, *91*(1-2), 129-141.

Galati, A., & Samuel, A. G. (2011). The role of speech-gesture congruency and delay in remembering action events. *Language and Cognitive Processes*, *26*(3), 406-436.

Grassmann, S., Kaminski, J., & Tomasello, M. (2012). How two word-trained dogs integrate pointing and naming. *Animal Cognition*, *15*(4), 657-665.

Grassmann, S., & Tomasello, M. (2010). Young children follow pointing over words in interpreting acts of reference. *Developmental Science*, *13*(1), 252-263.

Gibson, J. M., Scavelli, S. A., Udell, C. J., & Udell, M. A. R. (2014). Domestic dogs (*Canis lupus familiaris*) are sensitive to the “human” qualities of vocal commands. *Animal Behavior and Cognition*, *1*(3), 281.

Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, *298*(5598), 1634-1636.

Hare, B., Call, J., & Tomasello, M. (1998). Communication of food location between human and dog (*Canis familiaris*). *Evolution of communication*, *2*(1), 137-159.

Hare, B., & Tomasello, M. (2005). Human-like social skills in dogs? *Trends in cognitive sciences*, *9*(9), 439-444.

Hare, B., & Tomasello, M. (1999). Domestic dogs (*Canis familiaris*) use human and conspecific social cues to locate hidden food. *Journal of Comparative Psychology*, *113*(2), 173.

Horn, L., Range, F., & Huber, L. (2013). Dogs' attention towards humans depends on their relationship, not only on social familiarity. *Animal cognition*, *16*(3), 435-443.

Hostetter, A. B. (2011). When do gestures communicate? A meta-analysis. *Psychological bulletin*, *137*(2), 297.

Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for" fast mapping". *Science*, *304*(5677), 1682-1683.

Kelly, S. D., Özyürek, A., & Maris, E. (2010). Two sides of the same coin: Speech and gesture mutually interact to enhance comprehension. *Psychological Science*, *21*(2), 260-267.

Kelly, S. D., Ward, S., Creigh, P., & Bartolotti, J. (2007). An intentional stance modulates the integration of gesture and speech during comprehension. *Brain and language*, *101*(3), 222-233.

Kerepesi, A., Dóka, A., & Miklósi, Á. (2015). Dogs and their human companions: the effect of familiarity on dog–human interactions. *Behavioral processes*, *110*, 27-36.

Lakatos, G., Gácsi, M., Topál, J., & Miklósi, Á. (2012). Comprehension and utilisation of pointing gestures and gazing in dog–human communication in relatively complex situations. *Animal cognition*, *15*(2), 201-213.

Lakatos, G., Soproni, K., Dóka, A., & Miklósi, Á. (2009). A comparative approach to dogs' (*Canis familiaris*) and human infants' comprehension of various forms of pointing gestures. *Animal cognition*, *12*(4), 621-631.

Langton, S. R., & Bruce, V. (2000). You must see the point: Automatic processing of cues to the direction of social attention. *Journal of Experimental Psychology: Human Perception and Performance*, *26*(2), 747.

Langton, S. R., O'Malley, C., & Bruce, V. (1996). Actions speak no louder than words: Symmetrical cross-modal interference effects in the processing of verbal and gestural information. *Journal of Experimental Psychology: Human Perception and Performance*, *22*(6), 1357.

Marshall-Pescini, S., Passalacqua, C., Barnard, S., Valsecchi, P., & Prato-Previde, E. (2009). Agility and search and rescue training differently affects pet dogs' behavior in socio-cognitive tasks. *Behavioral processes*, *81*(3), 416-422.

Miklósi, Á., Polgárdi, R., Topál, J., & Csányi, V. (1998). Use of experimenter-given cues in dogs. *Animal cognition*, *1*(2), 113-121.

Miklósi, Á., Pongrácz, P., Lakatos, G., Topál, J., & Csányi, V. (2005). A comparative study of the use of visual communicative signals in interactions between dogs (*Canis familiaris*) and humans and cats (*Felis catus*) and humans. *Journal of comparative psychology*, *119*(2), 179.

Miklósi, Á., & Soproni, K. (2006). A comparative analysis of animals' understanding of the human pointing gesture. *Animal cognition*, *9*(2), 81-93.

Mills, D. S. (2005). What's in a word? A review of the attributes of a command affecting the performance of pet dogs. *Anthrozoös*, *18*(3), 208-221.

Molnár, C., Pongrácz, P., Faragó, T., Dóka, A., & Miklósi, Á. (2009). Dogs discriminate between barks: the effect of context and identity of the caller. *Behavioral processes*, *82*(2), 198-201.

Mongillo, P., Bono, G., Regolin, L., & Marinelli, L. (2010). Selective attention to humans in companion dogs, *Canis familiaris*. *Animal Behavior*, *80*(6), 1057-1063.

Özyürek, A., Willems, R. M., Kita, S., & Hagoort, P. (2007). On-line integration of semantic information from speech and gesture: Insights from event-related brain potentials. *Journal of cognitive neuroscience*, *19*(4), 605-616.

Pilley, J. W., & Reid, A. K. (2011). Border collie comprehends object names as verbal referents. *Behavioral processes*, *86*(2), 184-195.

Pongrácz, P., Miklósi, Á., Dóka, A., & Csányi, V. (2003). Successful application of video-projected human images for signalling to dogs. *Ethology*, *109*(10), 809-821.

Previde, E. P., Valsecchi, P. (2014). The immaterial cord: the dog-human attachment bond. In: *The Social Dog: Behavior and Cognition* (pp. 165-89).

Rossano, F., Nitzschner, M., & Tomasello, M. (2014). Domestic dogs and puppies can use human voice direction referentially. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1785), 20133201.

Scandurra, A., Alterisio, A., & D'Aniello, B. (2016). Behavioral effects of training on water rescue dogs in the Strange Situation Test. *Applied Animal Behavior Science*, *174*, 121-127.

Shepherd, K. (2002). Development of behavior, social behavior and communication in dogs. *BSAVA manual of canine and feline behavioral medicine*, 8-20.

Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2002). Dogs' (*Canis familaris*) responsiveness to human pointing gestures. *Journal of comparative psychology*, *116*(1), 27.

Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2001). Comprehension of human communicative signs in pet dogs (*Canis familiaris*). *Journal of comparative psychology*, *115*(2), 122.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2008a). Wolves outperform dogs in following human social cues. *Animal Behavior*, *76*(6), 1767-1773.

Udell, M. A., Giglio, R. F., & Wynne, C. D. (2008b). Domestic dogs (*Canis familiaris*) use human gestures but not nonhuman tokens to find hidden food. *Journal of Comparative Psychology*, *122*(1), 84.

Virányi, Z., Topál, J., Gácsi, M., Miklósi, Á., & Csányi, V. (2004). Dogs respond appropriately to cues of humans' attentional focus. *Behavioral processes*, *66*(2), 161-172.

Willems, R. M., & Hagoort, P. (2007). Neural evidence for the interplay between language, gesture, and action: A review. *Brain and language*, *101*(3), 278-289.

Willems, R. M., Özyürek, A., & Hagoort, P. (2006). When language meets action: The neural integration of gesture and speech. *Cerebral Cortex*, *17*(10), 2322-2333.

CHAPTER 7

SHOULD I FETCH ONE OR THE OTHER? A STUDY ON DOGS ON THE OBJECT CHOICE IN THE BIMODAL CONTRASTING PARADIGM

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Abstract

The present study assessed how dogs weigh gestural versus verbal information communicated to them by humans in transitive actions. The dogs were trained by their owners to fetch an object under three conditions: a bimodal congruent condition characterized by using gestures and voices simultaneously; a unimodal gestural condition characterized by using only gestures; and a unimodal verbal condition characterized by using only voices. An additional condition, defined as a bimodal incongruent condition was later added, in which the gesture contrasted with the verbal command, that is, the owner indicated an object while pronouncing the name of the other object visible to dogs. In the incongruent condition, seven out of nine dogs choose to follow the gestural indication and performed above chance, two were at chance, whereas none of the dogs followed the verbal cues above chance. The dogs, as a group, performed above chance the gestural command in 73.6% of cases. The analysis of latencies in the above-mentioned four conditions exhibited significant differences. The unimodal verbal and the gestural conditions recorded a slower performance than both the bimodal incongruent and congruent conditions. No statistical differences were observed between the unimodal and bimodal conditions. Our results demonstrate that dogs, trained to respond equally well to gestural and verbal commands, choose to follow the indication provided by the gestural command than the verbal one to a significant extent in transitive actions. Furthermore, the responses to bimodal conditions were found to be quicker than the unimodal ones.

Keywords: gestural cue, bimodal communication, contrasting paradigm, transitive actions, verbal cue, unimodal communication

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7.1 Introduction

In humans, the bimodal communication is defined as a phenomenon manifested by coupling of spoken language with gestures to communicate a particular message (Messing, 1994). The experiments in children who undertook an object-choice task demonstrated that they relied most heavily on the pointing gestures compared to words when these two sources of information conflicted, indicating the importance of visual cues in the communication transfer (Grassmann and Tomasello, 2010). With respect to interspecies interactions, humans rely on bimodal communication and utilize both gestures and words when interacting with dogs (Miklósi, 2007). On the other side, dogs are well equipped and skilled animals capable of receiving and responding to human cues during the normal social interactions or in working and collaborative contexts (Miklósi, 2007, 2009). They are able to understand and utilize efficiently the information from specific human signs, such as gazing, head-nodding, head turning, and glancing at a target (see Kaminski and Nitzschner, 2013 for a review). Several studies have pointed out on the importance of the ability of dogs to recognize human-given cues and pointing gestures (Hare and Tomasello, 1999; Lakatos et al., 2009, 2012; Miklósi and Soproni, 2006; Soproni et al., 2001, 2002; Udell et al., 2008a,b). On the other hand, dogs actively seek for intervention by and interaction with humans, especially when they encounter some trouble, unexpected situations, or unsolvable tasks (Miklósi et al., 2003; D'Aniello et al., 2015; Prato-Previde and Marshall-Pescini, 2014).

The dogs, once trained, also possess the ability to efficiently understand human verbal communication (Mills, 2005) and can quickly learn the meaning of several different words. For example, two dogs learned to fetch hundreds of objects upon hearing their names (Kaminski et al., 2004; Pilley and Reid, 2011). They also demonstrated a "fast mapping" ability, which is, associating a new word to a new object when asked to fetch it among many other already known objects (Kaminski et al., 2004; Pilley and Reid, 2011). Dogs may also moderate their responses to a vocal input discriminating between live-spoken and recorded sounds (Fukuzawa et al., 2005), indicating their ability to perceive some of the vocal characteristics of human speech (Gibson et al., 2014).

Various studies have investigated the relative relevance of human verbal and gestural messages for dogs in the contrasting paradigm, in which dogs were provided verbal messages indicating an action, while the gestural cues indicated an alternative action. Specifically, in our previous study (D'Aniello et al., 2016), we assessed how dogs weighed gestural versus verbal information when their owner asked to perform an intransitive action (i.e., ‘‘sit,’’ ‘‘lie down,’’ ‘‘stay,’’ ‘‘come’’). The owner provided the dogs a single source of information (gestural and verbal) or incongruent information (i.e., gestural and verbal commands referred to two different actions). In the incongruent condition, the dogs preferred to execute the action required by the gesture and not through the one communicated by words, suggesting that for them, the human gestures were more salient than words. In a follow-up study using the same paradigm, we underlined that for highly trained dogs, gestural signals were less dependent upon the familiarity of the signal-giver, whereas verbal signals were less effective when they were provided by an unfamiliar person (Scandurra et al., 2017). The above-mentioned findings were in sharp contrast with the outcomes obtained in a study dealing with transitive actions (Grassmann et al., 2012). In the latter, the authors tested the dogs in an object-choice task, in which an experimenter asked the dogs to fetch an object by name while pointing and watching to another one. The results demonstrated that the dogs were clearly biased toward the verbal indication (Grassmann et al., 2012). However, it should be pointed out that the two dogs tested by the group were specifically “word-trained,” which could have induced the dogs to choose the object indicated by name rather than by the object being pointed to.

In order to testing and analyzing the ability of dogs in the contrasting paradigm, a suitable protocol to investigate the relative power of gestural and verbal indications should be applied to dogs capable of responding equally well to either verbal or gestural command. With this aim, we specifically trained a group of dogs for object-choice tasks, that is, to fetch some objects by name and pointing gestures, using the cue-isolation procedure (Uetz and Roberts, 2002). In this study, stimuli from one or more channels were presented either in isolation (unimodal presentation) or in combination (bimodal presentation). This protocol further provided information about the preferences of dogs in choosing verbal or gestural messages in the bimodal incongruent condition, thereby allowing to compare the effectiveness of the response by recording the latencies (i.e., the amount of time in which a subject responds to a given stimulus) in all conditions (unimodal and the bimodal presentations).

Because of the conflicting data in literature, it is difficult to make a clear hypothesis. If dogs tend to prefer the verbal indications, it could mean that the processing of cues in transitive and intransitive actions varies, thereby triggering different responses. On the contrary, if dogs choose the gestural signs more frequently, then the processing of the two stimuli by brain produces the same outcome in both transitive and intransitive actions. However, different results obtained in the study by Grassmann et al. (2012) could be attributed to specific word-training that the dogs received during ontogenesis.

In our previous study on intransitive actions, no significant differences could be observed in the latencies of dogs to the commands of the owners in the unimodal and bimodal incongruent conditions, except for the command "lie down." In the latter, the dogs took a longer time to respond to the unimodal verbal presentation than the unimodal gestural and bimodal incongruent presentations (Scandurra et al., 2017). These results indicated that the dogs exhibited a certain difference in the response time to the verbal communication depending on the type of actions requested to them. Thus, in the current study, we compared the response latencies in the transitive actions to verify whether and how the time responses in the unimodal (i.e., verbal and gestural) and the bimodal (i.e., congruent and incongruent) conditions of cue presentation could be affected. According to the intersensory facilitation phenomenon (Colonius and Diederich, 2012), humans respond to bimodal audiovisual compound stimuli faster than the isolated component (Miller, 1982; Glelen et al., 1983). In dogs, one study on the aggressive bimodal communication found a higher value in the latency reaction to the unimodal visual presentation, followed by unimodal audio and bimodal audio-visual presentations. The authors suggested that the acoustic modality may additionally function to increase the detectability of the signal (Déaux et al., 2015). In the light of the above-mentioned studies, we expect that the response latencies of our dogs would be higher in the unimodal presentations compared to the bimodal congruent condition. On the other hand, there are no similar studies under incongruent condition; therefore, we have no suggestions about the latency of dogs' responses in this condition.

The original contribution of this work is to test how and with what latency the dogs, specifically trained for the study, respond to their owner's commands in transitive actions, in relationship to the unimodal or bimodal nature of communication.

7.2 Materials and methods

7.2.1 Participants

Six male and seven female dogs of different breeds (mean age + SD = 4.0 + 0.5 years) and their owners were enrolled in the study. All subjects were recruited at the training center, “La Voce del Cane” (Naples, Italy) where the owners followed a course to become the dog trainers. The dogs did not receive previous training.

7.2.2 Procedure

Training phase: The owners were asked to train their dogs to fetch three objects by name and gestures. These objects included a piece of wood, a half-liter plastic bottle, and a pencil case; these were associated with the Italian words, “legno,” “acqua,” and “borsa,” respectively. The words were chosen as they consisted of the same number of letters, and could be pronounced at the same time to the presentation of the pointing gesture (i.e. distal and momentary pointing). The training was provided at home according to the owner daily opportunity; during the weekend, it was also carried out in the room predisposed for the test to accustom the dogs with the place. The training was performed with the help of a clicker device and food.

The dogs were trained to respond to commands under following three conditions:

1. *Bimodal congruent condition*: The owner directed the dogs to one of the two objects with the combined use of voice and gesture, thereby coupling the name of the object with the pointing gesture. It was requested to give the verbal and the gestural cues at the same time. The pointing gesture was provided after calling the attention of dogs.
2. *Unimodal gestural condition:* The owner directed the dogs to one of the two objects with the use of only gesture. The procedure was the same as for the congruent condition, but

the name of the object was not stated.

3. *Unimodal verbal condition*: The owner directed the dogs to one of the two objects using only voice. The owners took a neutral position with arms extended along the body and the head directed forward without moving the head or looking at the dogs and objects.

The experimental protocol included periodic checks by researchers to verify the progress of training of dogs. After three months from the beginning of the training, the dogs were subjected to the pre-test phase.

Pre-test phase: The pre-tests were conducted indoors at the training center, in a large room known to dogs, and involved three people: the owner giving the commands, one researcher who positioned the objects, and another researcher who handled the video cameras, took notes, and randomly assigned trials to the owner. In each trial, the experimental setting consisted of two of the three objects that were placed randomly along a horizontal axis at 2 m from each other. The dog and the owner were positioned at 2.5 m away, paying attention to position the dog equidistant from the two objects (Fig. 7.1). The owner was requested to give the commands to dogs in three different conditions described above (see "*training phase"* section). The choice was considered correct when the dog fetched the requested object and incorrect when the dog fetched the wrong object. Only one repetition was allowed in case the owner was unable to restrain their body gestures in the verbal condition or if the dog refused the command wandering into the room.

A total of eight trials, for each condition, organized in a completely random manner, were performed leading to a total of 24 trials. The position of the two objects in the experimental setting was changed at least two times in a session of 8 trials randomly (i.e. without a precise schedule). After every eight consecutive trials, the test was stopped for around 10 minutes, during which the dogs were allowed to relax in an open space outside the testing room. Meanwhile, one of the two objects was substituted in the experimental room. The dogs were request to respond correctly to at least six out of the eight trials for each condition. Of the 13 dogs tested, nine met the requirements and were admitted to the testing phase: four males and five females (mean age + SD: 4.0 + 0.6 years; Table 7.1).

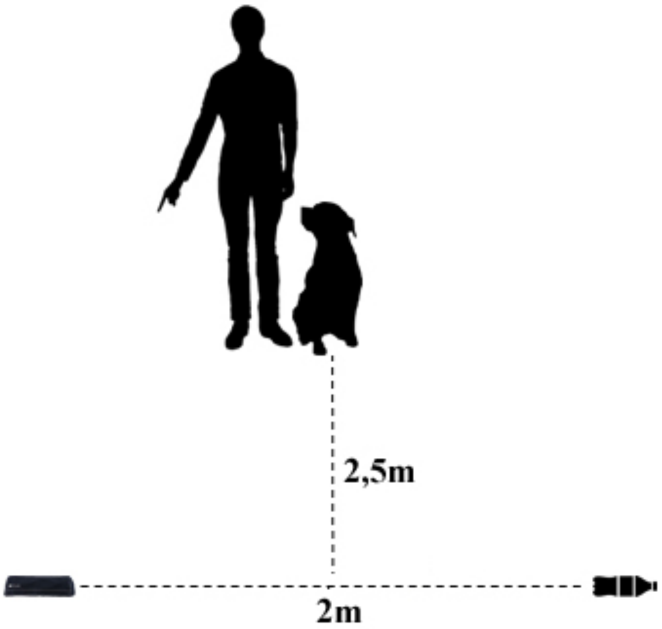

Fig 7.1 Experimental setting.

Test phase: The test phase was held after a week from the pretest phase, during which the owner continued the training. It was identical to the pre-test, but another condition was added, leading to a total of randomly proposed 32 trials.

Bimodal incongruent condition: The owner directed the dogs to one of the two objects by simultaneously utilizing voice and gesture in the same way as the bimodal congruent condition. However, in this case, the owner pointed at one object and named the other.

All tests were video recorded using a Sony Handycam HDR-PJ260VE placed in a corner behind the owner and a QUMOX SJ4000 placed in front of the dogs at 3 m.

Table 1 Dogs involved in the Test phase.

Name	Sex	Age (years)	Breed
Micla	F	4	Mixed breed
Negrita	F	9	Mixed breed
Thelma	F	4	Dachshund
Colì	M	2	Mixed breed
Yaki	M	4	Jack Russell Terrier
Lucky	M	4	Mixed breed
Matisse	M	4	Labrador Retriever
Way	F	3	Mixed breed
Reginella	F	3	Pointer

7.2.3 Data scoring and analysis

For the analysis of the frequencies of responses in the bimodal incongruent condition, only the test phase was considered, evaluating whether the dog responded to the verbal or gestural message. An analysis was first conducted at the individual level to check if each dog followed the gestural commands at above-chance levels. Since the Kolmogorov–Smirnov test revealed that the data were not normally distributed, one-sample Wilcoxon signed-rank test was employed. The frequency of responses was scored, in which score 1 was used for the gestural choice and 0 for the verbal choice. Pairwise Wilcoxon signed-rank test was applied for the analysis at the group level as well.

To evaluate the speed of the responses, the latencies in all four conditions (i.e., bimodal congruent and incongruent, unimodal gestural and verbal) were recorded. The latencies were registered from the end of the owner's cue until the choice of the dog. Inter-observer agreement performed on 3 of the 9 dogs tested, showed no disagreement on frequency (100% agreement). For the latency, the agreement was highly significant for all conditions (bimodal congruent: $r_s = 0.98$, $P < 0.001$; unimodal gestural: $r_s = 0.97$, $P < 0.001$; unimodal verbal: $r_s = 0.99$, $P < 0.001$; bimodal incongruent: $r_s = 0.98$, $P < 0.001$). Since the data were not normally distributed, the statistical comparison was performed applying Friedman's test, followed by a post hoc Wilcoxon signed-rank test with Bonferroni correction.

To verify eventual differences in the latencies of the verbal and gestural choices in the bimodal incongruent condition, the percentile bootstrap confidence intervals of the mean of latencies were calculated, using 1000 bootstrap replications (Wilcox and Keselman, 2003). All analyses were performed using SPSS Statistics 22 and R (version 3.3.3).

7.3 Results

The results of one-sample Wilcoxon signed-rank test for individual dogs in choosing the gestural rather than the verbal commands during the bimodal incongruent condition are reported in Table 7.2.

Table 7.2 Wilcoxon signed-rank test results for the gestural choice during the bimodal incongruent condition.

Dog	Total Trials	Gestural Choice	Observe Value (%)	P-value
Micla	8	7	88	0.006
Negrita	8	5	63	0.048
Thelma	8	3	38	0.359
Colì	8	7	88	0.006
Yaki	8	8	100	0.003
Lucky	8	7	88	0.006
Matisse	8	6	75	0.017
Way	8	3	38	0.359
Reginella	8	7	88	0.006

Seven out of the nine dogs significantly followed the pointing gesture rather than the verbal indications against chance performance. The other two dogs responded similar performance to the chance level. None of the dogs were biased toward the verbal indication in our group.

Overall, at the group level, dogs preferred to follow the gestural rather than the verbal commands during the bimodal incongruent condition ($W = 1.431$, $P < 0.001$; Fig. 7.2).

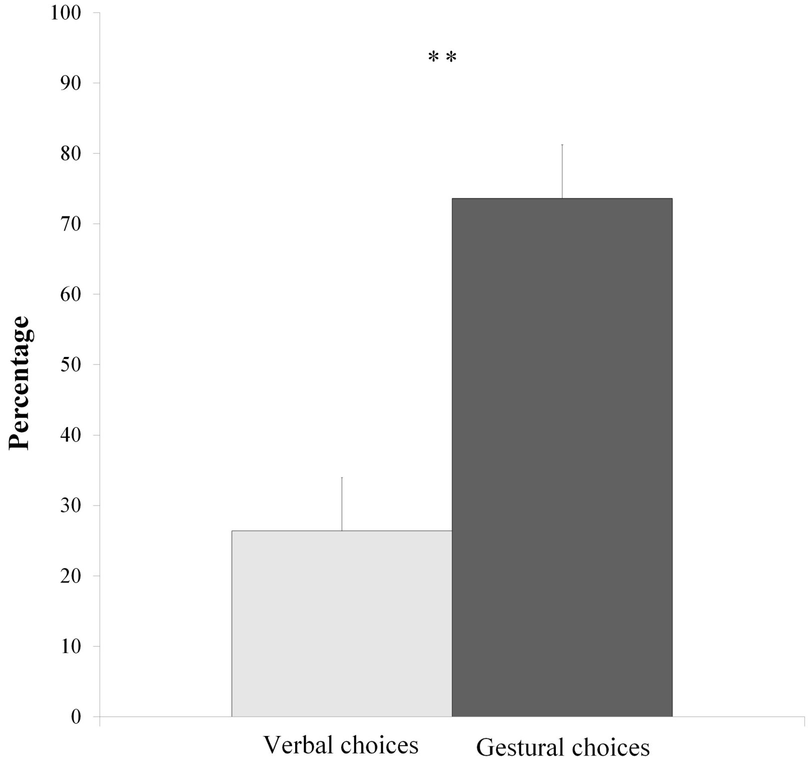

Fig 7.2 The choice of dogs as a group during the bimodal incongruent condition, expressed as a percentage. Pairwise Wilcoxon signed-rank test was applied. ***$P < 0.001$.

The analysis of latencies demonstrated statistical differences under four conditions ($\chi^2 = 23.23$, $df = 3$, $P < 0.001$; Fig. 7.3). The Wilcoxon signed-rank post hoc test showed that dogs reacted with lower latencies toward visual and gestural cues (bimodal congruent condition) than toward the unimodal verbal ($W = 1.723$, $P < 0.001$) and the gestural ($W = 1.668$, $P = 0.015$) conditions. Furthermore, the latencies of dogs were lower in the bimodal incongruent conditions than the unimodal verbal ($W = 1.489$, $P < 0.001$) and gestural ($W = 1.322$, $P = 0.006$) conditions. No statistical differences were detected between the bimodal congruent and the incongruent conditions ($W = 1.104$, $P = 1$), as well as between the unimodal verbal and unimodal gestural conditions ($W = 1.239$, $P = 1$).

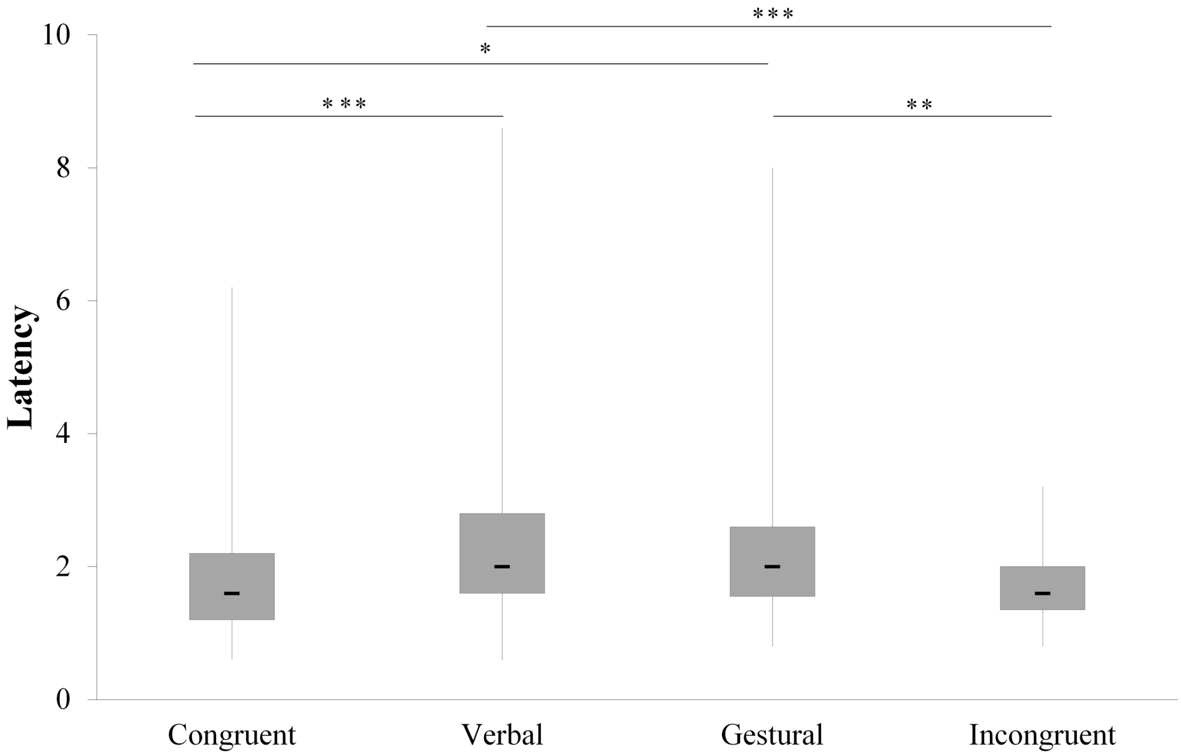

Fig 7.3 The latency of responses in the four different conditions. Friedman's test was followed by a post hoc Wilcoxon signed-rank test with Bonferroni correction. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

In the bimodal incongruent condition, the confidence intervals of the verbal and the gestural choices largely overlapped, owing to the fact that dogs weighed gestural and verbal communication similarly and answered with similar latencies, independently of the command they preferred to follow (Table 7.3, Fig. 7.4).

Table 7.3 Percentile bootstrap confidence intervals of the mean of latencies.

	Original sample mean	**Bootstrap mean**	**Bootstrap SD**	**95% Percentile confidence interval**
Gestural	1.75	1.75	0.07	1.63 – 1.86
Verbal	1.74	1.74	0.09	1.59 – 1.88

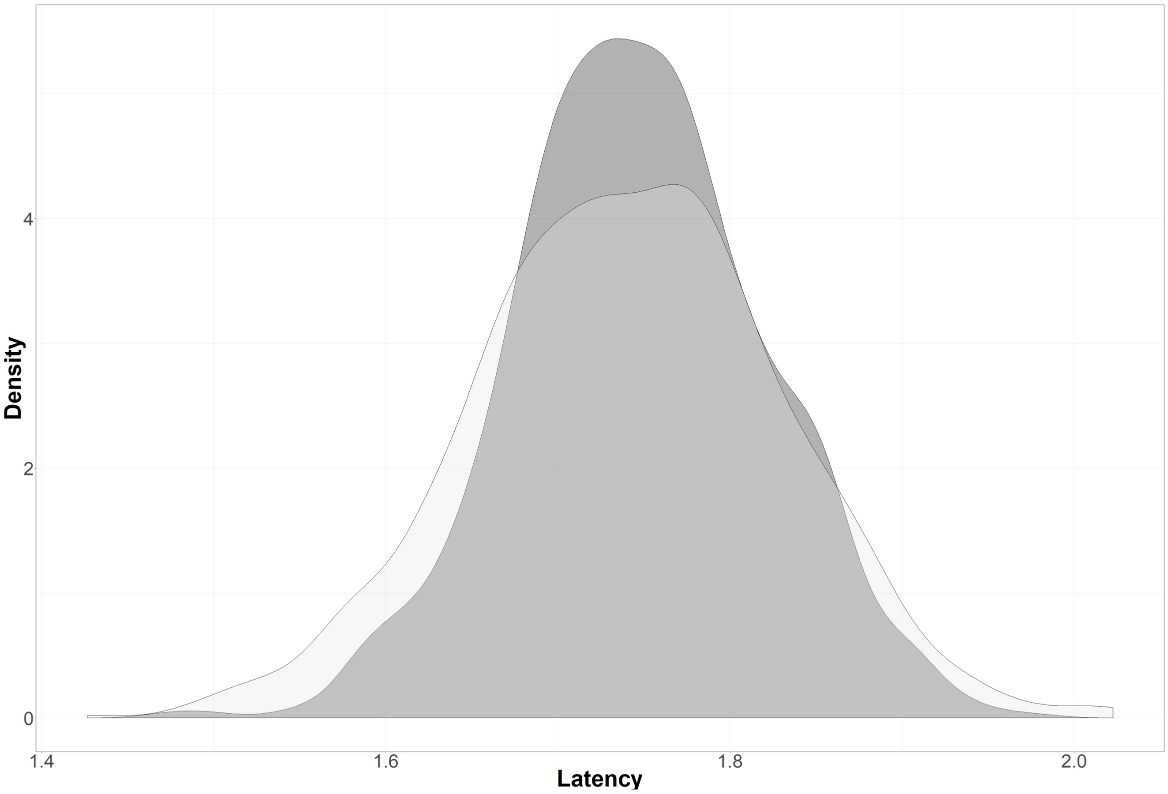

Fig 7.4 The densities of the latencies in the verbal (light gray) and gestural (dark gray) choices. The overlapping area is shown in intermediate gray.

7.4 Discussion

In the current study, we evaluated how dogs rate gestural versus verbal information in transitive actions through their response to object-choice task. Dogs were asked to react to four different conditions, namely, bimodal congruent, unimodal gestural, unimodal verbal, and bimodal incongruent both at individual and group levels. In the bimodal incongruent condition, seven out of nine dogs significantly followed the pointing gestures of their owners rather than the verbal indications when asked by their owners to fetch an object. The performance of other two dogs was at the chance level and thus none of the dogs displayed any biasness toward verbal indication in our group. Furthermore, at the level of the group, the dogs clearly demonstrated a preference toward following gestures than words in the fetching game. Previous data on assessment of transitive actions by dogs have demonstrated that the dogs responded to a verbal aspect of the communication better than the pointing and gazing gestures (Grassmann et al., 2012). Beyond the differences of the experimental settings (e.g.

the distance of the cue-giver from position of the dog), it's important to underline that the dogs tested in the above-mentioned study had a different ontogenetic background compared to the dogs tested in the present study. In particular, the two dogs tested by Grassmann et al. (2012) underwent a specific word-based training, and owners were not provided any kind of gesture-based cues during the training. This procedure might have resulted in dogs who obey only the verbal commands, as the authors themselves underlined. On the other hand, the results of their study appear to reflect that the gestural indication was not irrelevant at all since the dogs approached and fetched the object pointed-to them before fetching the one that was pointed to them through verbal communication. This observation may indicate that these dogs failed to learn that the pointing gesture meant fetching an object, which they might always consider only as a directional signal (Grassmann et al., 2012; however, also see Kaminski, 2008; Topál et al., 2009; Scheider et al., 2011; Wobber and Kaminski, 2011). Thus, the contrary results we obtained in our study underline the importance of the ontogenetic processes in shaping the behavioral responses of dogs (Scandurra et al., 2015; D'Aniello and Scandurra, 2016; D'Aniello et al., 2017).

Our data on the latencies of responses depicted that the bimodal congruent and the incongruent conditions resulted in same response time. In addition, both the conditions provided faster responses when compared to the unimodal verbal and the unimodal gestural conditions. This is an interesting outcome implying that in a unimodal communication, verbal and gestural commands are less effective in terms of response speed. However, the utilization of both verbal and gestural cues would help in the efficient socialization of dogs with the humans. These results are in line with the literature in demonstrating that multisensory integration can reduce the time interval between sensory encoding and motor control, and therefore the response time as depicted in monkeys (Bell et al., 2005). This phenomenon results in a significantly shorter latency in the multimodal mode as compared to the two unisensorial responses that compose it (Rowland et al., 2007).

In the context of communication, although visual and acoustic signals could be redundant (Déaux et al., 2015), multiple redundant signal components may be beneficial for the animals, providing insurance that the message would be transmitted smoothly despite the presence of environmental noise (backup signals) and increase the likelihood of detecting and identifying events or objects of interest without errors and confusion (Partan and Marler, 1999, 2005; Stein and Stanford, 2008). Indeed, when signals containing both verbal and pointed gestures are provided together, they enhance the responses in many cases, as shown in humans (Partan and Marler, 1999) and in spiders (Uetz et al., 2009). Furthermore, the multisensory integration is crucial for rapid responses to dynamic events within the environment (Calvert et al., 2004). Thus, despite the prevalence of identification of gestural responses in our data, the verbal inputs were also significant in triggering a quicker response, regardless of the mode of communication (congruent or incongruent). Another noteworthy observation was that the gestural and the verbal responses in the bimodal incongruent condition displayed the same confidence intervals, suggesting that the latencies of the verbal and gestural choices of dogs were similar. In our previous research in intransitive actions, we recorded a shorter latency in the unimodal gestural condition compared to the verbal one in the command “lie down” (Scandurra et al., 2017), but no differences were found for the latencies of other commands used (e.g. “sit”, “come”). Therefore, the present findings in transitive actions are partially in line with our previous study (Scandurra et al., 2017). This discrepancy could be due to the type of actions requested to dogs. In conclusion, our data support the observation that when dogs are equally accustomed to responding to gestural and verbal commands, the gestural communication is preferred both in intransitive (D’Aniello et al., 2016; Scandurra et al., 2017) and transitive actions (present data). This indicates that dogs domesticated and trained by humans could be more visually than acoustically focused during their communication with humans. These results have implications in the field of applied training, giving scientific support to the well-established belief that non-verbal signals are more salient than verbal commands for dogs.

The importance of verbal communication becomes undeniable in circumstances where the handler is out of sight and the only communicative channel is the verbal one. Furthermore the verbal cue in the bimodal communication triggers faster responses. Finally, the ontogenetic background can also induce a bias such that dogs choose the verbal mode as the main cue as opposed to gestures (Grassmann et al., 2012).

7.5 References

Bell, A. H., Meredith, M. A., Van Opstal, A. J., & Munoz, D. P. (2005). Crossmodal integration in the primate superior colliculus underlying the preparation and initiation of saccadic eye movements. *Journal of Neurophysiology*, *93*(6), 3659-3673.

Colonius, H., Diederich, A. (2012). Intersensory facilitation. In: Seel, M. L. (Ed.), *Encyclopedia of the Sciences of Learning* (pp. 1635-1638). Springer, US.

D'Aniello, B., & Scandurra, A. (2016). Ontogenetic effects on gazing behavior: a case study of kennel dogs (Labrador Retrievers) in the impossible task paradigm. *Animal cognition*, *19*(3), 565-570.

D'Aniello, B., Alterisio, A., Scandurra, A., Petremolo, E., Iommelli, M. R., & Aria, M. (2017). What's the point? Golden and Labrador retrievers living in kennels do not understand human pointing gestures. *Animal cognition*, *20*(4), 777-787.

D'Aniello, B., Scandurra, A., Alterisio, A., Valsecchi, P., & Prato-Previde, E. (2016). The importance of gestural communication: a study of human–dog communication using incongruent information. *Animal cognition*, *19*(6), 1231-1235.

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

Déaux, É. C., Clarke, J. A., & Charrier, I. (2015). Aggressive bimodal communication in domestic dogs, *Canis familiaris*. *PloS one*, *10*(11), e0142975.

Fukuzawa, M., Mills, D. S., & Cooper, J. J. (2005). More than just a word: non-semantic command variables affect obedience in the domestic dog (*Canis familiaris*). *Applied Animal Behavior Science*, *91*(1-2), 129-141.

Gibson, J. M., Scavelli, S. A., Udell, C. J., & Udell, M. A. R. (2014). Domestic dogs (*Canis lupus familiaris*) are sensitive to the "human" qualities of vocal commands. *Animal Behavior and Cognition*, *1*(3), 281.

Gielen, S. C., Schmidt, R. A., & Van Den Heuvel, P. J. (1983). On the nature of intersensory facilitation of reaction time. *Perception & Psychophysics*, *34*(2), 161-168.

Grassmann, S., & Tomasello, M. (2010). Young children follow pointing over words in interpreting acts of reference. *Developmental Science*, *13*(1), 252-263.

Grassmann, S., Kaminski, J., & Tomasello, M. (2012). How two word-trained dogs integrate pointing and naming. *Animal Cognition*, *15*(4), 657-665.

Hare, B., & Tomasello, M. (1999). Domestic dogs (*Canis familiaris*) use human and conspecific social cues to locate hidden food. *Journal of Comparative Psychology*, *113*(2), 173.

Kaminski, J. (2008). Dogs (*Canis familiaris*) are adapted to receive human communication. In: Berthoz, A., & Christen, Y. (Eds.), *Neurobiology of ''Umwelt'': how living beings perceive the world, research and perspectives in neurosciences.* Springer, Heidelberg.

Kaminski, J., & Nitzschner, M. (2013). Do dogs get the point? A review of dog–human communication ability. *Learning and Motivation*, *44*(4), 294-302.

Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for "fast mapping". *Science*, *304*(5677), 1682-1683.

Lakatos, G., Gácsi, M., Topál, J., & Miklósi, Á. (2012). Comprehension and utilisation of pointing gestures and gazing in dog–human communication in relatively complex situations. *Animal cognition*, *15*(2), 201-213.

Lakatos, G., Soproni, K., Dóka, A., & Miklósi, Á. (2009). A comparative approach to dogs' (*Canis familiaris*) and human infants' comprehension of various forms of pointing gestures. *Animal cognition*, *12*(4), 621-631.

Messing, L. (1994). Bimodal Communication, Signing Skill & Tenseness. *Sign Language Studies*, *84*(1), 209-220.

Miklósi, A. (2009). Evolutionary approach to communication between humans and dogs. *Veterinary research communications*, *33*(1), 53-59.

Miklósi, Á. (2007). Dog behavior, evolution and cognition. Oxford University Press, Oxford.

Miklósi, Á., & Soproni, K. (2006). A comparative analysis of animals' understanding of the human pointing gesture. *Animal cognition*, *9*(2), 81-93.

Miklósi, Á., Kubinyi, E., Topál, J., Gácsi, M., Virányi, Z., & Csányi, V. (2003). A simple reason for a big difference: wolves do not look back at humans, but dogs do. *Current biology*, *13*(9), 763-766.

Miller, J. (1982). Divided attention: Evidence for coactivation with redundant signals. *Cognitive psychology*, *14*(2), 247-279.

Mills, D. S. (2005). What's in a word? A review of the attributes of a command affecting the performance of pet dogs. *Anthrozoös*, *18*(3), 208-221.

Partan, S., & Marler, P. (1999). Communication goes multimodal. *Science*, *283*(5406), 1272-1273.

Partan, S. R., & Marler, P. (2005). Issues in the classification of multimodal communication signals. *The American Naturalist*, *166*(2), 231-245.

Pilley, J. W., & Reid, A. K. (2011). Border collie comprehends object names as verbal referents. *Behavioral processes*, *86*(2), 184-195.

Prato-Previde, E., Marshall-Pescini, S. (2014). Social looking in the domestic dog. In: Horowitz, A. (Ed.), *Domestic Dog Cognition and Behavior* (pp. 101-131). Springer-Verlag, BerlinHeidelberg.

Rowland, B. A., Quessy, S., Stanford, T. R., & Stein, B. E. (2007). Multisensory integration shortens physiological response latencies.

Scandurra, A., Alterisio, A., Marinelli, L., Mongillo, P., Semin, G. R., & D'Aniello, B. (2017). Effectiveness of verbal and gestural signals and familiarity with signal-senders on the performance of working dogs. *Applied Animal Behavior Science*, *191*, 78-83.

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D'Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behavior. *Animal cognition*, *18*(4), 937-944.

Scheider, L., Grassmann, S., Kaminski, J., & Tomasello, M. (2011). Domestic dogs use contextual information and tone of voice when following a human pointing gesture. *PLoS One*, *6*(7), e21676.

Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2001). Comprehension of human communicative signs in pet dogs (*Canis familiaris*). *Journal of comparative psychology*, *115*(2), 122.

Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2002). Dogs' (*Canis familaris*) responsiveness to human pointing gestures. *Journal of comparative psychology*, *116*(1), 27.

Stein, B. E., & Stanford, T. R. (2008). Multisensory integration: current issues from the perspective of the single neuron. *Nature Reviews Neuroscience*, *9*(4), 255.

Topál, J., Gergely, G., Erdőhegyi, Á., Csibra, G., & Miklósi, Á. (2009). Differential sensitivity to human communication in dogs, wolves, and human infants. *Science*, *325*(5945), 1269-1272.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2008a). Wolves outperform dogs in following human social cues. *Animal Behavior*, *76*(6), 1767-1773.

Udell, M. A., Giglio, R. F., & Wynne, C. D. (2008b). Domestic dogs (*Canis familiaris*) use human gestures but not nonhuman tokens to find hidden food. *Journal of Comparative Psychology*, *122*(1), 84.

Uetz, G. W., & Roberts, J. A. (2002). Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. *Brain, Behavior and Evolution*, *59*(4), 222-230.

Uetz, G. W., Roberts, J. A., & Taylor, P. W. (2009). Multimodal communication and mate choice in wolf spiders: female response to multimodal versus unimodal signals. *Animal Behavior*, *78*(2), 299-305.

Wilcox, R. R., & Keselman, H. J. (2003). Modern robust data analysis methods: measures of central tendency. *Psychological methods*, *8*(3), 254.

Wobber, V., & Kaminski, J. (2011). What do dogs understand about human communicative signals? A novel synthesis. In: deGiovine, V. (Ed.), *Dogs: biology, behavior, and health disorders* (pp, 93-110). Nova Press, New York.

CHAPTER 8

INTERSPECIES TRANSMISSION OF EMOTIONAL INFORMATION VIA CHEMOSIGNALS: FROM HUMANS TO DOGS (*CANIS LUPUS FAMILIARIS*)

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Abstract

We report a study examining interspecies emotion transfer via body odors (chemosignals). Do human body odors (chemosignals) produced under emotional conditions of happiness and fear provide information that is detectable by pet dogs (Labrador and Golden retrievers)? The odor samples were collected from the axilla of male donors not involved in the main experiment. The experimental setup involved the co-presence of the dog's owner, a stranger and the odor dispenser in a space where the dogs could move freely. There were three odor conditions (fear, happiness, and control [no sweat]) to which the dogs were assigned randomly. The dependent variables were the relevant behaviors of the dogs (e.g., approaching, interacting and gazing) directed to the three targets (owner, stranger, sweat dispenser) aside from the dogs' stress and heart rate indicators. The results indicated with high accuracy that the dogs manifested the predicted behaviors in the three conditions. There were fewer and shorter owner directed behaviors and more stranger directed behaviors when they were in the 'happy odor condition' compared to the fear odor and control conditions. In the fear odor condition they displayed more stressful behaviors. The heart rate data in the control and happy conditions were significantly lower than in the fear condition. Our findings suggest that interspecies emotional communication is facilitated by chemosignals.

Keywords: dogs, human emotional smell, interspecies emotional transfer, emotional communication, dog's heart rate, dog-human bond

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8.1 Introduction

Body odors constitute chemical signals that have evolved for species-specific communication (e.g., McClintock, 2000; Stevenson, 2010; Wyatt, 2015). Research has shown that in humans, chemosignals can carry compound information ranging from genetic relatedness (Jacob et al., 2002), gender, (Penn et al., 2007) to emotional states (e.g., Prehn et al., 2006; Mujica-Parodi et al., 2009; Zhou and Chen, 2009; de Groot et al., 2012; Mutic et al., 2015) and more (see de Groot et al., 2017). The transmission of olfactory information related to emotional states occurs without the requirement of communicative intent (Semin and de Groot, 2013) and is below the threshold of consciousness (Pause, 2012). Nevertheless, such transmission induces in the receiver a partial affective, behavioral, perceptual, and neural reproduction of the state of the sender (Semin, 2007). The question we addressed here was about interspecies transmission of emotional information. To this end, we employed an experimental paradigm used in our previous research (e.g., de Groot et al., 2012), whereby the signal was human body odor that was produced while the donors were experiencing experimentally induced emotional states (i.e. happy, fear). The receivers of the human chemosignals were pet dogs (Labrador retrievers and Golden retrievers). Thus, the communication paradigm we employed exposed pet dogs to chemosignals produced by humans and analyzed the dogs' reactions. In the following, we provide an overview of the relevant research to date with dogs and then outline the current study.

Dogs have an acute sensitivity to human gestures, which are chosen as landmarks when contrasted with other signals, such as verbal commands (D'Aniello et al., 2016a; Scandurra et al., 2017). There are two accounts regarding the origins of these abilities. One of them is the 'Domestication Hypothesis'. According to this view, dogs have evolved genetic predispositions allowing them to develop skills shared with humans (Hare et al., 2002; Hare and Tomasello, 2005; Topál et al., 2009; Miklósi and Topál, 2013). According to the other view, namely the 'Two-Stage Hypothesis' (Udell and Wynne, 2008, 2010; Wynne et al., 2008) the capacity to interact with humans is acquired after people are accepted as companions in early ontogeny. This close proximity provides the opportunity to learn from humans during ontogenesis and thus shape (D'Aniello et al., 2015; Scandurra et al., 2015) and improve social communicative skills (D'Aniello and Scandurra, 2016; D'Aniello et al., 2017). The two theories do not necessarily contradict each other. Indeed, the synergistic hypothesis suggests that the sensitivity to human gestural cues emerges both at the evolutionary and developmental level (Gácsi et al., 2009). In any case, dogs and humans went through a convergent evolution, whereby one of the most important consequences is that the two species have become social partners (Udell et al., 2010). In such a context, the reciprocal reading of the emotional status would be a very useful tool in many situations. The ability to recognize and respond appropriately to emotional messages has biological fitness benefits for both signaler and the receiver. Particularly, reading others emotions is very important for facilitating group cohesion (Racca et al., 2012) and it allows observers to use others' emotions to cope flexibly with events in the environment (Nelson and Russell, 2011). It has been shown that dogs can discriminate between smiling and neutral human faces (Nagasawa et al., 2011), between their owner's facial expression of sadness and happiness (Morisaki et al., 2009) and a range of emotional facial expressions (anger, joy, disgust and fear) when compared with neutral ones (Deputte and Doll, 2011). Furthermore, other findings show that dogs can extract and integrate bimodal sensory-emotional information. Indeed, dogs looked significantly longer at the expression of human faces (happy/playful versus angry/aggressive) that were congruent in expressive valence to either positive or negative vocalisations (Albuquerque et al., 2016). Moreover, there are indications that dogs, aside from recognizing human emotions, adjust their behavior according to the expressed emotion (Merola et al., 2012, 2013). Dogs are also found to be sensitive and respond accordingly to differences in the emotional content of a voice (gentle vs. harsh) used by humans in obedience tasks (Fukuzawa et al., 2005).

Throughout this research on the social communicative interactions between dogs and humans, the focus has been on the visual and acoustic systems as they mediate emotional responses. The contribution that the olfactory system may have has barely been studied. Dogs have an extraordinary ability to detect airborne odors and not surprisingly their olfactory system is a significant contributor to the regulation of their social relations (Thesen et al., 1993; Miklósi, 2007). For instance, male dogs can recognize kin, probably to avoid inbreeding (Hamilton and Vonk ,2015). They can discriminate odors from different parts of the body of the same person or from human twins (Hepper, 1988; Schoon and De Bruin, 1994). Social smells have been shown to activate specific brain areas differentially in dogs, such as the caudate nucleus (Berns et al., 2015), which is involved in positive expectations in many species (Schultz et al., 1997; Knutson et al., 2001; Montague and Berns, 2002; Berns et al., 2012, 2013), including social rewards (Rilling et al., 2002; Izuma et al., 2008). A dog's caudate nucleus is activated more strongly when it is exposed to the body odor of a familiar human compared to odors from a familiar or a strange dog, an unfamiliar human and even the dog's own odor (Berns et al., 2015) suggesting a positive emotional response to the odor of a familiar human (Panksepp, 2004; Bekoff, 2007).

One of the early olfactory transfer of emotion studies (Siniscalchi et al., 2011) showed that a veterinarian's sweat increased a dog's arousal. While this finding does not demonstrate the ability of dogs to perceive olfactory emotional messages from humans, a later study by the same research group (Siniscalchi et al., 2016) examined asymmetries in nostril use while the dogs were sniffing different emotive stimuli. The emotive stimuli were human (i.e. axillary sweat samples) and canine (i.e. perianal, interdigital and salivary secretions) odors produced under different emotional (fear, joy, stress) conditions. This research revealed that nostril use during sniffing of canine versus human odors varied systematically. Particularly, dogs consistently used their right nostril (right hemisphere) to sniff conspecific odors collected during a stressful situation. On the other hand, they preferred to use the left nostril to sniff human odors (left hemisphere) collected during fearful situations.

Siniscalchi et al. (2016) found that human fear chemosignals induced higher cardiac activation in dogs than neutral odors. Samples collected in humans during the induced state of joy emotion did not trigger a different cardiac effect than control condition. Furthermore, dogs appeared more stressed when they sniffed human chemosignals in the emotional fear condition than those obtained in the neutral or joy conditions.

The research we report here was designed to examine a new perspective, namely the transmission of emotional states from humans to dogs via human body odors produced during happiness and fear. The experimental setup for this examination was in a space in which the dogs could move and were free to manifest any behavior. Moreover, both the owner and a stranger were present in the room in which the examination took place. This interpersonal context was created to examine if dogs, when exposed to odors of happiness and fear, would display systematic differences in their social interest behavior towards their owner and a stranger. An important question is: do dogs manifest attachment behavior when exposed to fear odors? Dogs are known to form an affective bond with their owners fulfilling all attachment criteria described between parents and offspring in humans (Ainsworth and Bell, 1970). This is expressed behaviorally through a preference for the attachment figure over other individuals and through behaviors aimed at obtaining and maintaining proximity to the caregiver during worrisome or dangerous situations (Topál et al., 1998; Prato-Previde et al., 2003; Palestrini et al., 2005; Palmer and Custance, 2008; Rehn et al., 2013; Scandurra et al., 2016). We expected that the attachment system of dogs would be activated when they are exposed to fear chemosignals and lead dogs to look more to their owner as a “secure base”. In contrast, we expected happiness chemosignals to make dogs more confident in the environment and toward the stranger.

8.2 Material and methods

8.2.1 Odor collection

Odor donors were heterosexual males (see de Groot et al., 2012, 2015). They watched fear or happiness-inducing videos in two sessions separated by 1 week. They followed a strict protocol. Two days prior to the donation, odorous food, alcohol, smoking, and excessive exercise was prohibited. They were provided with scent-free personal care products and detergents. The sweat was collected with sterile absorbent compresses (Cutisorb, BSN Medical, Hamburg, Germany) from both armpits. Donors, who were students at ISPA University, Lisbon, with an average age of 21 watched 25-min videos. Before and after the videos, donors completed Spielberger’s State-Trait Anxiety Inventory (Van der Ploeg et al., 1980). Afterwards, sweat pads were removed and stored at −22 °C. The sweat pads were transferred to the Italian laboratory in dry ice. The odors were sent in two tranches: the first were from 4 Caucasian males, in Spring 2016; the second from a further 4 Caucasian males, in Spring 2017. To rule out interindividual differences in body odor, the pads of four different individuals were cut in four pieces and matched in a glass tube, thereby creating a pooled sample (see Mitro et al., 2012).

8.2.2 Subjects

The subjects were 40 pet dogs (17 males and 23 females; 31 Labrador retrievers and 9 Golden retrievers mean age in months 43.7 ± 5.0), recruited through personal contacts and advertisements in public places, veterinary surgeons and through the internet. All dogs lived in a household with at least two people. Dogs were randomly allocated to one of the three odor conditions: Happiness (7 males and 8 females; 10 Labrador retrievers and 5 Golden retrievers, mean age in months 45.3 ± 9.7), Fear (6 males and 9 females; 12 Labrador retrievers and 3 Golden retrievers, mean age in months 40.1 ± 7.9) and Empty (i.e. unused sweat pads) (4 males and 6 females; 9 Labrador retrievers and 1 Golden retrievers). The overall mean age for the pet dogs was 46.7 ± 7.6 months.

8.2.3 Apparatus and procedure

The study was conducted at the University of Naples "Federico II" (Naples) in a 4 x 3 m room that was new to the dogs. The room contained a water bowl in a corner and two chairs in two opposite corners (one for the owner and the other for a researcher (E_1), unknown to dogs). The chairs were equidistant from the apparatus placed in the centre of the room. The apparatus consisted of a wooden board of 39.5 x 30 cm with a semitransparent plastic container fixed at centre. The vial, without a cap, containing the samples was inserted in the container. The lid in the upper part of the container had a circular hole (diameter 3 cm), which allowed the dogs to engage in the olfactory exploration of the contents while preventing the dog from contaminating the substances by direct contact.

Before starting, the owner had been informed about the testing procedure, while the dogs were free to move in a space outside of the room where the trials took place. Subsequently, a heart rate monitor was attached to the dog (Polar RS800CX) and the dog was free again for about 10 minutes before the experimental procedure started. Generally, the dogs showed some anxious behavior when the heart rate monitor was applied, but they adapted to the device after a while. At the end of this procedure, the owner entered the experimental room with the dog, where the E_1 was already present. At this stage, the owner was free to interact with the dog while E_1 ignored both, reading a magazine. After 5 minutes, in which the dog had finished exploration and its arousal due to the new environment was lowered, the owner was asked to hold the dog, while a second researcher (E_2), entered the room to fix the experimental apparatus in the centre of the room. When E_2 left the room, the owner released the dog and the trial started. From this moment on, the owner and E_1 didn't interact with each other or the dog, and did not respond to any eventual solicitation by the dog. During this phase, the owner also begins to read a magazine to avoid looking at the dog or the experimenter. Both people in the room were not aware of the condition provided to the dog, so as not to influence accidentally their behavior. Each dog was allocated randomly to only one condition which lasted two minutes.

At the end of each trial, the bowl, the apparatus and the room were cleaned with a non-toxic disinfectant. This was done to eliminate the odors of dogs that had previously performed the trial and the vials with the samples were frozen again. Each sample was not used more than 4 times. The trial was recorded with a camera (HDR-PJ260VE) that was placed at a height of 220 cm in a corner of the room.

8.2.4 Behavioral parameters

All behaviors that were related to the apparatus and the people, such as approaching, interacting and gazing were grouped and categorised as apparatus, owner and stranger directed behaviors. All the stressful signals have been summarised and categorised as stressful behaviors (see Table 8.1 for the ethogram adopted). The frequency of all stressful behaviors was recorded, as well as the duration of the stressful behaviors. When two or more stressful behaviors co-occurred, we recorded the one that lasted longer.

The frequency and duration of each behavior were recorded using Solomon Coder® beta 16.06.26 (ELTE TTK, Hungary).

Table 8.1 Ethogram adopted**.**

Categories	Behaviors	Description
Owner directed behaviors	Approach owner	The dog's approach is clearly oriented toward the owner (visually).
	Interaction with owner	The dog engages in physical contact with or sniffing the owner regardless of visual orientation.
	Gazing at owner	The dog looks at the owner's face from a stationary position.
Stranger directed behaviors	Approach stranger	The dog's approach is clearly oriented toward the stranger (visually).
	Interaction with stranger	The dog engages in physical contact with or sniffs the stranger regardless of visual orientation.
	Gazing at stranger	The dog looks at the stranger's face from a stationary position.
Apparatus directed behaviors	Approach apparatus	The dog approaches and is clearly visually oriented toward the apparatus.
	Interaction with apparatus	The dog engages in physical contact or sniffs the apparatus regardless of visual orientation.
	Gazing at apparatus	The dog looks at the apparatus from a stationary position.
Stressful behaviors	All behaviors indicating a stressful situation	Mouth Licking (the dog licks its lips or nose, except after drinking), locomotion (the dog walking, pacing or running around without a distinguishable target), shaking off, scratching itself, yawning, barking, yapping, panting, putting its ears back, drinks water.

8.2.5 Heart rate monitoring

A Polar® RS800CX heart rate (HR) measuring system was used since it is an instrument scientifically validated for dogs (Jonckheer-Sheehy et al., 2012; Essner et al., 2013). Polar® heart rate monitor consisted of electrode belt and transmitter W.I.N.D. and heart rate monitor RS800CX. Following Essner et al. (2013), the coat was clipped at all electrode sites and Cogel® ECG electrode transmission gel was applied liberally to promote conductivity. We used two different electrode belts depending on the animal's size (S-M or L-XL). These were strapped around the chest of the dogs with the transmitter placed ventrally and the electrodes on each side of the sternum. This instrument allows storing the R-R interval recordings, as well as the time data, automatically in the watch-computer for later analysis. The R-R intervals are the inter-beat intervals and they are obtained as differences between successive R-wave occurrence times (Tarvainen et al., 2014).

The cardiac activity analysis was performed using the Polar Pro Trainer 5^{TM} 5.40.170 software (Polar®Electro Öy, Kempele, Finland).

8.2.6 Data analysis

To evaluate the responses recorded during the 2 minutes of the trial a point sampling approach was used. The data (the behavioral categories, i.e. owner directed behaviors; stranger directed behaviors; apparatus directed behaviors; stressful behaviors and heart rate) were recorded every 5 seconds, for a total of 24 sample points per dog. Each sample point contained all behavioral data displayed during the 5 seconds preceding the sample point. Similarly, the average heart rate during the five seconds prior to the chosen sample point was obtained. The averages of each sample point were obtained from 10 dogs exposed to the Empty condition (E), 15 the Happiness (H) and 15 the Fear (F) conditions.

To demonstrate response differences as a function of sweat sampled under different conditions, we adopted a linear discriminant analysis (LDA) to examine how well the measured variables (i.e. behavioral categories and the physiological parameter) could predict the specific odor condition in which each dog was.

The averages of the frequency and the durations of sample points obtained across the three different conditions were used to monitor the effect of the odors during the time on the behavioral parameters. The same approach was applied to the HR sample points. The temporal pattern was represented by a smoothing spline approach. The smoothing parameter was automatically selected, minimizing the residuals.

The distributions of the sample points of the behavioral parameters and of the HR were subsequently compared. If they were normally distributed, as showed by Shapiro-Wilk test, then they were analyzed with repeated measures ANOVA test with Tukey's pairwise post hoc comparisons. In the case of not normally distributed data, a Kruskal-Wallis test was used to compare medians.

All statistical analyses were performed with IBM SPSS Statistics 22 and R (3.3.3).

8.3 Results

8.3.1 Linear discriminant analysis (LDA)

The LDA revealed two discriminant functions. As can be seen in Table 8.2 the first function is represented by variables related to fear (stress frequency and duration; heart rate) and the second function by approach-avoidance variables (owner and stranger directed behaviors). As can be seen in Table 8.2 these functions discriminate successfully between the three conditions (Table 8.3).

Table 8.2 Correlation with discriminant functions.

Variables	Functions	
	1	**2**
Stressful behaviors frequency	**0.763**	-0.057
Stressful behaviors duration	**0.533**	-0.265
Average heart rate	**0.522**	0.191
Apparatus directed behaviors frequency	0.064	0.056
Owner directed behaviors duration	-0.116	**-0.529**
Owner directed behaviors frequency	0.006	**-0.509**
Stranger directed behaviors duration	-0.120	**0.468**
Stranger directed behaviors frequency	-0.024	**0.267**
Apparatus directed behaviors duration	0.018	0.107

Table 8.3 LDA Goodness of fit.

Function test	Wilks λ	$\chi2$	*df*	*P*	Eigenvalue
From 1 to 2	0.220	98.391	18	<0.001	1.561
2	0.564	37.270	8	<0.001	0.774

The first function mainly discriminated the F condition from the others. The second function highlighted the differences between H and E conditions (Fig. 8.1).

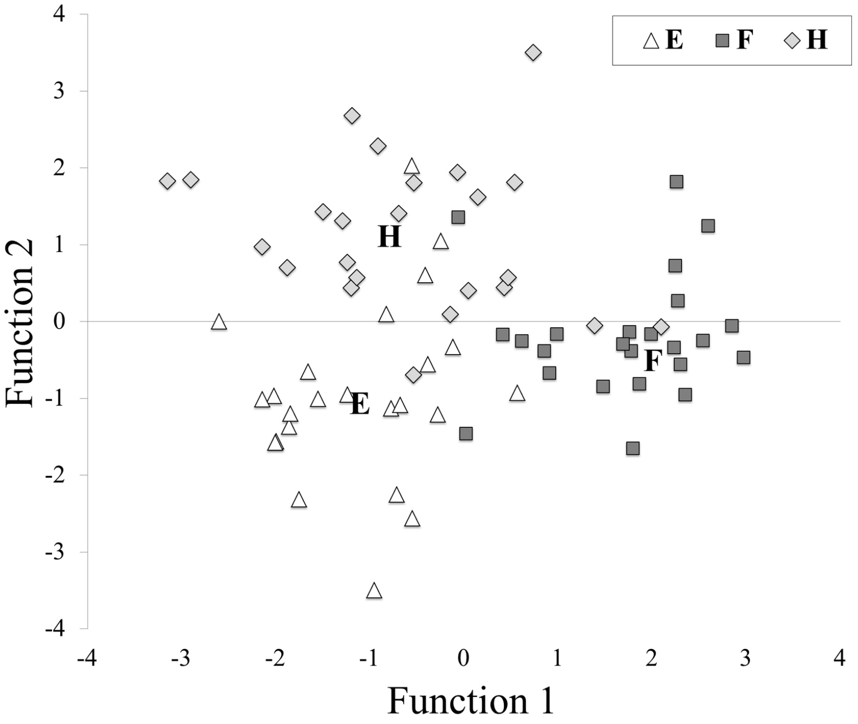

Fig 8.1 Graphical representation of the LDA. E, F and H represent the centroids of the clusters.

As can be seen in Table 8.4, the classification predicted by the two functions overlapped with a high degree of accuracy (87.5%) with the a priori classifications of the dogs to the odor conditions.

Table 8.4 A priori and predicted classification overlap LDA.

		Responses	**Belonging to the expected group**			**TOTAL**
			E	**F**	**H**	
A priori	Count	E	20	1	3	24
		F	1	22	1	24
		H	1	2	21	24
	Percent	E	83.3	4.2	12.5	100
		F	4.2	91.7	4.2	100
		H	4.2	8.3	87.5	100

8.3.2 Temporal unfolding

In the following section we describe the trends of the behavioral categories and the HR along the two minutes of test as a function of the sample points recorded for the frequency and the duration. At the same time, we compared the sample points across the different conditions.

8.3.3 Owner directed behaviors

The frequency of the *owner directed behaviors* in the E condition increased progressively, peaking at 35 seconds and decreasing 95 seconds. It remained stable subsequently and was constantly lower than the fear condition until the end of the trial. The F condition showed the highest initial value for the *owner directed behaviors*, and then decreased in the first 30 seconds and remained practically constant until the end of the trial. The H condition had a decreasing pattern until the 70th second, and remained constant thereafter, but was always below the F condition (Fig. 8.2a).

The statistical analysis of the sample points representing the curves of the *owner directed behaviors* were significantly different (ANOVA: $N_E = N_F = N_H = 24$, $F = 6.94$, $P = 0.002$), with the H condition lower than E (Tukey's post hoc: $Q = 5.18$, $P = 0.001$) and F (Tukey's post hoc: $Q = 3.42$, $P = 0.037$).

The duration of the *owner directed behaviors* follows the same pattern as in the case of the frequency measure. However, all curves converge on the same points after 90 seconds (Fig. 8.3a). The statistical comparison of sample points showed a significant difference (ANOVA: $N_E = N_F = N_H = 24$, $F = 8.45$, $P < 0.001$), with H lower than E (Tukey's post hoc: $Q = 5.81$, $P < 0.001$). Furthermore, there was a trend toward a lower duration of *owner directed behaviors* in E compared to F (Tukey's post hoc: $Q = 3.09$, $P = 0.081$).

8.3.4 Stranger directed behaviors

The frequency of the *stranger directed behaviors* showed an initial increase across all conditions. E and the F conditions showed a similar pattern, with a marked decrease after 45 seconds, whereas the H condition maintained higher values during the entire trial (Fig. 8.2b). The statistical analysis revealed no significant differences among the sample points representing the conditions (Kruskal-Wallis test: $N_E = N_F = N_H = 24$, $H = 4.56$, $P = 0.097$).

The duration of the *stranger directed behaviors* showed a pattern similar to the frequency one (Fig. 8.3b). However, the sample points were significantly different for duration (ANOVA: $N_E = N_F = N_H = 24$, $F = 6.59$; $P = 0.002$), with the H condition higher than both F (Tukey's post hoc: $Q = 4.42$, $P = 0.007$) and E (Tukey's post hoc: $Q = 4.47$, $P = 0.007$).

8.3.5 Apparatus directed behaviors

The trend of the frequency of the *apparatus directed behaviors* was very similar across all three conditions, decreasing during the first minute and remaining constant during the second minute (Fig. 8.2c). No statistical differences were recorded among the sample points of the conditions (Kruskal-Wallis test: $N_E = N_F = N_H = 24$, $H = 4.55$, $P = 0.097$).

The pattern described for the frequency can be applied unchanged for the duration (Fig. 8.3c), including no statistical differences (Kruskal-Wallis test: $N_E = N_F = N_H = 24$, $H = 4.34$, $P = 0.109$).

8.3.6 Stressful behaviors

The frequency of the *stressful behaviors* showed a similar trend in E and H conditions, showing a growing trend at the beginning, peaking at the 35th (H) and 50th (E) seconds, then decreasing progressively. The F condition differed from the other two conditions revealing constantly a higher level, with a peak around 55th second that held until the end of the trial (Fig. 8.2d). A significant difference was recorded for the sample points of *stressful behaviors* (ANOVA: $N_E = N_F = N_H = 24$, $F = 31.51$, $P < 0.001$), with F giving a higher value than both the H (Tukey's post hoc: $Q = 9.02$, $P < 0.001$) and E (Tukey's post hoc: $Q = 10.30$, $P < 0.001$) conditions.

The duration of the *stressful behaviors* showed a similar pattern to the frequency variable (Fig. 8.3d), with statistical differences between the sample points of the conditions (ANOVA: $N_E = N_F = N_H = 24$, $F = 17.14$, $P < 0.001$). Post hoc test showed that F was higher than both H (Tukey's post hoc: $Q = 7.77$, $P < 0.001$) and E (Tukey's post hoc: $Q = 6.36$, $P = 0.001$).

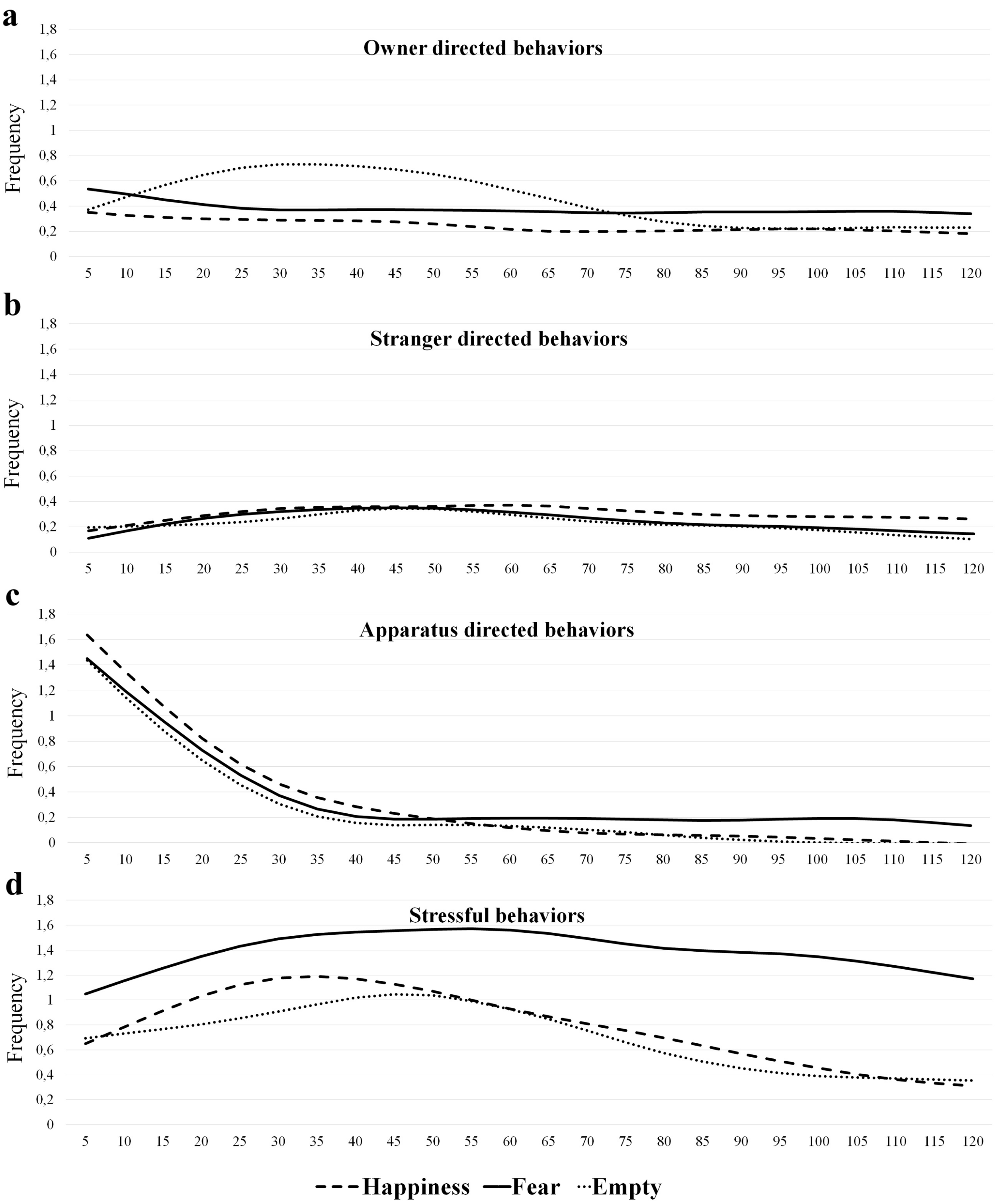

Fig 8.2 Temporal unfolding of the frequency of the recorded behaviors during the 2 minutes of trial. The temporal pattern was represented by a smoothing spline approach where the smoothing parameter was automatically selected minimizing the residuals. In Y axes the frequencies; in X axes the sample points.

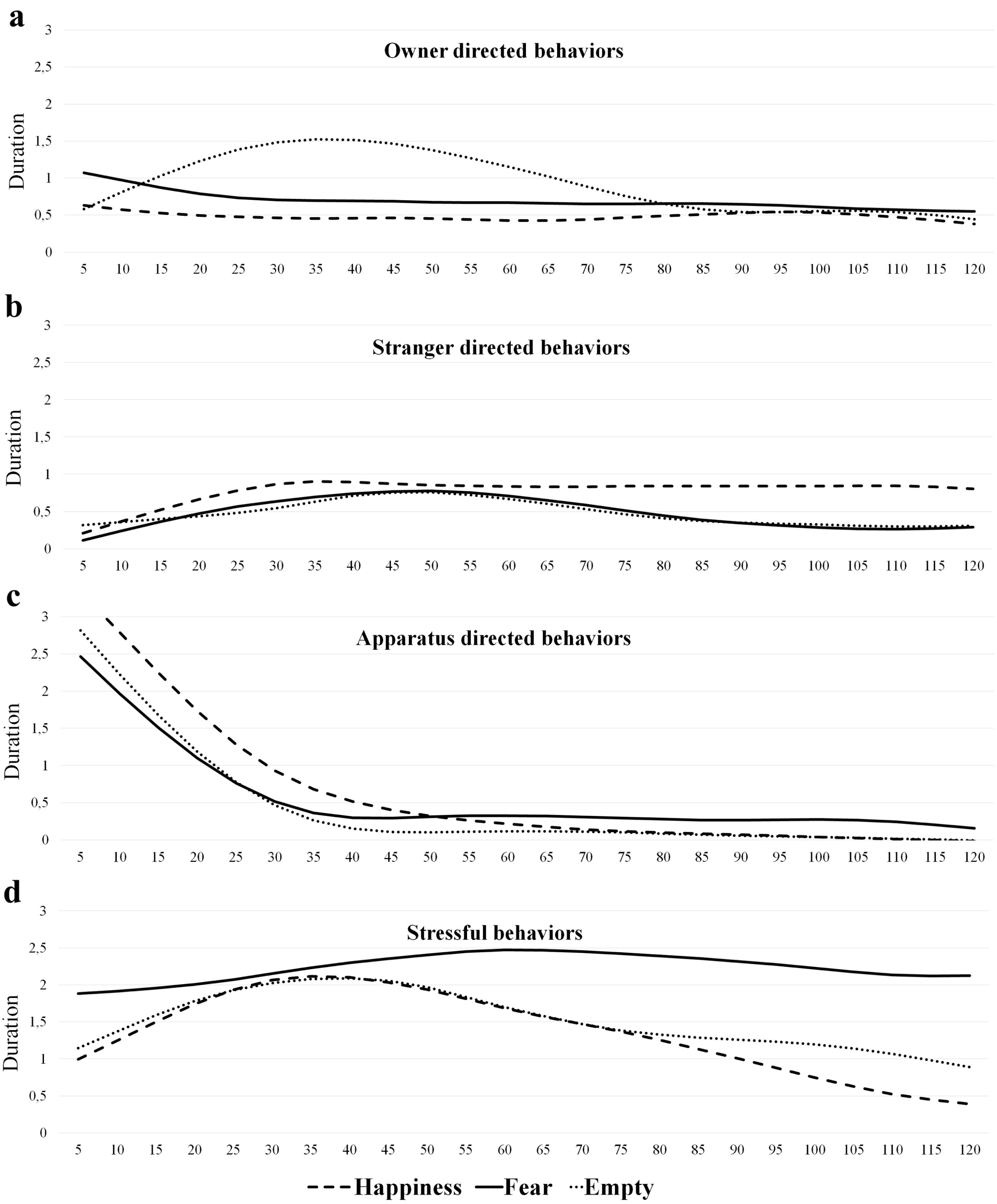

Fig 8.3 Temporal unfolding of the duration of the recorded behaviors during the 2 minutes of trial. The temporal pattern was represented by a smoothing spline approach where the smoothing parameter was automatically selected minimizing the residuals. In Y axes the durations in seconds; in X axes the sample points.

8.3.7 Heart rate monitoring

In the E condition, the heart rate (HR) was initially high, starting with a value comparable to the F condition. It then decreased as the trial progressed. In the F condition, the HR remained constantly higher than the other conditions. In the H condition the values were initially lower than the F and E conditions, then increasing in the first 40 seconds and decreasing until the end of the trial. During the phase when it was decreasing, the HR values remained above condition E and below condition F (Fig. 8.4). The dog's HR sample points differed (ANOVA: $N_E = N_F = N_H = 24$, $F = 15.91$, $P < 0.001$). Data from E were significantly lower than F (Tukey's post hoc: $Q = 7.86$, $P < 0.001$). Moreover, the HR in the H condition was also significantly lower than in the F condition (Tukey's post hoc: $Q = 4.64$, $P = 0.005$). A tendency toward a higher H with respect E was also detected (Tukey's post hoc: $Q = 3.22$, $P = 0.066$).

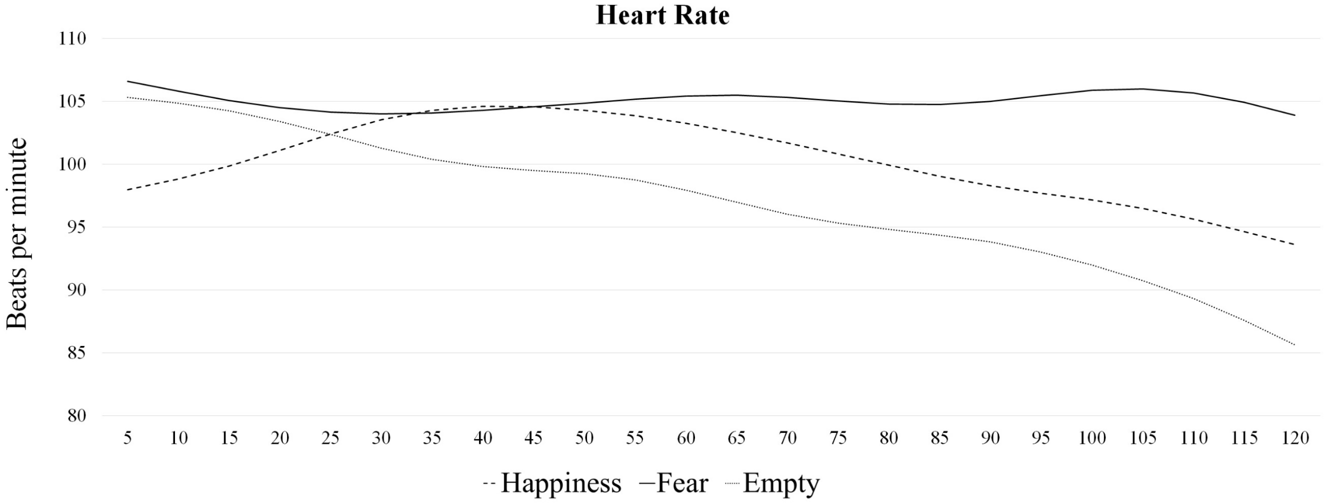

Fig 8.4 Temporal unfolding of the heart rate during the 2 minutes of trial. The temporal pattern was represented by a smoothing spline approach where the smoothing parameter was automatically selected minimizing the residuals. In Y axes the heartbeats per minutes; in X axes the sample points.

8.4 Discussion

The effect of the emotional responses triggered by the visual and acoustic signals in dogs has been widely studied, while the involvement of the olfactory system, which in dogs is probably the more reliable sensory system, has been barely studied. The reason for the paucity of research examining the communicative potential of the olfactory sensory system in dogs is probably due to the assumption that there is considerable difference in the sensitivities of the olfactory systems of dogs and humans (Marshall and Moulton, 1981). However, dogs and humans have a long co-evolutionary history and their communicative potential through visual and acoustic systems has been repeatedly demonstrated (Pongrácz et al., 2009; MacLean et al., 2017). It would have been surprising if their olfactory system, which has extraordinary potential to detect airborne odors, did not contribute to the regulation of their relations with humans. This would mean that they should display differential responses to the distinctive biochemical signatures of the body odors humans excrete under different emotional states.

To examine the interspecies communicative potential of human chemosignals of fear and happiness, we analyzed the behavioral changes and the physiological heart activation in dogs as a consequence of the exposition to these odors. Our data showed that the human chemosignals affect the physiological status of dogs and induce systematically different behaviors. Indeed, the discriminant analysis showed that the responses displayed by the dogs allow the identification of the body odor conditions the dogs were in, with a near perfect accuracy.

The time monitoring analysis of the data showed how the behavioral patterns unfolded over the duration of trials and revealed that the patterns were different, except for the apparatus interest, which followed a similar trend across all 3 conditions. The statistical analyses revealed that the dog's heart activation was significantly higher in the fear condition compared to both the happiness and the empty condition, thus confirming the previous results reporting a cardiac effect on dogs exposed the human emotional fear chemosignals (Siniscalchi et al., 2016). Interestingly, the pattern observed for the stressful behaviors was very much like that of cardiac activation, with higher levels of stress displayed only in the fearful condition, which is again in line with an earlier study (Siniscalchi et al., 2016). Although a behavioral analysis alone may be regarded as insufficient to elucidate what dogs experience or even to argue that their emotional responses are like human ones (Panksepp, 2004; Bekoff, 2007), the heart rate indicator has the advantage of showing the emotional activity of the dogs. Indeed, it is well-established that heart rate in well controlled conditions is a suitable measure of behavioral states associated with sympathetic stimulation, as has been shown across a variety of species (rats: Ashida, 1972; sheep: Baldock et al., 1988; chicken: Candland et al., 1969; pigs: Dantzer and Baldwin, 1974; rabbits: Eisermann, 1992; wolves: Fox and Andrews, 1973; monkeys: Weisbard and Graham, 1971). Thus, our finding of an enhanced heart rate in our dogs reflects a higher arousal when exposed to fear chemosignals.

As proposed by Siniscalchi et al. (2016), human fear chemosignals could have activated a predatory instinct of dogs, which could also explain anecdotal observations of a dog attacking people when they are afraid of dogs. This type of hypothetical situation would be the result of dogs using their left nostril when monitoring the human chemosignals of fear, which would be expected to lead to the activation of the left hemisphere. This in turn is involved in the control of predatory behavior (Siniscalchi et al., 2013). However, while an increase of the heartbeat rate is a sensible response in these circumstances, the physical expression of stressful signals is not necessarily a precursor of a predatory act, but could be regarded as an indicator of an emotional state of fear (together with the increased heartbeat). Thus, an alternative argument would suggest that stress signals are evidences of emotional contagion rather predatory behavior. Indeed, evidence of interspecies emotional contagion from dogs to humans has been shown on numerous occasions (Zahn-Waxler et al., 1984; Custance and Mayer, 2012; Sümegi et al., 2014; Yong and Ruffman, 2014; Huber et al., 2017). These studies demonstrate the role of visual and/or acoustic stimuli, whereas here for the first time, we show the involvement of the olfactory system in emotional transfer. Notably, the chemosignals used in our research came from a composite sweat stimulus pooled over 4 individuals unknown to dogs. It is worthwhile considering the possible effect of the emotional chemosignals from the owners themselves.

This is the first study revealing the effects of distinctive body odors on social behavioral responses. In line with our prevision, dogs adjusted their social interests after being exposed to different chemosignals, increasing the interest toward the stranger, as revealed by the increased duration of *stranger directed behaviors* in the happiness condition. On the other hand, our hypothesis was also supported for fearful chemosignals, which increased the owner interest above the other conditions as secure base effect, as showed by the higher frequency and duration of the *owner directed behaviors* in the fear. It should be pointed out that dogs have different attachment styles (see Udell and Brubaker, 2016), which may have influenced trends in the fear condition, especially with respect to the time-based measures, as insecure attachment styles would likely drive greater differences between conditions than secure ones.

In closing, we should note that the majority of domesticated dogs in our samples were Labrador retrievers, which are ranked very high in hereditable behavioral traits related to sociability and curiosity/fearlessness (Svartberg, 2006). Brain changes take place rapidly given highly responsive evolutionary plasticity resulting in considerable differences between closely related species (Pinelli et al., 2014; D'Aniello et al., 2016b). These types of differences can be seen in the course of the domestication of dog breeds that came to evolve with considerable differences in their behavioral traits (Svartberg, 2006). For this reason, it is possible that other breeds with a different selective history could show different behavioral reactivity when exposed to human emotional smells. Hence, further studies on the matter in different breeds are likely to shed more light to the issues we examined here. The important message that the current study provides is the remarkable symmetry that human chemosignals of fear and happiness induces in the pet dogs we have examined. The fact that the oldest sensory system is tuned across these two species may suggest that the specific biochemical signature of chemosignals has remained a relatively invariable carrier of information that although susceptible to contextual variations remains a major medium of interspecies communication.

8.5 References

Ainsworth, M. D. S., & Bell, S. M. (1970). Attachment, exploration, and separation: Illustrated by the behavior of one-year-olds in a strange situation. *Child development*, 49-67.

Albuquerque, N., Guo, K., Wilkinson, A., Savalli, C., Otta, E., & Mills, D. (2016). Dogs recognize dog and human emotions. *Biology letters*, *12*(1), 20150883.

Ashida, S. (1972). Development changes in the basal and evoked heart rate in neonatal rats. *Journal of Comparative and Physiological Psychology*, *78*(3), 368.

Baldock, N. M., Sibly, R. M., & Penning, P. D. (1988). Behavior and seasonal variation in heart rate in domestic sheep, *Ovis aries*. *Animal Behavior*, *36*(1), 35-43.

Bekoff, M. (2010). The emotional lives of animals: A leading scientist explores animal joy, sorrow, and empathy—and why they matter. New World Library, Novato.

Berns, G. S., Brooks, A. M., & Spivak, M. (2012). Functional MRI in awake unrestrained dogs. *PloS one*, *7*(5), e38027.

Berns, G. S., Brooks, A., & Spivak, M. (2013). Replicability and heterogeneity of awake unrestrained canine fMRI responses. *PloS one*, *8*(12), e81698.

Berns, G. S., Brooks, A. M., & Spivak, M. (2015). Scent of the familiar: an fMRI study of canine brain responses to familiar and unfamiliar human and dog odors. *Behavioral processes*, *110*, 37-46.

Candland, D. K., Taylor, D. B., Dresdale, L., Leiphart, J. M., & Solow, S. P. (1969). Heart rate, aggression, and dominance in the domestic chicken. *Journal of comparative and physiological psychology*, *67*(1), 70.

Custance, D., & Mayer, J. (2012). Empathic-like responding by domestic dogs (*Canis familiaris*) to distress in humans: an exploratory study. *Animal cognition*, *15*(5), 851-859.

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

D'Aniello, B., & Scandurra, A. (2016). Ontogenetic effects on gazing behavior: a case study of kennel dogs (Labrador Retrievers) in the impossible task paradigm. *Animal cognition*, *19*(3), 565-570.

D'Aniello, B., Scandurra, A., Alterisio, A., Valsecchi, P., & Prato-Previde, E. (2016a). The importance of gestural communication: a study of human–dog communication using incongruent information. *Animal cognition*, *19*(6), 1231-1235.

D'Aniello, B., Polese, G., Luongo, L., Scandurra, A., Magliozzi, L., Aria, M., & Pinelli, C. (2016b). Neuroanatomical relationships between FMRFamide-immunoreactive components of the nervus terminalis and the topology of olfactory bulbs in teleost fish. *Cell and tissue research*, *364*(1), 43-57.

D'Aniello, B., Alterisio, A., Scandurra, A., Petremolo, E., Iommelli, M. R., & Aria, M. (2017). What's the point? Golden and Labrador retrievers living in kennels do not understand human pointing gestures. *Animal cognition*, *20*(4), 777-787.

Dantzer, R., & Baldwin, B. A. (1974). Changes in heart rate during suppression of operant responding in pigs. *Physiology & behavior*, *12*(3), 385-391.

de Groot, J. H., Smeets, M. A., Kaldewaij, A., Duijndam, M. J., & Semin, G. R. (2012). Chemosignals communicate human emotions. *Psychological science*, *23*(11), 1417-1424.

de Groot, J. H., Smeets, M. A., Rowson, M. J., Bulsing, P. J., Blonk, C. G., Wilkinson, J. E., & Semin, G. R. (2015). A sniff of happiness. *Psychological science*, *26*(6), 684-700.

de Groot, J. H., Semin, G. R., & Smeets, M. A. (2017). On the communicative function of body odors: a theoretical integration and review. *Perspectives on Psychological Science*, *12*(2), 306-324.

Deputte, B. L., & Doll, A. (2011). Do dogs understand human facial expressions? *Journal of Veterinary Behavior: Clinical Applications and Research*, *6*(1), 78-79.

Eisermann, K. (1992). Long-term heartrate responses to social stress in wild European rabbits: predominant effect of rank position. *Physiology & behavior*, *52*(1), 33-36.

Essner, A., Sjöström, R., Ahlgren, E., & Lindmark, B. (2013). Validity and reliability of Polar® RS800CX heart rate monitor, measuring heart rate in dogs during standing position and at trot on a treadmill. *Physiology & behavior*, *114*, 1-5.

Fox, M. W., & Andrews, R. V. (1973). Physiological and biochemical correlates of individual differences in behavior of wolf cubs. *Behavior*, *46*(1), 129-139.

Fukuzawa, M., Mills, D. S., & Cooper, J. J. (2005). More than just a word: non-semantic command variables affect obedience in the domestic dog (*Canis familiaris*). *Applied Animal Behavior Science*, *91*(1-2), 129-141.

Gácsi, M., Gyoöri, B., Virányi, Z., Kubinyi, E., Range, F., Belényi, B., & Miklósi, Á. (2009). Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills. *PLoS One*, *4*(8), e6584.

Hamilton, J., & Vonk, J. (2015). Do dogs (*Canis lupus familiaris*) prefer family? *Behavioral processes*, *119*, 123-134.

Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, *298*(5598), 1634-1636.

Hare, B., & Tomasello, M. (2005). Human-like social skills in dogs? *Trends in cognitive sciences*, *9*(9), 439-444.

Hepper, P. G. (1988). The discrimination of human odour by the dog. *Perception*, *17*(4), 549-554.

Huber, A., Barber, A. L., Faragó, T., Müller, C. A., & Huber, L. (2017). Investigating emotional contagion in dogs (*Canis familiaris*) to emotional sounds of humans and conspecifics. *Animal cognition*, *20*(4), 703-715.

Izuma, K., Saito, D. N., & Sadato, N. (2008). Processing of social and monetary rewards in the human striatum. *Neuron*, *58*(2), 284-294.

Jacob, S., McClintock, M. K., Zelano, B., & Ober, C. (2002). Paternally inherited HLA alleles are associated with women's choice of male odor. *Nature genetics*, *30*(2), 175.

Jonckheer-Sheehy, V. S., Vinke, C. M., & Ortolani, A. (2012). Validation of a Polar® human heart rate monitor for measuring heart rate and heart rate variability in adult dogs under stationary conditions. *Journal of Veterinary Behavior: Clinical Applications and Research*, *7*(4), 205-212.

Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *Journal of Neuroscience*, *21*(16), RC159-RC159.

MacLean, E. L., Herrmann, E., Suchindran, S., & Hare, B. (2017). Individual differences in cooperative communicative skills are more similar between dogs and humans than chimpanzees. *Animal Behavior*, *126*, 41-51.

Marshall, D. A., & Moulton, D. G. (1981). Olfactory sensitivity to α-ionone in humans and dogs. *Chemical senses*, *6*(1), 53-61.

McClintock, M. K. (2000). Human pheromones: Primers, releasers, signalers, or modulators? In: Wallen, K., & Schneider, J. E. (Eds.), *Reproduction in context* (pp. 355-420). MIT Press, Cambridge.

Merola, I., Prato-Previde, E., & Marshall-Pescini, S. (2012). Social referencing in dog-owner dyads? *Animal cognition*, *15*(2), 175-185.

Merola, I., Marshall-Pescini, S., D'Aniello, B., & Prato-Previde, E. (2013). Social referencing: water rescue trained dogs are less affected than pet dogs by the stranger's message. *Applied animal behavior science*, *147*(1-2), 132-138.

Miklósi, Á. (2007). Human-animal interactions and social cognition in dogs. In: Jensen, P. (Ed.), *The behavioral biology of dogs* (pp. 205-222). CAB International, Wallingford.

Miklósi, Á., & Topál, J. (2013). What does it take to become 'best friends'? Evolutionary changes in canine social competence. *Trends in cognitive sciences*, *17*(6), 287-294.

Mitro, S., Gordon, A. R., Olsson, M. J., & Lundström, J. N. (2012). The smell of age: perception and discrimination of body odors of different ages. *PloS one*, *7*(5), e38110.

Montague, P. R., & Berns, G. S. (2002). Neural economics and the biological substrates of valuation. *Neuron*, *36*(2), 265-284.

Morisaki, A., Takaoka, A., & Fujita, K. (2009). Are dogs sensitive to the emotional state of humans? *Journal of Veterinary Behavior: Clinical Applications and Research*, *4*(2), 49.

Mujica-Parodi, L. R., Strey, H. H., Frederick, B., Savoy, R., Cox, D., Botanov, Y., ... & Weber, J. (2009). Chemosensory cues to conspecific emotional stress activate amygdala in humans. *PLoS One*, *4*(7), e6415.

Mutic, S., Parma, V., Brünner, Y. F., & Freiherr, J. (2015). You smell dangerous: communicating fight responses through human chemosignals of aggression. *Chemical senses*, *41*(1), 35-43.

Nagasawa, M., Murai, K., Mogi, K., & Kikusui, T. (2011). Dogs can discriminate human smiling faces from blank expressions. *Animal cognition*, *14*(4), 525-533.

Nelson, N. L., & Russell, J. A. (2013). Universality revisited. *Emotion Review*, *5*(1), 8-15.

Palestrini, C., Previde, E. P., Spiezio, C., & Verga, M. (2005). Heart rate and behavioral responses of dogs in the Ainsworth's Strange Situation: a pilot study. *Applied Animal Behavior Science*, *94*(1-2), 75-88.

Palmer, R., & Custance, D. (2008). A counterbalanced version of Ainsworth's Strange Situation Procedure reveals secure-base effects in dog–human relationships. *Applied animal behavior science*, *109*(2-4), 306-319.

Panksepp, J. (2004). Affective neuroscience: The foundations of human and animal emotions. Oxford university press, New York

Pause, B. M. (2012). Processing of body odor signals by the human brain. *Chemosensory perception*, *5*(1), 55-63.

Penn, D. J., Oberzaucher, E., Grammer, K., Fischer, G., Soini, H. A., Wiesler, D., ... & Brereton, R. G. (2007). Individual and gender fingerprints in human body odour. *Journal of the Royal society interface*, *4*(13), 331-340.

Pinelli, C., Rastogi, R. K., Scandurra, A., Jadhao, A. G., Aria, M., & D'Aniello, B. (2014). A comparative cluster analysis of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase histochemistry in the brains of amphibians. *Journal of Comparative Neurology*, *522*(13), 2980-3003.

Pongrácz, P., Molnár, C., & Miklósi, Á. (2009). Dog barking: A specific way of dog–human communication. *Journal of Veterinary Behavior: Clinical Applications and Research*, *4*(2), 54.

Prato-Previde, E., Custance, D. M., Spiezio, C., & Sabatini, F. (2003). Is the dog-human relationship an attachment bond? An observational study using Ainsworth's strange situation. *Behavior*, *140*(2), 225-254.

Prehn, A., Ohrt, A., Sojka, B., Ferstl, R., & Pause, B. M. (2006). Chemosensory anxiety signals augment the startle reflex in humans. *Neuroscience letters*, *394*(2), 127-130.

Racca, A., Guo, K., Meints, K., & Mills, D. S. (2012). Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *PLoS one*, *7*(4), e36076.

Rehn, T., McGowan, R. T., & Keeling, L. J. (2013). Evaluating the strange situation procedure (SSP) to assess the bond between dogs and humans. *PloS one*, *8*(2), e56938.

Rilling JK, Gutman DA, Zeh TR, Pagnoni G, Berns GS, Kilts CD (2002) A neural basis for social cooperation. Neuron 35: 395-405

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D’Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behavior. *Animal cognition*, *18*(4), 937-944.

Scandurra, A., Alterisio, A., & D’Aniello, B. (2016). Behavioral effects of training on water rescue dogs in the Strange Situation Test. *Applied Animal Behavior Science*, *174*, 121-127.

Scandurra, A., Alterisio, A., Marinelli, L., Mongillo, P., Semin, G. R., & D’Aniello, B. (2017). Effectiveness of verbal and gestural signals and familiarity with signal-senders on the performance of working dogs. *Applied Animal Behavior Science*, *191*, 78-83.

Schoon, G. A., & De Bruin, J. C. (1994). The ability of dogs to recognize and cross-match human odours. *Forensic science international*, *69*(2), 111-118.

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science*, *275*(5306), 1593-1599.

Semin, G. R. (2007). Grounding communication: Synchrony. In: Kruglanski, A. W., Higgins, E. T. (Eds.), *Social psychology: Handbook of basic principles* (pp. 630–649). Guilford Press, New York.

Semin, G. R., & De Groot, J. H. (2013). The chemical bases of human sociality. *Trends in Cognitive Sciences*, *17*(9), 427-429.

Siniscalchi, M., Sasso, R., Pepe, A. M., Dimatteo, S., Vallortigara, G., & Quaranta, A. (2011). Sniffing with the right nostril: lateralization of response to odour stimuli by dogs. *Animal behavior*, *82*(2), 399-404.

Siniscalchi, M., Pergola, G., & Quaranta, A. (2013). Detour behavior in attack-trained dogs: Left-turners perform better than right-turners. *Laterality: Asymmetries of Body, Brain and Cognition*, *18*(3), 282-293.

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2016). The dog nose "KNOWS" fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli. *Behavioral brain research*, *304*, 34-41.

Stevenson, R. J. (2009). The psychology of flavour. Oxford University Press, Oxford.

Sümegi, Z., Oláh, K., & Topál, J. (2014). Emotional contagion in dogs as measured by change in cognitive task performance. *Applied Animal Behavior Science*, *160*, 106-115.

Svartberg, K. (2006). Breed-typical behavior in dogs—historical remnants or recent constructs? *Applied Animal Behavior Science*, *96*(3-4), 293-313.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Computer methods and programs in biomedicine*, *113*(1), 210-220.

Thesen, A., Steen, J. B., & Doving, K. B. (1993). Behavior of dogs during olfactory tracking. *Journal of Experimental Biology*, *180*(1), 247-251.

Topál, J., Miklósi, Á., Csányi, V., & Dóka, A. (1998). Attachment behavior in dogs (*Canis familiaris*): a new application of Ainsworth's (1969) Strange Situation Test. *Journal of comparative psychology*, *112*(3), 219.

Topál, J., Gergely, G., Erdőhegyi, Á., Csibra, G., & Miklósi, Á. (2009). Differential sensitivity to human communication in dogs, wolves, and human infants. *Science*, *325*(5945), 1269-1272.

Udell, M. A., & Brubaker, L. (2016). Are Dogs Social Generalists? Canine Social Cognition, Attachment, and the Dog-Human Bond. *Current Directions in Psychological Science*, *25*(5), 327-333.

Udell, M. A., & Wynne, C. D. (2008). A review of domestic dogs' (*Canis familiaris*) human-like behaviors: or why behavior analysts should stop worrying and love their dogs. *Journal of the experimental analysis of behavior*, *89*(2), 247-261.

Udell, M. A., & Wynne, C. D. (2010). Ontogeny and phylogeny: both are essential to human-sensitive behavior in the genus *Canis*. *Animal Behavior*, *79*(2), e9-e14.

Udell, M. A., Dorey, N. R., & Wynne, C. D. (2010). What did domestication do to dogs? A new account of dogs' sensitivity to human actions. *Biological reviews*, *85*(2), 327-345.

Van der Ploeg, H. M. (1980). Validity of the Zelf-Beoordelings-Vragenlijst (A Dutch version of the Spielberger State-Trait Anxiety Inventory). *Nederlands Tijdschrift voor de Psychologie en haar Grensgebieden*.

Weisbard, C., & Graham, F. K. (1971). Heart-rate change as a component of the orienting response in monkeys. *Journal of comparative and physiological psychology*, *76*(1), 74.

Wyatt, T. D. (2015). How animals communicate via pheromones. *American Scientist*, *103*(2), 114.

Wynne, C. D., Udell, M. A., & Lord, K. A. (2008). Ontogeny's impacts on humanedog communication. *Age (weeks)*, *6*(8), 16.

Yong, M. H., & Ruffman, T. (2014). Emotional contagion: Dogs and humans show a similar physiological response to human infant crying. *Behavioral processes*, *108*, 155-165.

Zahn-Waxler, C., Hollenbeck, B., & Radke-Yarrow, M. (1984). The origins of empathy and altruism. In: Fox, M. W., & Mickley, L. D. (Eds.). *Advances in animal welfare science* (pp. 21-41). Kluwer Academic, Norwell.

Zhou, W., & Chen, D. (2009). Fear-related chemosignals modulate recognition of fear in ambiguous facial expressions. *Psychological science*, *20*(2), 177-183.

CHAPTER 9

BEHAVIORAL AND PERCEPTUAL DIFFERENCES BETWEEN SEXES IN DOGS: AN OVERVIEW

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Abstract

In this paper, we review the scientific reports of sex-related differences in dogs as compared to the outcomes described for wild animals. Our aim was to explore whether the differences in male and female dogs were affected by the domestication process, in which artificial selection is the main driver. For this purpose, we used information regarding personality traits, cognitive processes, and perception, for which there is a wide theoretical framework in behavioral ecology. Aggressiveness and boldness, described as a behavioral syndrome, were reported as being higher in males than females. Females also seemed more inclined to interspecific social interactions with humans in tasks that require cooperative skills, whereas males appeared more inclined to social play, thus implying different levels of social engagement between the sexes, depending on the context. Studies on cognitive processes underlined a greater flexibility in resorting to a particular navigation strategy in males. Most lateralization studies seem to support the view that males are preferentially left-handed and females are preferentially right-handed. Reports on visual focusing coherently rank females as superior in focusing on single social and physical stimuli. Only male dogs are able to discriminate kin; however, the timing of the olfactory recording in sexes is related to the stimulus relevance. Dogs are largely in line with life-history theories, which indicate that sex differences in dogs are mainly rooted in their biological and evolutionary heritage, remaining unchanged despite artificial selection. In contrast, the higher intraspecific sociability in wild male animals was not replicated in dogs.

Keywords: dog behavior, aggressiveness, boldness, navigation strategy, distractibility, lateralization, sociability, excitability, olfactory skill

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9.1 Introduction

In behavioral ecology, there has been increased interest in studies on individual behavioral differences in animals. Examples include studies of behavioral phenotypes, temperaments, or personalities in both vertebrate and non-vertebrate species (refer to Sih et al., 2004, for a review). These studies have underlined several inter-individual differences in different traits, such as aggressiveness (Budaev et al., 1999; Kralj-Fišer et al., 2007), activity levels (Øverli et al., 2002; Pavlova ey al., 2007), sociability (Kralj-Fišer et al., 2007), and boldness (Lopez et al., 2005). Individual competition may favor the expression of traits that improve reproductive fitness, although these specific adaptations are often costly in terms of energy and survival at both the morpho-physiological and behavioral levels (Fitzpatrick et al., 2005; Cain and Ketterson, 2012; Tobias et al., 2012).

Sex status is a biological trait that affects the determination of an individual's behavioral responses to physical and social environmental challenges, thus biasing the behavior of the sexes. The reproductive success of males and females depends on different factors. In the majority of studied species, females are more limited by the production and care of offspring, and their fitness is not enhanced by mating with multiple males, whereas males fitness is directly proportional to the number of females inseminated (Fitzpatrick et al., 2005; Bateman, 1948; Williams, 1996; Shuster and Wade, 2003; Rubenstein and Lovette, 2009; Rosvall, 2011). Moreover, specific traits may be actively selected and maintained by sexual selection. For example, differences in personality traits between males and females animals have been linked to sexual selection as an effect of intra-sexual competition and mate choice in both humans and non-human animals (Schuett et al., 2010).

In humans, cognitive processes such as visual-spatial perceptions and verbal and mathematical approaches follow different brain processing in men and women, although disputes remain with respect to this observation (Kimura, 1999; Halpern, 2000). Ethological studies also underline many behavioral sex differences in other animals (Becker and Koob, 2016). Prominent observations related to reproductive behaviors, such as parental care, mating strategies, and courtship displays, are almost exclusively expressed by only one of the sexes. These traits have been tagged as real "sexual dimorphism" (McCarthy et al., 2012) or "qualitative differences" (Becker and Koob, 2016). However, differences in behaviors not exclusive to reproduction are less obvious and may differ in magnitude between the sexes. Odor detection and stress responses, for example, fall in this category and are simply considered "sex differences" (McCarthy et al., 2012) or "quantitative differences" (Becker and Koob, 2016). In some cases, both sexes appear to exhibit the same behavior; however, the underlying neural substrate differs between them such that, under particular conditions, one sex might display a different behavior (sex convergence and divergence, McCarthy et al., 2012). For example, Lighthall et al. (2012) reported there were no significant sex differences in a human decision-making task; however, under the influence of a cold pressor stress, men showed a faster reward-related decision-making speed than females, thus indicating a clear sexual divergence in behavior. This effect was attributed to differential brain functions in the dorsal striatum and anterior insula, with an increased activation in men compared to women after the stress event. Finally, there may also be "population differences" in behavior, which indicates that the frequency of display varies between the sexes, although the pattern is consistent (Becker and Koob, 2016). For example, in most social mammals, males tend to disperse more than females (Greenwood, 1980).

The dog (*Canis lupus familiaris*) has evolved into a synanthropic species via a very long domestication process over the ages, which involved both natural and artificial selection. Co-evolution with humans has shaped the dog's cognitive processes accordingly, favoring behaviors that aim to optimize their adaptation to various anthropogenic environments. Dogs implement appropriate behavioral strategies in response to communicative cues from humans through different sensory channels. They are responsive to both verbal and non-verbal vocal sounds (Mills, 2005) and can recognize up to several hundred words (Kaminski et al., 2004; Pilley and Reid, 2011), with specific neural mechanisms that analyze and integrate word meaning and intonation (Andics et al., 2016). They have also evolved an acute sensitivity to human gestures (D'Aniello et al., 2016, 2017; Scandurra et al., 2017, 2018a; Grassmann et al., 2012). Moreover, it has recently been demonstrated that dogs are able to perceive human emotions via chemosignals, which suggests a type of olfactory communication (Siniscalchi et al., 2016; D'Aniello et al., 2018).

How dogs acquired these skills remains a subject of debate. The "domestication hypothesis" emphasizes genetic predispositions that may have enabled dogs to develop communicative skills attuned to humans (Hare et al., 2002; Hare and Tomasello, 2005; Topál et al., 2009; Miklósi and Topál, 2013), whereas the "two-stage hypothesis" leans more on ontogenetic aspects (Udell and Wynne, 2008, 2010; Wynne at al., 2008), implying that dogs may have learned from humans during their ontogenesis, thus shaping their behavioral responses (D'Aniello et al., 2015; Scandurra et al., 2015) and improving their social communicative skills (D'Aniello et al., 2017; D'Aniello and Scandurra, 2016). These two theories have been integrated into the "synergistic hypothesis," which suggests that sensitivity to human gestural cues may have emerged at both the evolutionary and developmental levels (Gácsi et al., 2009), although individual contributions of genetic and ontogenetic inputs have yet to be determined. The switch from natural (and sexual) selection to artificial selection may have imposed several deviations from what might be regarded as the natural situation. Co-habitation with humans may have directly diminished the selective pressure in dogs for essential survival traits (Price, 2002). For example, although dogs are able to utilize intraspecific observational learning (Range and Virányi, 2014; Scandurra et al., 2016), they have been shown to be less skillful than wolves in this behavior (Range and Virányi, 2013). As a side effect of artificial selection, the differences between males and females, which are maintained in nature through natural (and sexual) selection, could have changed during and after domestication. Furthermore, living often in close proximity with and depending by humans could have make less necessary to maintain sex-specific traits. In such a context, the dog may be an interesting model to investigate the effect of human directed effects on the roles of males and females and their interactions. Thus, the aim of this review is to describe whether the sex-specific differences identified in wild animals were affected by living in the anthropogenic niche in dogs, which include the domestication process and the ontogenetic acquisitions.

Papers on sex differences in dogs were first selected in our literature database, which is monthly updated regarding studies on dog behavior, periodically checking the journals included in the "behavioral science," "zoology" and "multidisciplinary sciences" categories. We subsequently enlarged our sample by specifically searching in the main online academic databases for "dogs sex differences" or "sex differences" coupled with keywords related to the specific personality trait, behavior or perceptive channel (e.g., aggress; behav phenotype, behav syndrome, behav trait, bold, domestication, fear, neophobia, olfactory, perception, personality, predator, selective strategy, sociability, temperament and visual focusing). Papers of interest were selected (e.g., physiological differences were excluded), and duplicates were eliminated. A further search was performed by reviewing the references in the selected papers, which revealed other missing studies (Table 9.1).

The studies have been compared and summarized according to our experience and framed in the theories of behavioral ecology. Several specific sex-related differences in dogs regarding personality traits (i.e., excitability and distractibility) and cognitive processes (i.e., smartness) for which naturalistic frameworks do not yet exist have been excluded.

9.2 Personality traits

9.2.1 Aggressiveness

Aggressiveness is a hostile behavior (e.g., threatening gestures or real attacks) that may inflict physical or emotional harm to one or more different targets and is performed with the intention to modify the behavior of a recipient. Intraspecific aggression is directed toward members of the same species, whereas interspecific aggressiveness is directed toward members of different species. Bouts of aggression may also be elicited following a threatening event not related to another living being. Displays of aggression are linked to the instinct of preservation and are well documented in predation scenarios, often evoking an aggressive defensive response (fear-induced) in the prey species. Aggression may also be aimed at defending a territory for monopolizing resources (e.g., food and mating partners) or achieving and maintaining a higher social status. Thus, from a behavioral ecology perspective, aggression is a tool to achieve a competitive advantage; however, it is a behavior that is energetically expensive, time-consuming and potentially dangerous (Fitzpatrick et al., 1995; Shuster and Wade, 2003). The energy employed in the expression of the aggressive behavior is no longer available for other functions, such as pregnancy and caring for offspring. For males, the cost of the aggressiveness to defend a territory and obtain access to females is balanced by ameliorative reproductive success, whereas females have fewer direct advantages, considering that the energy invested in the expression of aggressive displays is detracted by the functions related to the sex-specific behaviors linked to reproduction (Andersson, 1994). Thus, it is expected that females would express fewer aggressive behaviors than males in several contexts. In many species, including humans, a higher incidence of aggressive behavior has been reported in males and is well documented (Johnson, 1972; Leshner, 1978). However, in some species (e.g., pigs), males and females show the same level of aggressive behaviors (D'Eath and Lawrence, 2004) and in some cases, a sex-reversed trait, with more aggressive females, has also been identified. For example, as an effect of the particular social structure in which females are dominant, female spotted hyenas (*Crocuta crocuta*) appear to be consistently more aggressive than males (Glickman et al., 1987). Considering that dogs do not belong to a species with a sex-reversed role, more aggressive behavior is expected in males unless the domestication process has affected sex differences related to aggression.

Dogs often live in close relationship to human families, in which aggression would be an unwanted behavioral trait. It has been established that aggression is one of the most complex canine behaviors to define in terms of context, intensity, and target (Mehrkam and Wynne, 2014). Intraspecific aggression in dogs has long been acknowledged (Scott and Fuller, 2007) and represents a major behavioral problem, together with interspecific aggression toward both unfamiliar and familiar humans (Mehrkam and Wynne, 2014). Furthermore, aggressiveness is required in specific working dogs, such as military and guard dogs, although they always work under human supervision. In dogs, displays of aggression generally involve barking, growling, and biting, which are often exhibited in an escalating sequence and are accompanied or preceded by threatening or defensive postures, such as standing-over, staring, lunging, ears being pulled back, and the tail held down. The tendency for aggression seems to be a result of both environmental and genetic factors (Houpt, 2007; Barsky, 2010) and may be modulated by life experiences. Dogs that are appropriately socialized as puppies are less likely to exhibit aggression (Howell et al., 2015; Serpell and Jagoe, 1995). One of the oldest studies addressing sex differences in aggressive behavior in dogs was based on direct interviews with the owners (Borchelt, 1983). Several hundred cases involving aggression in dogs kept as companion animals were considered. The study aimed to delineate motivations that elicit aggressive responses and examined differences in the reactivity of males and females on a case by case basis. The results indicated that intraspecific aggression is the major motivations influenced by sex, with males expressing higher levels than females. In intraspecific aggression, females appeared to be aggressive predominantly toward other females. Aggression incidences have been reported to be higher in males than in females in many other studies (Hart and Hart, 1985; Cameron, 1997; Pérez-Guisado et al., 2006, 2008ab, 2009; Wright and Nesselrote, 1987).

A more recent report, aimed to investigate the effects of early maternal and litter factors on different behavioral traits measured in adult German Shepherds, showed a principal component (i.e., aggression) with a loading of 0.62. Females scored lower than males on aggression (Foyer et al., 2013). Displays of aggression were the results of an environmentally threatening event not related to another living being; thus, this study could not provide information regarding intra- or interspecific aggressiveness. Furthermore, a study on the personalities of Labrador Retrievers demonstrated a lower tendency of females, compared to males, in engaging aggressive behaviors toward the owner, whereas no differences between sexes were found for stranger-directed and intraspecific aggressions (Lofgren et al., 2014). In the same study, male German Shepherd dogs were classified as generally more aggressive than females. Intraspecific aggressiveness was not specifically tested in the latter study, a matter addressed by Rooney and Bradshaw (2004), which demonstrated that English Springer Spaniel, Labrador Retriever, cross-breeds, and Border Collie males were more aggressive toward other male dogs than conspecific females. In another study with 20 different breeds, investigators found that male dogs generally showed more aggression toward both other dogs and human strangers (Asp et al., 2015).

The effect of sex hormones in regulating aggressive behaviors is somewhat more complex. Several studies have reported that castration reduces intraspecific aggressive behaviors between males (Borchelt, 1983; Wright and Nesselrote, 1987; Hopkins et al., 1976; Knol and Egberink-Alink, 1989; Maarschalkerweerd et al., 1997; Neilson et al., 1997) as well as interspecific aggressive behaviors toward humans (Gershman et al., 1994). These outcomes concur with data that indicate dominance-related aggressiveness correlates with the levels of androgens in pet dogs (Leshner, 1978). However, in other studies, observations that castration reduced intraspecific (Hopkins and Schubert, 1976; Guy et al., 2001; Le Boeuf, 1970; Podberscek and Serpell, 1996; Hart and Eckstein, 1997; Casey et al., 2013; Garde et al., 2016) or interspecific aggression in male dogs (Casey et al., 2014) could not be confirmed. Rather, increased aggression in castrated dogs was also identified in some cases (Guy et al., 2001; Slauterbeck et al., 2004; Bamberger and Houpt, 2006; Kaufmann et al., 2017). Studies on female dogs appear to be more coherent, as a majority of outcomes report a higher level of aggressive behaviors in spayed females (Borchelt, 1983; Wright and Nesselrote, 1987; Gershman et al., 1994; O' Farrell and Peachey, 1990; Reisner et al., 2005; Kim et al., 2006). However, a recent study failed to replicate these data and indicated a contradictory lower incidence of aggression toward people (i.e., both familiars and strangers) in spayed female dogs (Casey et al., 2013).

9.2.2 Boldness and courage

In laymen's terms, words such as fearlessness, courage, bravery, dauntlessness, intrepidity, or boldness are often considered synonyms that indicate a particular mindset that equips one to face difficulty or danger. In human psychology, each of these terms has a distinct meaning. Courage, for example, as opposed to fearlessness, is identified as a behavioral approach to a task despite the feeling of fear (Cox et al., 1983), in the sense that a courageous individual could complete the same action as a fearless individual, despite experiencing fear (McMillan and Rachman, 1988). Personality studies consider boldness a "super-trait" identifying higher-order personality traits in humans and other animals (Zuckerman, 1994; Krueger et al., 2001), belonging to one end of the shy-bold axis in dogs (Svartberg and Forkman, 2002; Svartberg, 2002). According to the risk-reward hypothesis (Sih and Del Guidice, 2012), boldness makes individuals more proactive and explorative, which enables greater potential to gather resources; at the same time, it enforces more risks. Shyer individuals take fewer risks; however, they lose opportunities for foraging and mating, thereby reducing fitness (Réale et al., 2007; Wolf et al., 2007; Biro and Stamps, 2008). In the species in which the mating success of males depends on the time spent on the courtship, searching for females, and competing for access to partners, higher boldness is required to face the risk of being detected and caught by predators. In contrast, females may choose mates according to boldness, thus advantaging bolder males through sexual selection (Shuett et al., 2010). In this scenario, it is expected that males will be bolder than females. Studies across vertebrates (fish: (Øverli et al., 2006; Shuett and Dall, 2009; Johnsson et al., 2001;

Piyapong et al., 2009); reptiles (Ward-Fear et al., 2018); birds (Van Oers et al., 2005; Atwell et al., 2012); mammals (Dammhahn, 2012; Petelle et al., 2013)) have coherently demonstrated a higher boldness in males compared to females. However, the direction of the sex difference for this personality trait is likely to depend upon ecological factors, as in the case of the hyena females that turned out to be bolder than the males (Gosling, 1998). In invertebrates, females appear systematically bolder than males (Gyuris et al., 2011; Hedrick and Kortet, 2012).

It has been observed in many species that boldness correlates with aggressiveness: individuals more likely to take risks by engaging in intraspecific aggressive fights also appear to risk more when confronted with environmental hazards, such as predators (Gosling, 1998; Koolhaas et al., 1999; Bell and Stamps, 2004; Groothuis and Carere, 2005). This recurrent correlation has enabled scholars to individuate a specific aggression–boldness syndrome (Sih et al., 2004). Thus, considering that most of the researches report a higher level of aggressiveness, it is expected that male dogs will exhibit greater boldness, which proved to be the case in the research on the matter. In dogs, boldness is described as an individual characteristic providing less aversion to risk or novelty that enables the subject to actively seek out and engage in social interactions (at both cooperative and competitive levels), as well as toward non-social objects or events (Svartberg and Forkman, 2002; Svartberg, 2002, 2005; Wilson et al., 1994; Turcsán et al., 2011; Carter and Feeney, 2012; Starling et al., 2013; McDermott et al., 2014). One of the main components to consider when defining boldness seems to be the level of neophobia (Toms et al., 2010), although it has not been considered as an indicator of some investigations (Beckmann and Biro, 2013).

We have attempted to consolidate all studies related to sex differences that specifically discuss boldness, including studies concerning fear responses and courage. One of the first studies conducted in relation to sex difference–related fear responses was an investigation of several components of behavior used to select Alsatian dogs (e.g., German shepherds) for the Swedish Army (Reuterwall and Ryman, 1973). Based on the trainers' evaluations, the authors reported that there were no differences between males and females in courage (measured as a response to an approaching man-shaped figure) and responses to sudden disturbances. However, it was noted as a sub-classification of the latter response that females were more susceptible than males to gunfire. In the following study aimed at determining the factors that affect the suitability of subjects as guides for blind people, female guide dogs (mostly Labrador and Golden Retrievers) were rejected more often than males because of problems related to fearfulness (Goddard and Beilharz, 1982, 1983). The latter studies were based on the trainer's scoring, in which the authors attempted to correlate behavioral and genetic traits with influencing environmental factors. Investigations based on trainer assessments are often considered inconsistent (McKenzie, 2010), inasmuch as scores vary greatly in consistency between trainers (Goddard and Beilharz, 1983). However, these findings were confirmed in a follow-up study based on experimental designs aimed at evaluating the quality of puppy walking that guide dogs experienced, correlated with measurements of exploration and activity (Goddard and Beilharz, 1984). The follow-up study was conducted on Labrador Retrievers, German Shepherds, Boxers, Kelpies, and F1 crosses, with dogs tested at six and 12 months of age (during the puppy walking time) and again when they were returned to the Guide Dog Center for training (final testing). The tests measured different behaviors related to the general activity of the dogs and the willingness to respond to commands. Goddard and Beilharz (1984) determined that in unfamiliar, crowded, and noisy places both olfactory exploration and neophobia were increased. In these circumstances, females showed higher levels of olfactory exploration than males, thus indicating a heightened fear response. In a study by Wilsson and Sundgren (1997) on different behavioral characteristics in Labrador Retrievers and German Shepherds, males scored higher than females in courage in both breeds, as evaluated by seven test situations. Results from further studies regarding boldness and fearlessness as behavioral traits fall along the same lines. One study investigated the correlation between the personality and performance of Belgian Tervurens and German Shepherds in working dog trials (Svartberg, 2002). The dogs were subjected to a variety of different tests, and the sexes were compared using a boldness score, which was extracted by a factor analysis of different behavioral outcomes. Males scored higher than females. Similar results were obtained in a study that aimed to examine the genetic covariation of behavioral traits in German Shepherds, in which males appeared bolder than females (Strandberg et al., 2005), as indicated by dog mentality assessment tests (Fält, 1997).

In another study, using 14,004 questionnaires on different breeds directed to owners in Germany, Kubinyi et al. (2009) applied a principal component analysis to 24 items, obtaining the boldness factor described by the traits reserved, aloof, and fearful with scores of up to 0.7. The following analyses demonstrated that boldness was age-dependent, with younger male dogs (younger than two years) scoring higher on the boldness factor scale than older dogs or female dogs. Overall, intact males were the boldest group, whereas spayed females were the least bold. Similar results obtained from personality surveys circulated among Australian dog owners (Starling et al., 2013) also confirmed the negative effect of neutering on boldness in both sexes. A principal component analysis produced "boldness" as a factor in which social traits scored higher positive loadings, whereas avoidance and other fear-related behaviors showed higher negative loadings. In a recent study (Asp et al., 2015), breed and grouping effects (working/non-working) on everyday behavior, in a sample of 20 different breeds of Swedish dogs, showed that male dogs exhibited fewer conspecific-directed (fearful response to unfamiliar dogs) and stranger-directed (fearful response to unfamiliar person) fears compared to female dogs, whereas no significant sex difference toward the owner was observed.

9.2.3 Sociability

Many species live in complex social structures in which affiliative interactions prevail against anti-social behaviors, such as aggression and territoriality. In humans, sociability is defined as an attitude of taking into account other individuals to achieve a goal. In the animal studies, Réale et al. (2007) provided a terminology for the sociability to be used as a working tool for ecological studies of temperament. According to the authors, the "sociability is an individual's reaction to the presence or absence of conspecifics (excluding aggressive behavior). Sociable individuals seek the presence of conspecifics, while unsociable individuals avoid conspecifics."

In the realm of behavioral ecology, it is proposed that the social behavior of males and females is differentially targeted by selective pressures. Males are principally devoted to access to females, whereas females privilege other resources (Van Schaik, 1996; Sterck et al., 1997). In this context, males should tend to be more aggressive than females in social behavior because the socioecological theory predicts that social contacts in males increase reproductive success, thus enabling the animal to reach a high rank in the hierarchy or establish alliances (Wrangham and Smuts, 1980; Muller and Mitani, 2005). In many species, such as primates (Furuichi and Ihobe, 1994; Watts, 1998) and dolphins (Connor et al., 1992), males establish alliances, whereas female dolphins have been observed to form more dynamic social bonds (Smolker et al., 1992; Frère et al., 2010). In some primate species, males appear to develop greater social behavior than females very early in life: male infant chimpanzees show more social interaction than females, and they also interact with more adult males than females (Lonsdorf et al., 2014). In the same way, human boys have been reported to be more social, playing in larger groups than girls (Maccoby and Jaklin, 1987; Fabes et al., 2003). In contrast, in a study on intraspecific sociability, male dogs appeared to be less sociable than females, with a pronounced effect in dogs belonging to the 4–8 years age group (Starling et al., 2013). In the latter study, sociability was extracted by four traits (i.e., friendly, quarreling, bullying, and kindness) that received high loading in a principal component analysis.

Although studies of animal intraspecific sociability in wild and captive animals have been abundant, interspecific sociability has barely been investigated, which may be a result of the difficulty of studying cooperating species. In this context, considering their long cooperative story with humans, dogs are very appropriate models. A recent study has suggested that canine sociability may be the result of the canine homologous Williams-Beuren syndrome (Shuldiner et al., 2017), a genetic disorder that in humans causes hyper-sociability, among other symptoms (Dykens et al., 1999). Despite the overall high sociability in dogs, there are indications that females may be more likely to interactions with humans. One of the first reports showing sex differences in sociability was performed in 20 intact pet dogs of different pure and mixed breeds (Lore and Eisenberg, 1986). The dogs' reactions to an unfamiliar person were assessed by their responses to male and female human-reaction tests. The results showed that female dogs were friendlier and would make physical contact with a stranger.

Support for the hypothesis that female dogs are more social in interspecific interactions was obtained in the context of results from tests that assessed decision-making mechanisms, such as the impossible task paradigm. The impossible task paradigm (Topál et al., 1997) is similar to the problem-solving paradigm: the subject initially learns to solve an easy task, which in the next phase of the test becomes impossible to solve, thus raising an expectancy violation that forces the subject to pursue the objective alone or ask for help from human counterparts (D'Aniello and Scandurra, 2017). This paradigm has been very useful to investigate canine social interactions with known people and strangers (D'Aniello et al., 2015; Scandurra et al., 2015; D'Aniello and Scandurra 2016). Researchers have applied this paradigm to investigate the responses of a Beagle population living in a kennel under standard conditions (Persson et al., 2015). The participants did not have a precise reference figure, as the researcher was the only human reference in the test. The results showed that females outperformed males when they encountered the impossible phase of the task, with higher social interactions with the experimenter, including alternating interactions between the apparatus and the experimenter. Although the study included only one breed, a large sample size ($n = 498$) was tested, indicating that the tendency for higher sociability observed in females may be genetically encoded (Persson et al., 2015). Similar results were obtained in our work with the impossible task paradigm applied to different breeds (D'Aniello and Scandurra, 2018). Our experimental setting provided an option of two human partners, the owner and a stranger, who did not touch the container or the food (for details refer to D'Aniello et al., 2015; Scandurra et al., 2015). Re-analyzing our database to filter for the sex of the dogs, we determined that, as previously reported (Persson et al., 2015), females were more willing to interact with the stranger than males, whereas no sex differences were identified for the owner. Further support for a higher tendency among female dogs for socialization with humans was obtained from a study on German Shepherds that aimed to evaluate the correlation of early maternal and litter traits with different behavioral traits measured in adult dogs at the Swedish Armed Forces (Foyer et al., 2013). Behavioral traits were extracted from a temperamental test used by the armed forces to select suitable work dogs. The Swedish Armed Forces and the researchers (Wilsson and Sundgren, 1997) used a modified version of the Dog Mentality Assessment test, including 12 standardized sub-tests. A principal component analysis showed that female German Shepherds scored significantly higher than males in social engagement.

Despite this body of evidence highlighting that female dogs engage more in social interaction with humans, other data seem to be contradictory. Using a questionnaire distributed to dog owners, Asp et al. (2015) investigated 20 breeds registered at the Swedish Kennel Club, limiting the bias from subjective descriptions of a dog by using a large number of independent discrete responses (refer to Jones and Gosling, 2005). In this study, male dogs were found to be more interested in human-directed play. These results mirrored the findings obtained in Dog Mentality Assessment standardized tests on German Shepherds used by the Swedish Working Dog Association, in which males scored higher in social play than females (Strandberg et al., 2005).

9.3 Cognitive processes

9.3.1 Spatial cognition

Spatial cognition is the internal understanding and recollection of space (Golledge, 2004) and concerns the study of our awareness of objects and events in the world (Montello, 2015). Spatial navigation is a process that enables animals to know their surroundings and identify the optimal path to their targets using multiple resources such as path integration, magnetic cues, and different landmarks (Brodbeck and Tanninen, 2012). This process involves memorizing specific landmarks, positions, and locations to create a cognitive map that enables one to orient and navigate oneself through the surrounding environment.

In mammals, males typically show greater prowess in spatial navigation tasks, likely because of a relevant function in reproduction (Astur et al., 2004; Shah et al., 2013). The males' advantage in solving spatial navigation tasks has been linked to the competition for mating, which often requires a larger territory (Pal et al., 1998; Ecuyer-Dab and Robert, 2004), whereas the females' major involvement in reproduction may have favored a superior spatial sense in more restricted areas (Ecuyer-Dab and Robert, 2004; Herman and Wallen, 2007). Males utilize an allocentric navigation strategy based on the relative positions of environmental landmarks, whereas females rely more on an egocentric navigation strategy, predominantly referring to their motor responses (Herman and Wallen, 2007; Hawley et al., 2012; Jonasson et al., 2004; Waller and Nadel, 2013).

Using a wide range of spatial skills, dogs can solve different spatial tasks, including both egocentric and allocentric signals depending on the task (Chapuis et al., 1983; Cattet and Etienne, 2004). They can integrate spatial signals during locomotion, continuously updating information on the distance from and direction to a particular object (path integration; Cattet and Etienne, 2004). Based on studies regarding mammals, it is expected that the use of navigation strategies differs in male and female dogs. In a study that investigated dogs' flexibility in the acquisition of spatial information through social learning (Fugazza et al., 2017), the dogs were tested in the "Do as I Do" paradigm (Topál et al., 2006) that required the dogs to reproduce actions demonstrated by humans. First, it was determined whether the dogs preferred an egocentric or allocentric strategy for recalling the demonstration of the owner. Once an allocentric strategy was confirmed in this context, dogs were forced to switch to an egocentric strategy by rotating the targets by 90% and withholding a reward until it touched the correct (egocentric) target. It was shown that male dogs switched from the preferred (allocentric) to non-preferred (egocentric) strategy in fewer trials than females. Another study tested the navigation skills of dogs in an indoor T-maze paradigm (Mongillo et al., 2017). The dogs' ability to learn the correct exit path from the maze was initially first tested, and a recall memory test was performed after two weeks to assess whether they retained the information acquired in the learning task. Finally, the dogs were tested in a reversal-learning task that aimed to evaluate the dogs' ability to modify previously acquired information regarding the correct exit path. The results showed that intact females had a better performance in the learning task than ovariectomized females and intact males. In a third study by the same group (Scandurra et al., 2018b) dogs' spatial skills were tested in an indoor plus-maze. After a learning phase to acquaint the dogs with the location of food in the maze, their preference toward egocentric or allocentric information was assessed. The dogs subsequently underwent a reversal-learning phase to force them to change their preferred navigation strategy. Sex-related differences were absent from the strategy preference in such a context. However, ovariectomized females were significantly more likely to prefer an egocentric strategy. An interesting result in the plus-maze study was that the probability of successfully resorting to the non-preferred strategy increases with age in females, whereas it decreases in males (Scandurra et al., 2018b).

9.3.2 Lateralization

Lateralization has been extensively investigated as a physical measure of the brain's asymmetry (Coren and Porac, 1977; Harris, 1983; Springer at al., 1989) and is manifested as a bias in performing motor or sensory tasks based on the dominant hemisphere (Batt et al., 2008). It has been established that the left hemisphere of the brain controls the expression of behavioral patterns in non-stressful situations, whereas the right hemisphere controls behaviors in unexpected or dangerous situations that require fight and flight responses (Rogers, 2010). In some vertebrates, it has been reported that aggressive responses toward conspecifics are performed predominantly from their left side (Vallortigara and Rogers, 2005; Ariyomo and Watt, 2013; Austin and Rogers, 2014).

In dogs, lateralization has been identified in different functions. Studies have shown that domestic dogs display a left gaze bias when viewing human faces (Guo et al., 2009) and that the emotional valence of facial expressions affected this behavior (Racca et al., 2012). Similarly, dogs show a lateralization of tail-wagging (Quaranta et al., 2006) and olfactory (Siniscalchi et al., 2016) and acoustic processing (Siniscalchi et al., 2008) following negative and positive stimuli, thus providing support for hemispheric specialization.

A paw preference test paradigm is a common tool adopted for the study of motor function lateralization. Sex differences in paw preferences have been reported in cats, with females showing greater use of their right paw, whereas males preferred their left paw (Tan et al., 1990; Yetkin, 2002; Wells and Millsopp, 2009). Similar outcomes were also reported in primates (Hopkins and Leavens, 1998) and horses (Murphy et al., 2005). In humans, a meta-analysis of studies on handedness indicated more left-handedness in males (Sommer et al., 2008). This pattern of different lateralization in males and females is challenging to interpret, particularly in light of the theory that bilateral symmetry is an evolutionary adaptation (Corballis, 1983). Most environmental actions could be better solved by having the opportunity to use both paws indifferently, without the need to change the position of the body to enable the use of a dominant limb. Thus, it should be expected that animals would not show paw preferences, irrespective of sex. However, considering the previous findings in domesticated species and primates it is expected that there is a higher number of left-pawed male dogs than females.

One of the first studies that addressed paw preferences in dogs demonstrated that more than 50% were right biased, approximatively 18% were left-biased, and 25% were ambidextrous (Tan, 1987). The subjects were blindfolded with adhesive plaster and subsequently allowed to attempt to remove it from the eyes using their preferred paw. In another study, dogs were tested in three different tasks (Wells, 2003). The first task was an arbitrary action in which the participants were instructed to give a paw after sitting, the second task involved an action directed toward a flannel blanket that the dogs had to remove from over their heads, and the third task was a food retrieval task from a metal can. Interestingly, males and females showed contrasting paw preferences, in which females preferred their right paw, whereas males were biased to their left paw. These results were confirmed in a follow-up study in which dogs were required to remove a piece of adhesive paper from the snout (Quaranta et al., 2004). In a more recent study, paw preferences were tested using a Kong toy, a hollow conical-shaped rubber toy stuffed with food (Wells et al., 2016). However, in this case, the results were contradictory to previous studies, as significantly more male dogs were classified as right-pawed, whereas females were shown to be ambidextrous. Despite the assumption that the paw used to stabilize the Kong was the dominant paw, the researchers claimed that the participants used their non-preferred paw to stabilize the toy, which mirrors the results obtained by similar work on lateral limb use in humans (Bagesteiro and Sainburg, 2002). Although this interpretation of the results is in line with the previous analogous human studies, other research groups have not yet been able to replicate these data. Branson and Rogers (2006) and Schneider et al. (2013) also investigated paw preferences using the same Kong paradigm of Wells et al. (2016); however, they did not substantiate differences in paw preference between males and females. Poyser et al. (2006) also tested dogs in different tasks that replicated the paradigms of Wells [189] and Quaranta et al. (2004) and did not identify a significant association between paw preference and sex. In line with previous studies, Poyser et al. (2006) underlined a tendency of males to use the left paw in the first trial with a significantly shorter latency of usage. However, this effect was less pronounced with repeated presentations of the test and was not recorded in females, thus prompting the interpretation that behavioral lateralization was labile in dogs and might be influenced by hemispheric effects only responding to novel stimuli.

In an attempt to interpret the inconsistency in the literature, Tomkins et al. (Tomkins et al., 2010) considered that different tests could produce different results regarding lateralization. For example, the Kong test focused on food retrieval and tape-removal with the aim to relieve discomfort may have posed different challenges to the participants and thereby influenced the effects (Batt et al., 2008). Moreover, studies have suggested that task complexity may influence the results of lateralization paradigms (Corp and Byrne, 2004; Trouillard and Blois-Heulin, 2005).

9.4 Perception

9.4.1 Visual focusing

Visual focusing is an animal's ability to concentrate and distinguish significant perceptual cues in the immediate physical environment at any given time. In humans, a study aimed to reveal sex differences in exploratory eye movements measured the exploratory eye movements of normal subjects (39 male and 39 female) using an eye-mark recorder. A wide set of the picture was projected onto a screen (e.g., open circle, happy face, different animals, the sun, an airplane, five trees, a house, two mountains, and a river). The results revealed that the mean gazing time of adult women looking at pictures was consistently longer than that of men, while the environmental scanning length of adult men was higher (Miyahira et al., 2000). This finding may be an effect of the greater need for vigilant behavior in men (Giambla and Quilter, 1989) as well as in other male animals (Cowlishaw, 1997; Cameron and du Toit, 2005). Based on the results obtained in humans, it is expected that female dogs will have a greater tendency to focus on a single target.

In the context of social cognition, a study on shepherds and Molossoid dogs showed that when an unfamiliar person approached, females displayed more referential gazing and gaze alterations between the owner and the stranger (Duranton et al., 2016). Similarly, Mongillo et al. (2016) reported that females looked more at their owners in a room where the owner was talking to a stranger. Although castrated males were not included in the cited study, intact females showed more gazing behavior than spayed females, thus also underlining a potential effect of female sex hormones in gazing behavior. Females also seem to be responsive to intranasal oxytocin increasing the gazing behavior toward the owner (Nagasawa et al., 2015; Kovács et al., 2016); however, sex differences with regards to oxytocin have not been reported in other studies (e.g., Kis et al., 2015). Female dogs also seem to rely more on visual signals than males in their behavioral regulation. A study that aimed to assess the preferred communicative channel between verbal and gestural messages underlined a preference for visual cues in dogs (D'Aniello et al., 2016). A group of Labrador and Golden Retrievers, after acclimatization to four common actions (i.e., sit, down, stay, and come) using bimodal gestural and verbal messages, were subjected to three different conditions. The dogs were required to perform the actions using verbal commands only, gestural commands only, and then in conflicting bimodal condition in which the gestural and verbal messages were directed toward opposite actions. The results showed that in the absence of visual signals, females made more mistakes than males, which indicates a greater dependence of female dogs on visual cues.

Physical cognition is concerned with the understanding of and interaction with the physical world and the different objects in it. The studies in this domain typically focus on an animal's problem-solving skills with tools and processing the considerable complexity of their environment. In a study by Müller et al. (2011), male and female dogs were tested in an object permanence task in the expectancy-violation paradigm. Unexpected and expected events were presented to the dogs: a ball disappearing behind a screen and another ball of different (unexpected) or the same (expected) size reappearing on the other side. Females reported longer gazing times than males in the unexpected than in the expected conditions, thus demonstrating that females respond better to object permanence violation. The effect was independent of sterilization status in both sexes. In another study, dogs and different ape species were compared in their physical cognitive abilities using a transposition task under the same expectancy-violation paradigm (Rooijakkers et al., 2009). No significant differences were identified between males and females; however, females showed a trend ($p < 0.069$) toward better performance in the object permanence task. In contrast to social cognition (Mongillo et al., 2016), sex differences identified in physical cognition appeared to be independent of sex hormones, as sterilization had no effect on performance (Müller et al., 2011). These differences may be related to different information-processing strategies between the sexes that are acquired as a brain organizational effect in early life (Müller et al., 2011).

9.4.2 Olfactory skills

Kin discrimination in animals is closely linked to various social and genetic benefits, including preferential treatment of kin (nepotism) in parental care or cooperative behavior, which facilitates a functional social structure (Tang-Martinez, 2001). Furthermore, kin discrimination avoids inbreeding that reduces individual fitness resulting from reproduction among relatives, a well-established phenomenon in both natural and experimental populations (Charlesworth and Charlesworth, 1999; Keller and Waller, 2002), although kin are preferred mate partners in some species (e.g., Cichlid fish *Pelvicachromis taeniatus,* refer to Szulkin et al., 2013).

Hepper (1994) demonstrated that both puppies and adults can discriminate their siblings and mother, and mothers can discriminate their offspring by olfactory cues. Adult siblings could discriminate one another only if they had co-habited. Although poorly represented in subsequent works, Hamilton and Vonk (2015) specifically addressed sex differences by demonstrating that male dogs were able to discriminate kin without the prerequisite of familiarity, whereas females did not show such abilities. Although the reason for this difference remains uncertain, it should be underlined that females in the diestrus phase were tested, so the ability to discriminate kin may be activated during the estrus phase. Other studies on olfactory use have reported that male dogs tend to sniff the vaginal secretion odor more than females, whereas females investigated for longer periods of time with respect to food odor (Siniscalchi et al., 2011).

9.5 Discussion

This paper is the first comprehensive review reporting sex differences in dogs regarding personality traits, cognitive processes and perception. Although the literature is ample for some traits, thus enabling us to draw several patterns, only limited indications are present for other traits. From the data included in this review, it appears that males tend to be more aggressive and bolder than females, whereas a lower level of intraspecific sociability in males was reported. Females seem more inclined to interspecific social interactions with humans in tasks that require cooperative skills, whereas males appear more likely to interspecific social play. Studies of spatial skills underlined a higher flexibility in resorting to a particular navigation strategy in males in an outdoor environment; however, females appear to be better at spatial learning tasks in restricted areas. Lateralization studies seem to support the view that males are preferentially left-pawed and females are preferentially right-pawed; however, some studies have failed to replicate these results. Reports on visual focusing rank females as superior in focus on specific social and physical stimuli. In olfactory monitoring activity, only male dogs are able to discriminate kin. For other stimuli, the use of olfactory recording may be related to the differential relevance that olfactory signals have for males and females.

With regard to aggressiveness, it should be noted that the number of different contexts in which the dogs were tested appears to be limited. For example, it is expected that, in some circumstances (e.g., in defense of offspring), females will be more aggressive than males. Furthermore, because of the inherent differences in aggressive scores between breeds (Asp et al., 2015, Duffy et al., 2008), females of some breeds might appear to be more aggressive than males of other breeds. Moreover, some studies on aggressive behaviors have been obfuscated by the methodological limitations that result from the involvement of samples that are not representative of the complete dog population (refer to McKenzie, 2010) or because they are based on owner surveys. Direct and indirect methods for measuring aggression have been shown to have a low reliability (Mehrkam and Wynne, 2014). In contrast to the trend, a study on bite incidences toward people surprisingly indicated that female dogs were almost three times more likely to have bitten humans than male dogs, particularly in cases of small-sized species (Guy et al., 2001). However, it should be emphasized that, in this study, a high percentage of sterilized dogs (87.5%) were used, with significantly more spayed females than castrated males. As ovariectomy results in increased aggressive behaviors in females (Borchelt, 1983; Wright and Nesselrote, 1987; Gershman et al., 1994; O' Farrell and Peachey, 1990; Reisner et al., 2005; Kim et al., 2006), neutered females may have partially biased the results of Guy et al. (2001). Because of these limitations, the conclusion that male dogs are more aggressive would seem not robust. On the other side, male dogs were also reported systematically bolder than females, coherently with the behavioral syndrome with which boldness and aggressiveness positively correlate (Sih et al., 2004). Despite the limitations in some studies, the patterns described in most of the studies are consistent with the theory of behavioral ecology, predicting that the higher level of aggression had greater positive consequences in terms of fitness for males (Andersson, 1994).

Male and female dogs show different levels of sociability. Engagement in dog-human interspecific social play male dogs show more social contact than females, whereas in cooperative behavior in trying to solve a problem, the opposite trend has been found. An increased interest in intraspecific social play was identified in male primates (Maccoby and Jacklin, 1987; Maccoby, 1988; Meredith, 2013); however, it was not reported in dogs, in contrast to the sociobiological theories predicting higher social behavior in males.

Studies on spatial navigation underline that male dogs outperform females in reverting the navigation strategy in a "Do as I do" paradigm (Topál et al., 2006); however, no sex differences were identified in reversal-learning in the T- and plus-maze paradigms (Mongillo et al., 2017; Scandurra et al., 2018). In a T-maze paradigm, the better performance of the intact females in the learning task may be linked to the superior spatial ability of females in restricted areas reported for other mammals (Ecuyer-Dab and Robert, 2004; Hamilton and Vonk, 2015). An interesting outcome in a plus-maze study is the different effect of age between sexes, with a positive correlation in females and the opposite trend in males (Scandurra et al., 2018). Consistent with the reports in most mammals, females tend to disperse from their natal group less frequently than males (Greenwood, 1980). Thus, dogs likely require more experiences across the lifespan to learn to resort to navigation strategies. In contrast, younger male dogs tend to disperse more frequently than adults (Pal et al., 1998). Thus, it is possible that they are predisposed to use flexibly different spatial information in the early years of life to cope with unpredictable environments, in line with the major skill in the navigation strategies identified in males in other mammals (Astur et al., 2004; Shah et al., 2013) and the theoretical predictions (Pal et al., 1998; Ecuyer-Dab and Robert, 2004; Herman and Wallen, 2007). Sexual hormones seem to affect the spatial cognition since ovariectomized females are more likely to prefer an egocentric strategy than an allocentric one (Scandurra et al., 2018). An effect also underlined in rats in which a bias toward the use of egocentric rather than allocentric strategies was observed (Gold et al., 2001; Karol and Manning 2001; Karol and Kolo, 2002).

Most studies reported male dogs to be prevalently left-pawed, whereas females more frequently use the right paw. These outcomes are in line with the study of paw preferences in other domesticated animal species (Tan et al., 1990; Wells and Millsopp, 2009; Hopkins and Leavens, 1998; Murphy et al., 2005) and in captive primates (Hopkins and Leavens, 1998). The observation of no paw preference in dogs may be the result of a weak effect, making it difficult to obtain statistical significance. The right paw preference in males reported in Tan (1987) may be because there were near twice as many females as males in their study and the sex as a factor was not controlled or accounted for in the analysis.

Studies on visual focusing in dogs are in line with the results from similar studies in humans and other mammals (Jones and Healy, 2006), which indicate that females reserve more attention for specific visual cues than males. The lower attention for single visual signals in males may be the result of greater vigilant behavior, which leads males to switch from one visual stimulus to another more often (e.g., giraffes: Cameron and du Toit, 2005; baboons: Cowlishaw, 1997; humans: Giambla and Quilter, 1989), thus not allowing them to focus their attention on a single target for a long time. Moreover, it is theorized that in the wild, male animals with a higher level of vigilance may be more attractive to choosy females because they can offer more security against predation (Dahlgren, 1990; Ridley and Hill, 1987). There is also a male advantage from higher vigilance in preventing the behavior of sexual competitors Cowlishaw, 1997; Cameron and du Toit, 2005). Alternatively, the lower rate of visual focusing in male dogs may also be a side effect of their higher distractibility (Goddard and Beilharz, 1982, 1984). The higher visual focusing ability of females has direct implications for dog-human communications: owners and trainers could spend more effort on obtaining sustained visual attention from males, which is an important prerequisite to communication with dogs (Miklósi et al., 2005).

How do sex differences in dogs conform with the naturalistic scenario from which they originate? Along the domestication process, in which natural selection was replaced by the artificial selection, dogs seem to have maintained the sex differences in the aggressiveness-boldness syndrome described in wild animals (at least in the studied contexts). Researches in spatial cognition in restricted areas reporting better performance in females in the learning task agree with that reported for the other mammals (Ecuyer-Dab and Robert, 2004; Hamilton and Vonk, 2015). Also, studies reporting male dogs to be prevalently left-pawed and females right-pawed are in line with the study of paw preferences in not domesticated species (Hopkins and Leavens, 1998), but also with other domesticated animal species (Tan et al., 1990; Yetkin, 2002; Wells and Millsopp, 2009; Murphy et al., 2005). The sex differences in dog sociability seem not agree to the previsions requiring males more social, apart from the social play with humans in which male dogs seem to show a higher tendency than females. It should be emphasized that most parts of studies on sociability are centered on interspecific interaction, for which is not possible to make a comparison with wild animals. Thus, a more conservative view should be maintained for this personality trait.

In summary, the main outcome obtained by our work is that despite 30,000 years of domestication during which artificial selection was the main driver (Thalmann et al., 2013), dogs have largely maintained the sex differences described in wild animals. Overall, these reports suggest that sex differences in dogs are mainly rooted in their biological and evolutionary heritage. However, in contrast to the trend, these results failed to indicate an enhanced intraspecific sociability in male dogs, which may be a side effect of living in an anthropogenic niche.

9.6 Future directions

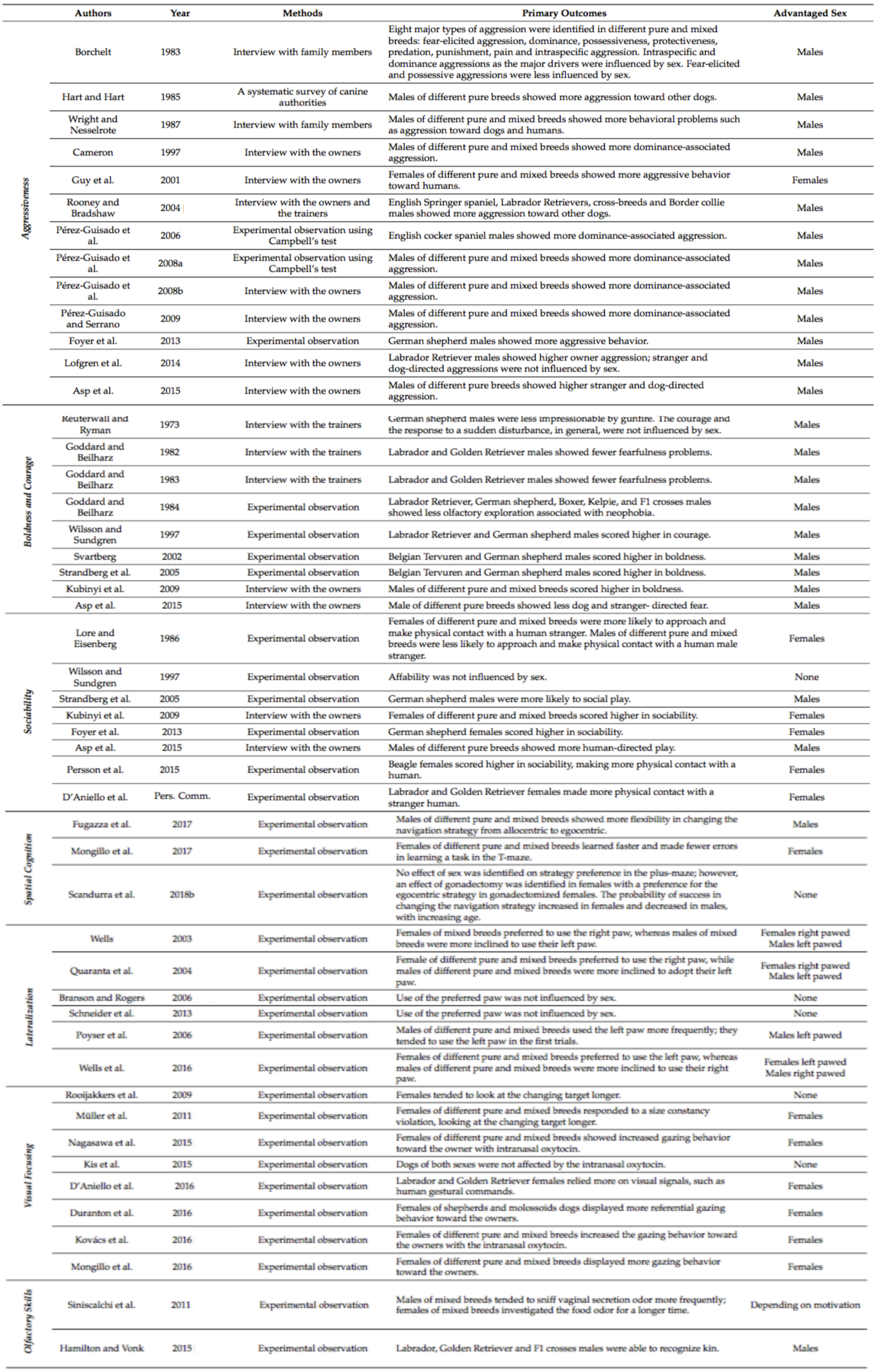
From our overview, it appears that studies on dog sex differences are largely biased, with some traits being largely ignored. This imposes a challenge in reaching more robust generalizations. For example, studies on olfactory skills are very limited, whereas olfactory exchanges are particularly important as communicative tools in dogs. In humans, a different use of olfactory monitoring is established, with females appearing to be more effective in the studied contexts (Brand and Millot, 2001), whereas in dogs, the field is completely open. This is a crucial aspect, particularly in light of the different strategies that male and female dogs adopt to achieve reproductive success.

Another field that requires exploration is the ontogenesis of personality traits. It is established that dogs appropriately socialized as puppies are less likely to exhibit aggression (Howell et al., 2015; Serpell and Jagoe, 1995); however, nothing is known about the other personality traits. The effect of the type of human-dog relationship in shaping dogs' personalities may be very important. For example, regarding the time spent together, living conditions (home, garden), and training, the inclusion of the dogs' sex in the variables may provide new insights and should be applied to all sex-specific variables. Some traits were more consistently investigated by many authors; however, many gaps remain to be filled, and inconsistencies in the results must be resolved.

With regard to aggressiveness, future studies should be targeted to gauge the aggressive behaviors directed toward different targets, such as familiar or unfamiliar humans, dogs, and other animals separately, because these factors seem to be uncorrelated (Asp et al., 2015; Casey et al., 2013). Different motivations (i.e., dominance, territorial dispute, fear, and defense) should also be explored separately. The interaction of these factors should be tested in structured studies to delineate a clearer pattern. Moreover, it should be noted that in the majority of studies, the authors concentrated exclusively on physical aggression, whereas incidences not culminating in real attacks, such as threat gestures, are largely underestimated. In humans, there are indications that female criminals are more likely to commit crimes during the menstrual phase, whereas aggression is reduced around the time of ovulation (Carlson, 1994). Thus, there is also the need to investigate the expression of bouts of aggression in different phases of the reproductive cycle in female dogs. The effect of fluctuating hormones may well condition other personality traits, which suggests the need for additional research in this area.

Finally, apart from the sex differences in dogs framed in a naturalist context, there are other dog-specific sex differences that require deeper exploration. One example is personality traits, such as excitability, in which females tend to be more excitable than males (Goddard and Beilharz, 1982, 1983), distractibility, which shows an opposite trend (Goddard and Beilharz, 1982, 1984), and cognitive abilities, in which males are reported to be smarter in a study on problem-solving (Duanton et al., 2015).

Table 9.1 List of scientific publications analyzing sex differences in dogs.



	Authors	Year	Methods	Primary Outcomes	Advantaged Sex
Aggressiveness	Borchelt	1983	Interview with family members	Eight major types of aggression were identified in different pure and mixed breeds: fear-elicited aggression, dominance, possessiveness, protectiveness, predation, punishment, pain and intraspecific aggression. Intraspecific and dominance aggressions as the major drivers were influenced by sex. Fear-elicited and possessive aggressions were less influenced by sex.	Males
	Hart and Hart	1985	A systematic survey of canine authorities	Males of different pure breeds showed more aggression toward other dogs.	Males
	Wright and Nesselrote	1987	Interview with family members	Males of different pure and mixed breeds showed more behavioral problems such as aggression toward dogs and humans.	Males
	Cameron	1997	Interview with the owners	Males of different pure and mixed breeds showed more dominance-associated aggression.	Males
	Guy et al.	2001	Interview with the owners	Females of different pure and mixed breeds showed more aggressive behavior toward humans.	Females
	Rooney and Bradshaw	2004	Interview with the owners and the trainers	English Springer spaniel, Labrador Retrievers, cross-breeds and Border collie males showed more aggression toward other dogs.	Males
	Pérez-Guisado et al.	2006	Experimental observation using Campbell's test	English cocker spaniel males showed more dominance-associated aggression.	Males
	Pérez-Guisado et al.	2008a	Experimental observation using Campbell's test	Males of different pure and mixed breeds showed more dominance-associated aggression.	Males
	Pérez-Guisado et al.	2008b	Interview with the owners	Males of different pure and mixed breeds showed more dominance-associated aggression.	Males
	Pérez-Guisado and Serrano	2009	Interview with the owners	Males of different pure and mixed breeds showed more dominance-associated aggression.	Males
	Foyer et al.	2013	Experimental observation	German shepherd males showed more aggressive behavior.	Males
	Lofgren et al.	2014	Interview with the owners	Labrador Retriever males showed higher owner aggression; stranger and dog-directed aggressions were not influenced by sex.	Males
	Asp et al.	2015	Interview with the owners	Males of different pure breeds showed higher stranger and dog-directed aggression.	Males
Boldness and Courage	Reuterwall and Ryman	1973	Interview with the trainers	German shepherd males were less impressionable by gunfire. The courage and the response to a sudden disturbance, in general, were not influenced by sex.	Males
	Goddard and Beilharz	1982	Interview with the trainers	Labrador and Golden Retriever males showed fewer fearfulness problems.	Males
	Goddard and Beilharz	1983	Interview with the trainers	Labrador and Golden Retriever males showed fewer fearfulness problems.	Males
	Goddard and Beilharz	1984	Experimental observation	Labrador Retriever, German shepherd, Boxer, Kelpie, and F1 crosses males showed less olfactory exploration associated with neophobia.	Males
	Wilsson and Sundgren	1997	Experimental observation	Labrador Retriever and German shepherd males scored higher in courage.	Males
	Svartberg	2002	Experimental observation	Belgian Tervuren and German shepherd males scored higher in boldness.	Males
	Strandberg et al.	2005	Experimental observation	Belgian Tervuren and German shepherd males scored higher in boldness.	Males
	Kubinyi et al.	2009	Interview with the owners	Males of different pure and mixed breeds scored higher in boldness.	Males
	Asp et al.	2015	Interview with the owners	Male of different pure breeds showed less dog and stranger- directed fear.	Males
Sociability	Lore and Eisenberg	1986	Experimental observation	Females of different pure and mixed breeds were more likely to approach and make physical contact with a human stranger. Males of different pure and mixed breeds were less likely to approach and make physical contact with a human male stranger.	Females
	Wilsson and Sundgren	1997	Experimental observation	Affability was not influenced by sex.	None
	Strandberg et al.	2005	Experimental observation	German shepherd males were more likely to social play.	Males
	Kubinyi et al.	2009	Interview with the owners	Females of different pure and mixed breeds scored higher in sociability.	Females
	Foyer et al.	2013	Experimental observation	German shepherd females scored higher in sociability.	Females
	Asp et al.	2015	Interview with the owners	Males of different pure breeds showed more human-directed play.	Males
	Persson et al.	2015	Experimental observation	Beagle females scored higher in sociability, making more physical contact with a human.	Females
	D'Aniello et al.	Pers. Comm.	Experimental observation	Labrador and Golden Retriever females made more physical contact with a stranger human.	Females
Spatial Cognition	Fugazza et al.	2017	Experimental observation	Males of different pure and mixed breeds showed more flexibility in changing the navigation strategy from allocentric to egocentric.	Males
	Mongillo et al.	2017	Experimental observation	Females of different pure and mixed breeds learned faster and made fewer errors in learning a task in the T-maze.	Females
	Scandurra et al.	2018b	Experimental observation	No effect of sex was identified on strategy preference in the plus-maze; however, an effect of gonadectomy was identified in females with a preference for the egocentric strategy in gonadectomized females. The probability of success in changing the navigation strategy increased in females and decreased in males, with increasing age.	None
Lateralization	Wells	2003	Experimental observation	Females of mixed breeds preferred to use the right paw, whereas males of mixed breeds were more inclined to use their left paw.	Females right pawed Males left pawed
	Quaranta et al.	2004	Experimental observation	Female of different pure and mixed breeds preferred to use the right paw, while males of different pure and mixed breeds were more inclined to adopt their left paw.	Females right pawed Males left pawed
	Branson and Rogers	2006	Experimental observation	Use of the preferred paw was not influenced by sex.	None
	Schneider et al.	2013	Experimental observation	Use of the preferred paw was not influenced by sex.	None
	Poyser et al.	2006	Experimental observation	Males of different pure and mixed breeds used the left paw more frequently; they tended to use the left paw in the first trials.	Males left pawed
	Wells et al.	2016	Experimental observation	Females of different pure and mixed breeds preferred to use the left paw, whereas males of different pure and mixed breeds were more inclined to use their right paw.	Females left pawed Males right pawed
Visual Focusing	Rooijakkers et al.	2009	Experimental observation	Females tended to look at the changing target longer.	None
	Müller et al.	2011	Experimental observation	Females of different pure and mixed breeds responded to a size constancy violation, looking at the changing target longer.	Females
	Nagasawa et al.	2015	Experimental observation	Females of different pure and mixed breeds showed increased gazing behavior toward the owner with intranasal oxytocin.	Females
	Kis et al.	2015	Experimental observation	Dogs of both sexes were not affected by the intranasal oxytocin.	None
	D'Aniello et al.	2016	Experimental observation	Labrador and Golden Retriever females relied more on visual signals, such as human gestural commands.	Females
	Duranton et al.	2016	Experimental observation	Females of shepherds and molossoids dogs displayed more referential gazing behavior toward the owners.	Females
	Kovács et al.	2016	Experimental observation	Females of different pure and mixed breeds increased the gazing behavior toward the owners with the intranasal oxytocin.	Females
	Mongillo et al.	2016	Experimental observation	Females of different pure and mixed breeds displayed more gazing behavior toward the owners.	Females
Olfactory Skills	Siniscalchi et al.	2011	Experimental observation	Males of mixed breeds tended to sniff vaginal secretion odor more frequently; females of mixed breeds investigated the food odor for a longer time.	Depending on motivation
	Hamilton and Vonk	2015	Experimental observation	Labrador, Golden Retriever and F1 crosses males were able to recognize kin.	Males

9.7 References

Andersson, M. B. (1994). *Sexual selection*. Princeton University Press.

Andics, A., Gábor, A., Gácsi, M., Faragó, T., Szabó, D., & Miklósi, Á. (2016). Neural mechanisms for lexical processing in dogs. *Science*, *353*(6303), 1030-1032.

Ariyomo, T. O., & Watt, P. J. (2013). Aggression and sex differences in lateralization in the zebrafish. *Animal behavior*, *86*(3), 617-622.

Asp, H. E., Fikse, W. F., Nilsson, K., & Strandberg, E. (2015). Breed differences in everyday behavior of dogs. *Applied Animal Behavior Science*, *169*, 69-77.

Astur, R. S., Tropp, J., Sava, S., Constable, R. T., & Markus, E. J. (2004). Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. Behavioral brain research, 151(1-2), 103-115.

Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W., & Ketterson, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, *23*(5), 960-969.

Austin, N. P., & Rogers, L. J. (2014). Lateralization of agonistic and vigilance responses in Przewalski horses (*Equus przewalskii*). *Applied Animal Behavior Science*, *151*, 43-50.

Bagesteiro, L. B., & Sainburg, R. L. (2002). Handedness: dominant arm advantages in control of limb dynamics. *Journal of neurophysiology*, *88*(5), 2408-2421.

Bamberger, M., & Houpt, K. A. (2006). Signalment factors, comorbidity, and trends in behavior diagnoses in dogs: 1,644 cases (1991–2001). *Journal of the American Veterinary Medical Association*, *229*(10), 1591-1601.

Barsky, P. I. (2010). Environment, genes, and experience: Lessons from behavior genetics. *Journal of Physiology-Paris*, *104*(5), 243-252.

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity*, *2*(Pt. 3), 349-368.

Batt, L., Batt, M., Baguley, J., & McGreevy, P. (2008). Stability of motor lateralisation in maturing dogs. *Laterality*, *13*(5), 468-479.

Becker, J. B., & Koob, G. F. (2016). Sex differences in animal models: focus on addiction. *Pharmacological reviews*, *68*(2), 242-263.

Beckmann, C., & Biro, P. A. (2013). On the validity of a single (boldness) assay in personality research. *Ethology*, *119*(11), 937-947.

Bell, A. M., & Stamps, J. A. (2004). Development of behavioral differences between individuals and populations of sticklebacks, Gasterosteus aculeatus. *Animal Behavior*, *68*(6), 1339-1348.

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, *23*(7), 361-368.

Borchelt, P. L. (1983). Aggressive behavior of dogs kept as companion animals: classification and influence of sex, reproductive status and breed. *Applied Animal Ethology*, *10*(1), 45-61.

Brand, G., & Millot, J. L. (2001). Sex differences in human olfaction: between evidence and enigma. *The Quarterly Journal of Experimental Psychology: Section B*, *54*(3), 259-270.

Branson, N. J., & Rogers, L. J. (2006). Relationship between paw preference strength and noise phobia in *Canis familiaris*. *Journal of Comparative Psychology*, *120*(3), 176.

Brodbeck, D. R., & Tanninen, S. E. (2012). Place learning and spatial navigation. In: *Encyclopedia of the Sciences of Learning* (pp. 2639-2641). Springer, Boston, MA.

Budaev, S. V., Zworykin, D. D., & Mochek, A. D. (1999). Consistency of individual differences in behavior of the lion-headed cichlid, *Steatocranus casuarius*. *Behavioral Processes*, *48*(1-2), 49-55.

Cain, K. E., & Ketterson, E. D. (2012). Competitive females are successful females; phenotype, mechanism, and selection in a common songbird. *Behavioral ecology and sociobiology*, *66*(2), 241-252.

Cameron, D. B. (1997). Canine dominance-associated aggression: concepts, incidence, and treatment in a private behavior practice. *Applied Animal Behavior Science*, *52*(3-4), 265-274.

Cameron, E. Z., & du Toit, J. T. (2005). Social influences on vigilance behavior in giraffes, *Giraffa camelopardalis*. *Animal Behavior*, *69*(6), 1337-1344.

Carlson, N.R. (1194). Physiology of Behavior. Allyn & Bacon, Needham Heights, MA, USA.

Carter, A. J., & Feeney, W. E. (2012). Taking a comparative approach: analysing personality as a multivariate behavioral response across species. *PLoS One*, *7*(7), e42440.

Casey, R. A., Loftus, B., Bolster, C., Richards, G. J., & Blackwell, E. J. (2013). Inter-dog aggression in a UK owner survey: prevalence, co-occurrence in different contexts and risk factors. *Veterinary Record*, *172*(127), 10-1136.

Casey, R. A., Loftus, B., Bolster, C., Richards, G. J., & Blackwell, E. J. (2014). Human directed aggression in domestic dogs (*Canis familiaris*): Occurrence in different contexts and risk factors. *Applied Animal Behavior Science*, *152*, 52-63.

Cattet, J., & Etienne, A. S. (2004). Blindfolded dogs relocate a target through path integration. *Animal Behavior*, *68*(1), 203-212.

Chapuis, N., Thinus-Blanc, C., & Poucet, B. (1983). Dissociation of mechanisms involved in dogs' oriented displacements. *The Quarterly Journal of Experimental Psychology Section B*, *35*(3b), 213-219.

Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genetics Research*, *74*(3), 329-340.

Connor, R.C., Smolker, R.A., & Richards, A.F. (1992). Dolphin alliances and coalitions. In: Harcourt, A.H., & De Waal, F.B.M. (Eds.), *Coalitions and Alliances in Humans and Other Animals* (pp. 419–440). Oxford University Press, New York, NY, USA.

Corballis, M.C. (1983). Human Handedness. In: Corballis, M.C. (Ed.), *Human Laterality* (pp. 11-27). Academic Press, New York, NY, USA.

Coren, S., & Porac, C. (1977). Fifty centuries of right-handedness: the historical record. *Science*, *198*(4317), 631-632.

Corp, N., & Byrne, R. W. (2004). Sex difference in chimpanzee handedness. *American journal of physical anthropology*, *123*(1), 62-68.

Cowlishaw, G. U. Y. (1997). Trade-offs between foraging and predation risk determine habitat use in a desert baboon population. *Animal Behavior*, *53*(4), 667-686.

Cox, D., Hallam, R., O'Connor, K., & Rachman, S. (1983). An experimental analysis of fearlessness and courage. *British Journal of Psychology*, *74*(1), 107-117.

D’Aniello, B., Alterisio, A., Scandurra, A., Petremolo, E., Iommelli, M. R., & Aria, M. (2017). What’s the point? Golden and Labrador retrievers living in kennels do not understand human pointing gestures. *Animal cognition*, *20*(4), 777-787.

D’Aniello, B., & Scandurra, A. (2018). (University of Naples “Federico II”, Naples, Italy). Personal communication.

D’Aniello, B., & Scandurra, A. (2017) Impossible task paradigm. In: Vonk, J., & Shackelford, T. (Eds.), *Encyclopedia of Animal Cognition and Behavior*. Springer International Publishing, Cham, Switzerland.

D’Aniello, B., & Scandurra, A. (2016). Ontogenetic effects on gazing behavior: a case study of kennel dogs (Labrador Retrievers) in the impossible task paradigm. *Animal cognition*, *19*(3), 565-570.

D’Aniello, B., Scandurra, A., Alterisio, A., Valsecchi, P., & Prato-Previde, E. (2016). The importance of gestural communication: a study of human–dog communication using incongruent information. *Animal cognition*, *19*(6), 1231-1235.

D’Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: a study on water rescue dogs using the impossible task paradigm. *Behavioral processes*, *110*, 68-73.

D’Aniello, B., Semin, G. R., Alterisio, A., Aria, M., & Scandurra, A. (2018). Interspecies transmission of emotional information via chemosignals: from humans to dogs (*Canis lupus familiaris*). *Animal cognition*, *21*(1), 67-78.

D'Eath, R. B., & Lawrence, A. B. (2004). Early life predictors of the development of aggressive behavior in the domestic pig. *Animal Behavior*, *67*(3), 501-509.

Dahlgren, J. (1990). Females choose vigilant males: an experiment with the monogamous grey partridge, *Perdix perdix*. *Animal Behavior*, *39*(4), 646-651.

Dammhahn, M. (2012). Are personality differences in a small iteroparous mammal maintained by a life-history trade-off? *Proceedings of the Royal Society of London B, Biological Sciences*.

Duffy, D. L., Hsu, Y., & Serpell, J. A. (2008). Breed differences in canine aggression. *Applied Animal Behavior Science*, *114*(3-4), 441-460.

Duranton, C., Bedossa, T., & Gaunet, F. (2016). When facing an unfamiliar person, pet dogs present social referencing based on their owners' direction of movement alone. *Animal behavior*, *113*, 147-156.

Duranton, C., Rödel, H. G., Bedossa, T., & Belkhir, S. (2015). Inverse sex effects on performance of domestic dogs (*Canis familiaris*) in a repeated problem-solving task. *Journal of Comparative Psychology*, *129*(1), 84.

Dykens, E. M., & Rosner, B. A. (1999). Refining behavioral phenotypes: Personality—motivation in Williams and Prader-Willi syndromes. *American Journal on Mental Retardation*, *104*(2), 158-169.

Ecuyer-Dab, I., & Robert, M. (2004). Spatial ability and home-range size: examining the relationship in Western men and women (*Homo sapiens*). *Journal of Comparative Psychology*, *118*(2), 217.

Fabes, R. A., Martin, C. L., & Hanish, L. D. (2003). Young children's play qualities in same-, other-, and mixed-sex peer groups. *Child development*, *74*(3), 921-932.

Fält, L. (1997). Kompendium Mentalitet. Svenska Brukshundklubben, Stockholm, Sweden.

Fitzpatrick, S., Berglund, A., & Rosenqvist, G. (1995). Ornaments or offspring: costs to reproductive success restrict sexual selection processes. *Biological Journal of the Linnean Society*, *55*(3), 251-260.

Foyer, P., Wilsson, E., Wright, D., & Jensen, P. (2013). Early experiences modulate stress coping in a population of German shepherd dogs. *Applied animal behavior science*, *146*(1-4), 79-87.

Frère, C. H., Krützen, M., Mann, J., Connor, R. C., Bejder, L., & Sherwin, W. B. (2010). Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proceedings of the National Academy of Sciences*, *107*(46), 19949-19954.

Fugazza, C., Mongillo, P., & Marinelli, L. (2017). Sex differences in dogs' social learning of spatial information. *Animal cognition*, *20*(4), 789-794.

Furuichi, T., & Ihobe, H. (1994). Variation in male relationships in bonobos and chimpanzees. *Behavior*, *130*(3), 211-228.

Gácsi, M., Gyoöri, B., Virányi, Z., Kubinyi, E., Range, F., Belényi, B., & Miklósi, Á. (2009). Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills. *PLoS One*, *4*(8), e6584.

Garde, E., Pérez, G. E., Vanderstichel, R., Dalla Villa, P. F., & Serpell, J. A. (2016). Effects of surgical and chemical sterilization on the behavior of free-roaming male dogs in Puerto Natales, Chile. *Preventive veterinary medicine*, *123*, 106-120.

Gershman, K. A., Sacks, J. J., & Wright, J. C. (1994). Which dogs bite? A case-control study of risk factors. *Pediatrics*, *93*(6), 913-917.

Giambra, L. M., & Quilter, R. E. (1989). Sex differences in sustained attention across the adult life span. *Journal of applied psychology*, *74*(1), 91.

Glickman, S. E., Frank, L. G., Davidson, J. M., Smith, E. R., & Siiteri, P. K. (1987). Androstenedione may organize or activate sex-reversed traits in female spotted hyenas. *Proceedings of the National Academy of Sciences*, *84*(10), 3444-3447.

Goddard, M. E., & Beilharz, R. G. (1982). Genetic and environmental factors affecting the suitability of dogs as guide dogs for the blind. *Theoretical and Applied genetics*, *62*(2), 97-102.

Goddard, M. E., & Beilharz, R. G. (1983). Genetics of traits which determine the suitability of dogs as guide-dogs for the blind. *Applied Animal Ethology*, *9*(3-4), 299-315.

Goddard, M. E., & Beilharz, R. G. (1984). The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. *Applied Animal Behavior Science*, *12*(3), 267-278.

Gold, E. B., Bromberger, J., Crawford, S., Samuels, S., Greendale, G. A., Harlow, S. D., & Skurnick, J. (2001). Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American journal of epidemiology*, *153*(9), 865-874.

Golledge, R.G. (2004). Spatial cognition. In: Spielberger, C (Ed.), *Encyclopedia of Applied Psychology* (pp. 443–452). Elsevier Ltd., Amsterdam, The Netherlands.

Gosling, S. D. (1998). Personality dimensions in spotted hyenas (*Crocuta crocuta*). *Journal of Comparative Psychology*, *112*(2), 107.

Grassmann, S., Kaminski, J., & Tomasello, M. (2012). How two word-trained dogs integrate pointing and naming. *Animal Cognition*, *15*(4), 657-665.

Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal behavior*, *28*(4), 1140-1162.

Groothuis, T. G., & Carere, C. (2005). Avian personalities: characterization and epigenesis. *Neuroscience & Biobehavioral Reviews*, *29*(1), 137-150.

Guo, K., Meints, K., Hall, C., Hall, S., & Mills, D. (2009). Left gaze bias in humans, rhesus monkeys and domestic dogs. *Animal cognition*, *12*(3), 409-418.

Guy, N. C., Luescher, U. A., Dohoo, S. E., Spangler, E., Miller, J. B., Dohoo, I. R., & Bate, L. A. (2001). Risk factors for dog bites to owners in a general veterinary caseload. *Applied Animal Behavior Science*, *74*(1), 29-42.

Gyuris, E., Feró, O., Tartally, A., & Barta, Z. (2010). Individual behavior in firebugs (*Pyrrhocoris apterus*). *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20101326.

Halpern, D.F. (2000). *Sex* Differences in Cognitive Abilities, 3rd ed., Lawrence Erlbaum Associates, London, UK.

Hamilton, J., & Vonk, J. (2015). Do dogs (*Canis lupus familiaris*) prefer family? *Behavioral processes*, *119*, 123-134.

Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, *298*(5598), 1634-1636.

Hare, B., & Tomasello, M. (2005). Human-like social skills in dogs? *Trends in cognitive sciences*, *9*(9), 439-444.

Harris, L.J. (1983). Laterality of function in the infant: Historical and contemporary trends in theory and research. In: Young, G. (Ed.). *Manual Specialization and the Developing Brain* (pp. 177–247). Academic Press, New York, NY, USA.

Hart, B. L., & Eckstein, R. A. (1997). The role of gonadal hormones in the occurrence of objectionable behaviors in dogs and cats. *Applied Animal Behavior Science*, *52*(3-4), 331-344.

Hart, B. L., & Hart, L. A. (1985). Selecting pet dogs on the basis of cluster analysis of breed behavior profiles and gender. *Journal of the American Veterinary Medical Association*, *186*(11), 1181-1185.

Hawley, W. R., Grissom, E. M., Barratt, H. E., Conrad, T. S., & Dohanich, G. P. (2012). The effects of biological sex and gonadal hormones on learning strategy in adult rats. *Physiology & behavior*, *105*(4), 1014-1020.

Hedrick, A. V., & Kortet, R. (2012). Sex differences in the repeatability of boldness over metamorphosis. *Behavioral Ecology and Sociobiology*, *66*(3), 407-412.

Hepper, P. G. (1994). Long-term retention of kinship recognition established during infancy in the domestic dog. *Behavioral processes*, *33*(1-2), 3-14.

Herman, R. A., & Wallen, K. (2007). Cognitive performance in rhesus monkeys varies by sex and prenatal androgen exposure. *Hormones and behavior*, *51*(4), 496-507.

Hopkins, S. G., Schubert, T. A., & Hart, B. L. (1976). Castration of adult male dogs: effects on roaming, aggression, urine marking, and mounting. *Journal of the American Veterinary Medical Association*, *168*(12), 1108-1110.

Hopkins, W. D., & Leavens, D. A. (1998). Hand use and gestural communication in chimpanzees (*Pan troglodytes*). *Journal of Comparative Psychology*, *112*(1), 95.

Houpt, K. A. (2007). Genetics of canine behavior. *Acta Veterinaria Brno*, *76*(3), 431-444.

Howell, T. J., King, T., & Bennett, P. C. (2015). Puppy parties and beyond: the role of early age socialization practices on adult dog behavior. *Veterinary Medicine: Research and Reports*, *6*, 143-153.

Johnson, R.N. (1972). Aggression in Man and Animals, 3th ed., W.B. Saunders Company: Philadelphia, PA, USA.

Johnsson, J. I., Sernland, E., & Blixt, M. (2001). Sex-specific aggression and antipredator behavior in young brown trout. *Ethology*, *107*(7), 587-599.

Jonasson, Z., Cahill, J. F., Tobey, R. E., & Baxter, M. G. (2004). Sexually dimorphic effects of hippocampal cholinergic deafferentation in rats. *European Journal of Neuroscience*, *20*(11), 3041-3053.

Jones, A. C., & Gosling, S. D. (2005). Temperament and personality in dogs (*Canis familiaris*): a review and evaluation of past research. *Applied Animal Behavior Science*, *95*(1-2), 1-53.

Jones, C. M., & Healy, S. D. (2006). Differences in cue use and spatial memory in men and women. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*(1598), 2241-2247.

Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for" fast mapping". *Science*, *304*(5677), 1682-1683.

Kaufmann, C. A., Forndran, S., Stau-ber, C., Woerner, K., & Gansloßer, U. (2017). The social behavior of neutered male dogs compared to intact dogs (*Canis lupus familiaris*): Video analyses, questionnaires and case studies. *Open Journal of Veterinary Medicine, 2*(1), 22-37.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in ecology & evolution*, *17*(5), 230-241.

Kim, H. H., Yeon, S. C., Houpt, K. A., Lee, H. C., Chang, H. H., & Lee, H. J. (2006). Effects of ovariohysterectomy on reactivity in German Shepherd dogs. *The Veterinary Journal*, *172*(1), 154-159.

Kimura, D. (1999). *Sex and Cognition*; MIT Press: Cambridge, MA, USA.

Kis, A., Hernádi, A., Kanizsár, O., Gácsi, M., & Topál, J. (2015). Oxytocin induces positive expectations about ambivalent stimuli (cognitive bias) in dogs. *Hormones and behavior*, *69*, 1-7.

Knol, B. W., & Egberink-Alink, S. T. (1989). Treatment of problem behavior in dogs and cats by castration and progestagen administration: a review. *Veterinary Quarterly*, *11*(2), 102-107.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., ... & Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925-935.

Korol, D. L., & Kolo, L. L. (2002). Estrogen-induced changes in place and response learning in young adult female rats. *Behavioral neuroscience*, *116*(3), 411.

Korol, D.L., & Manning, C.A. (2001). Effects of estrogen on cognition: Implications for menopause. In: Carroll, M.E., & Overmier, J.B. (Eds.), *Animal Research and Human Health: Advancing Human Welfare through Behavioral Science* (pp. 305–322). American Psychological Association, Washington, DC, USA.

Kovács, K., Kis, A., Kanizsár, O., Hernádi, A., Gácsi, M., & Topál, J. (2016). The effect of oxytocin on biological motion perception in dogs (*Canis familiaris*). *Animal cognition*, *19*(3), 513-522.

Kralj-Fišer, S., Scheiber, I. B., Blejec, A., Moestl, E., & Kotrschal, K. (2007). Individualities in a flock of free-roaming greylag geese: behavioral and physiological consistency over time and across situations. *Hormones and Behavior*, *51*(2), 239-248.

Krueger, R. F., Hicks, B. M., & McGue, M. (2001). Altruism and antisocial behavior: Independent tendencies, unique personality correlates, distinct etiologies. *Psychological Science*, *12*(5), 397-402.

Kubinyi, E., Turcsán, B., & Miklósi, Á. (2009). Dog and owner demographic characteristics and dog personality trait associations. *Behavioral Processes*, *81*(3), 392-401.

Le Boeuf, B. J. (1970). Copulatory and aggressive behavior in the prepuberally castrated dog. *Hormones and Behavior*, *1*(2), 127-136.

Leshner, A.I. (1978). Introduction to Behavioral Endocrinology. Oxford University Press, Oxford, UK.

Lighthall, N. R., Sakaki, M., Vasunilashorn, S., Nga, L., Somayajula, S., Chen, E. Y., ... & Mather, M. (2011). Gender differences in reward-related decision processing under stress. *Social Cognitive and Affective Neuroscience*, *7*(4), 476-484.

Lofgren, S. E., Wiener, P., Blott, S. C., Sanchez-Molano, E., Woolliams, J. A., Clements, D. N., & Haskell, M. J. (2014). Management and personality in Labrador Retriever dogs. *Applied Animal Behavior Science*, *156*, 44-53.

Lonsdorf, E. V., Anderson, K. E., Stanton, M. A., Shender, M., Heintz, M. R., Goodall, J., & Murray, C. M. (2014). Boys will be boys: sex differences in wild infant chimpanzee social interactions. *Animal behavior*, *88*, 79-83.

López, P., Hawlena, D., Polo, V., Amo, L., & Martín, J. (2005). Sources of individual shy–bold variations in antipredator behavior of male Iberian rock lizards. *Animal Behavior*, *69*(1), 1-9.

Lore, R. K., & Eisenberg, F. B. (1986). Avoidance reactions of domestic dogs to unfamiliar male and female humans in a kennel setting. *Applied Animal Behavior Science*, *15*(3), 261-266.

Maarschalkerweerd, R. J., Endenburg, N., Kirpensteijn, J., & Knol, B. W. (1997). Influence of orchiectomy on canine behavior. *Veterinary Record*, *140*, 617-619.

Maccoby, E. E. (1988). Gender as a social category. *Developmental psychology*, *24*(6), 755–765.

Maccoby, E.E., & Jaklin, C.N. (1987). Gender segregation in childhood. In: Reese, H.W. (Ed.), *Advances in Child Development and Behavior* (pp. 239–287). Academic Press, New York, NY, USA.

McCarthy, M. M., Arnold, A. P., Ball, G. F., Blaustein, J. D., & De Vries, G. J. (2012). Sex differences in the brain: the not so inconvenient truth. *Journal of Neuroscience*, *32*(7), 2241-2247.

McDermott, D. R., Chips, M. J., McGuirk, M., Armagost, F., DiRienzo, N., & Pruitt, J. N. (2014). Boldness is influenced by sublethal interactions with predators and is associated with successful harem infiltration in Madagascar hissing cockroaches. *Behavioral ecology and sociobiology*, *68*(3), 425-435.

McKenzie, B. (2010). Evaluating the benefits and risks of neutering dogs and cats. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *5*, 1-18.

McMillan, T. M., & Rachman, S. J. (1988). Fearlessness and courage in paratroopers undergoing training. *Personality and Individual Differences*, *9*(2), 373-378.

Mehrkam, L. R., & Wynne, C. D. (2014). Behavioral differences among breeds of domestic dogs (*Canis lupus familiaris*): Current status of the science. *Applied Animal Behavior Science*, *155*, 12-27.

Meredith, S.L. (2013). Identifying proximate and ultimate causation in the development of primate sex-typed social behavior. In: Clancy, K.B.H., Hinde, K., & Rutherford, J.N. (Eds.), *Building Babies: Primate Development in Proximate and Ultimate Perspective* (pp. 411–433). Springer, New York, NY, USA.

Miklósi, Á., Pongrácz, P., Lakatos, G., Topál, J., & Csányi, V. (2005). A comparative study of the use of visual communicative signals in interactions between dogs (*Canis familiaris*) and humans and cats (*Felis catus*) and humans. *Journal of comparative psychology*, *119*(2), 179.

Miklósi, Á., & Topál, J. (2013). What does it take to become 'best friends'? Evolutionary changes in canine social competence. *Trends in cognitive sciences*, *17*(6), 287-294.

Mills, D. S. (2005). What's in a word? A review of the attributes of a command affecting the performance of pet dogs. *Anthrozoös*, *18*(3), 208-221.

Miyahira, A., Morita, K., Yamaguchi, H., Nonaka, K., & Maeda, H. (2000). Gender differences of exploratory eye movements: a life span study. *Life sciences*, *68*(5), 569-577.

Mongillo, P., Pitteri, E., Candaten, M., & Marinelli, L. (2016). Can attention be taught? Interspecific attention by dogs (Canis familiaris) performing obedience tasks. *Applied Animal Behavior Science*, *182*, 30-37.

Mongillo, P., Scandurra, A., D'Aniello, B., & Marinelli, L. (2017). Effect of sex and gonadectomy on dogs' spatial performance. *Applied Animal Behavior Science*, *191*, 84-89.

Montello, D. R. (2015). Behavioral methods for spatial cognition research. *Research Methods for Environmental Psychology*, 161.

Müller, C. A., Mayer, C., Dörrenberg, S., Huber, L., & Range, F. (2011). Female but not male dogs respond to a size constancy violation. *Biology letters*, rsbl20110287.

Muller, M., & Mitani, J.C. (2005). Conflict and cooperation in wild chimpanzees. In: Slater, P.J.B., Rosenblatt, J., Snowdon, C., Roper, T., & Naguib, M., (Eds.), *Advances in the Study of Behavior* (pp. 275–331). Elsevier, New York, NY, USA.

Murphy, J., Sutherland, A., & Arkins, S. (2005). Idiosyncratic motor laterality in the horse. *Applied Animal Behavior Science*, *91*(3-4), 297-310.

Nagasawa, M., Mitsui, S., En, S., Ohtani, N., Ohta, M., Sakuma, Y., ... & Kikusui, T. (2015). Oxytocin-gaze positive loop and the coevolution of human-dog bonds. *Science*, *348*(6232), 333-336.

Neilson, J. C., Eckstein, R. A., & Hart, B. L. (1997). Effects of castration on problem behaviors in male dogs with reference to age and duration of behavior. *Journal of the American Veterinary Medical Association*, *211*(2), 180-182.

O'farrell, V., & Peachey, E. (1990). Behavioral effects of ovariohysterectomy on hitches. *Journal of Small Animal Practice*, *31*(12), 595-598.

Øverli, Ø., Sørensen, C., & Nilsson, G. E. (2006). Behavioral indicators of stress-coping style in rainbow trout: do males and females react differently to novelty? *Physiology & Behavior*, *87*(3), 506-512.

Øverli, Ø., Pottinger, T. G., Carrick, T. R., Øverli, E., & Winberg, S. (2002). Differences in behavior between rainbow trout selected for high-and low-stress responsiveness. *Journal of Experimental Biology*, *205*(3), 391-395.

Pal, S. K., Ghosh, B., & Roy, S. (1998). Dispersal behavior of free-ranging dogs (Canis familiaris) in relation to age, sex, season and dispersal distance. *Applied Animal Behavior Science*, *61*(2), 123-132.

Pavlova, D. Z., Pinxten, R., & Eens, M. (2007). Seasonal singing patterns and individual consistency in song activity in female European starlings (*Sturnus vulgaris*). *Behavior*, *144*(6), 663-680.

Pérez-Guisado, J., Lopez-Rodríguez, R., & Muñoz-Serrano, A. (2006). Heritability of dominant–aggressive behavior in English Cocker Spaniels. *Applied Animal Behavior Science*, *100*(3-4), 219-227.

Perez-Guisado, J., & Munoz-Serrano, A. (2009). Factors linked to dominance aggression in dogs. *Journal of Animal and Veterinary Advances*, *8*(2), 336-342.

Pérez-Guisado, J., Muñoz-Serrano, A., & López-Rodríguez, R. (2008a). Evaluation of the Campbell test and the influence of age, sex, breed, and coat color on puppy behavioral responses. *Canadian Journal of Veterinary Research*, *72*(3), 269.

Pérez-Guisado, J., Muñoz-Serrano, A., & López-Rodríguez, R. (2008b). Perros peligrosos, la agresividad por dominancia (parte1): factores asociados.

Persson, M. E., Roth, L. S. V., Johnsson, M., Wright, D., & Jensen, P. (2015). Human-directed social behavior in dogs shows significant heritability. *Genes, Brain and Behavior*, *14*(4), 337-344.

Petelle, M. B., McCoy, D. E., Alejandro, V., Martin, J. G., & Blumstein, D. T. (2013). Development of boldness and docility in yellow-bellied marmots. *Animal Behavior*, *86*(6), 1147-1154.

Pilley, J. W., & Reid, A. K. (2011). Border collie comprehends object names as verbal referents. *Behavioral processes*, *86*(2), 184-195.

Piyapong, C., Krause, J., Chapman, B. B., Ramnarine, I. W., Louca, V., & Croft, D. P. (2009). Sex matters: a social context to boldness in guppies (*Poecilia reticulata*). *Behavioral Ecology*, *21*(1), 3-8.

Podberscek, A. L., & Serpell, J. A. (1996). The English Cocker Spaniel: preliminary findings on aggressive behavior. *Applied Animal Behavior Science*, *47*(1), 75-89.

Poyser, F., Caldwell, C., & Cobb, M. (2006). Dog paw preference shows lability and sex differences. *Behavioral processes*, *73*(2), 216-221.

Price, E.O. (2002). Animal Domestication and Behavior. CABI, Wallingford, UK.

Quaranta, A., Siniscalchi, M., Frate, A., Iacoviello, R., Buonavoglia, C., & Vallortigara, G. (2006). Lateralised behavior and immune response in dogs: Relations between paw preference and interferon-γ, interleukin-10 and IgG antibodies production. *Behavioral brain research*, *166*(2), 236-240.

Quaranta, A., Siniscalchi, M., Frate, A., & Vallortigara, G. (2004). Paw preference in dogs: relations between lateralised behavior and immunity. *Behavioral brain research*, *153*(2), 521-525.

Racca, A., Guo, K., Meints, K., & Mills, D. S. (2012). Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *PLoS one*, *7*(4), e36076.

Range, F., & Virányi, Z. (2013). Social learning from humans or conspecifics: differences and similarities between wolves and dogs. *Frontiers in psychology*, *4*, 868.

Range, F., & Virányi, Z. (2014). Wolves are better imitators of conspecifics than dogs. *PLoS One*, *9*(1), e86559.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological reviews*, *82*(2), 291-318.

Reisner, I. R., Houpt, K. A., & Shofer, F. S. (2005). National survey of owner-directed aggression in English Springer Spaniels. *Journal of the American Veterinary Medical Association*, *227*(10), 1594-1603.

Reuterwall, C., & Ryman, N. (1973). An estimate of the magnitude of additive genetic variation of some mental characters in Alsatian dogs. *Hereditas*, *73*(2), 277-283.

Ridley, M. W., & Hill, D. A. (1987). Social organization in the pheasant (*Phasianus colchicus*): harem formation, mate selection and the role of mate guarding. *Journal of Zoology*, *211*(4), 619-630.

Rogers, L. J. (2010). Relevance of brain and behavioral lateralization to animal welfare. *Applied Animal Behavior Science*, *127*(1-2), 1-11.

Rooijakkers, E. F., Kaminski, J., & Call, J. (2009). Comparing dogs and great apes in their ability to visually track object transpositions. *Animal Cognition*, *12*(6), 789.

Rooney, N. J., & Bradshaw, J. W. (2004). Breed and sex differences in the behavioral attributes of specialist search dogs—a questionnaire survey of trainers and handlers. *Applied Animal Behavior Science*, *86*(1-2), 123-135.

Rosvall, K. A. (2011). Intrasexual competition in females: evidence for sexual selection? *Behavioral Ecology*, *22*(6), 1131-1140.

Rubenstein, D. R., & Lovette, I. J. (2009). Reproductive skew and selection on female ornamentation in social species. *Nature*, *462*(7274), 786.

Scandurra, A., Alterisio, A., Aria, M., Vernese, R., & D'Aniello, B. (2018a). Should I fetch one or the other? A study on dogs on the object choice in the bimodal contrasting paradigm. *Animal cognition*, *21*(1), 119-126.

Scandurra, A., Alterisio, A., Marinelli, L., Mongillo, P., Semin, G. R., & D'Aniello, B. (2017). Effectiveness of verbal and gestural signals and familiarity with signal-senders on the performance of working dogs. *Applied Animal Behavior Science*, *191*, 78-83.

Scandurra, A., Marinelli, L., Lõoke, M., D'Aniello, B., & Mongillo, P. (2018b). The effect of age, sex and gonadectomy on dogs' use of spatial navigation strategies. *Applied Animal Behavior Science*.

Scandurra, A., Mongillo, P., Marinelli, L., Aria, M., & D'Aniello, B. (2016). Conspecific observational learning by adult dogs in a training context. *Applied Animal Behavior Science*, *174*, 116-120.

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D'Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behavior. *Animal cognition*, *18*(4), 937-944.

Schneider, L. A., Delfabbro, P. H., & Burns, N. R. (2013). Temperament and lateralization in the domestic dog (*Canis familiaris*). *Journal of Veterinary Behavior: Clinical Applications and Research*, *8*(3), 124-134.

Schuett, W., & Dall, S. R. (2009). Sex differences, social context and personality in zebra finches, *Taeniopygia guttata*. *Animal Behavior*, *77*(5), 1041-1050.

Schuett, W., Tregenza, T., & Dall, S. R. (2010). Sexual selection and animal personality. *Biological Reviews*, *85*(2), 217-246.

Scott, J.P., & Fuller, J.L. (1965). Dog Behavior: The Genetic Basis. University of Chicago Press, Chicago, IL, USA.

Serpell, J., & Jagoe, J.A. (1995). Early experience and the development of behavior. In: Serpell, J. (Ed.), *The Domestic Dog: Its Evolution, Behavior and Interactions with People* (pp. 79–102). Cambridge University Press, Cambridge, UK.

Shah, D. S., Prados, J., Gamble, J., De Lillo, C., & Gibson, C. L. (2013). Sex differences in spatial memory using serial and search tasks. *Behavioral brain research*, *257*, 90-99.

Shuster, S.M., & Wade, M.J. (2003). Mating Systems and Strategies: (Monographs in Behavior and Ecology). Princeton University Press: Princeton, NJ, USA.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: an integrative overview. *The quarterly review of biology*, *79*(3), 241-277.

Sih, A., & Del Giudice, M. (2012). Linking behavioral syndromes and cognition: a behavioral ecology perspective. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *367*(1603), 2762-2772.

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2016). The dog nose "KNOWS" fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli. *Behavioral brain research*, *304*, 34-41.

Siniscalchi, M., Quaranta, A., & Rogers, L. J. (2008). Hemispheric specialization in dogs for processing different acoustic stimuli. *PLoS One*, *3*(10), e3349.

Siniscalchi, M., Sasso, R., Pepe, A. M., Dimatteo, S., Vallortigara, G., & Quaranta, A. (2011). Sniffing with the right nostril: lateralization of response to odour stimuli by dogs. *Animal behavior*, *82*(2), 399-404.

Slauterbeck, J. R., Pankratz, K., Xu, K. T., Bozeman, S. C., & Hardy, D. M. (2004). Canine ovariohysterectomy and orchiectomy increases the prevalence of ACL injury. *Clinical Orthopaedics and Related Research (1976-2007)*, *429*, 301-305.

Smolker, R. A., Richards, A. F., Connor, R. C., & Pepper, J. W. (1992). Sex differences in patterns of association among Indian Ocean bottlenose dolphins. *Behavior*, *123*(1), 38-69.

Sommer, I. E., Aleman, A., Somers, M., Boks, M. P., & Kahn, R. S. (2008). Sex differences in handedness, asymmetry of the planum temporale and functional language lateralization. *Brain research*, *1206*, 76-88.

Springer, S.P., & Deutsch, G. (1989). Left Brain, Right Brain: Perspectives from Cognitive Neuroscience. Freeman, New York, NY, USA.

Starling, M. J., Branson, N., Thomson, P. C., & McGreevy, P. D. (2013). "Boldness" in the domestic dog differs among breeds and breed groups. *Behavioral processes*, *97*, 53-62.

Starling, M. J., Branson, N., Thomson, P. C., & McGreevy, P. D. (2013). Age, sex and reproductive status affect boldness in dogs. *The Veterinary Journal*, *197*(3), 868-872.

Sterck, E. H., Watts, D. P., & van Schaik, C. P. (1997). The evolution of female social relationships in nonhuman primates. *Behavioral ecology and sociobiology*, *41*(5), 291-309.

Strandberg, E., Jacobsson, J., & Saetre, P. (2005). Direct genetic, maternal and litter effects on behavior in German shepherd dogs in Sweden. *Livestock Production Science*, *93*(1), 33-42.

Svartberg, K. (2005). A comparison of behavior in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. *Applied Animal Behavior Science*, *91*(1-2), 103-128.

Svartberg, K. (2002). Shyness–boldness predicts performance in working dogs. *Applied Animal Behavior Science*, *79*(2), 157-174.

Svartberg, K., & Forkman, B. (2002). Personality traits in the domestic dog (*Canis familiaris*). *Applied animal behavior science*, *79*(2), 133-155.

Szulkin, M., Stopher, K. V., Pemberton, J. M., & Reid, J. M. (2013). Inbreeding avoidance, tolerance, or preference in animals?. *Trends in Ecology & Evolution*, *28*(4), 205-211.

Tan, Ü. (1987). Paw preferences in dogs. *International Journal of Neuroscience*, *32*(3-4), 825-829.

Tan, Ü., Yaprak, M., & Kutlu, N. (1990). Paw preference in cats: Distribution and sex differences. *International Journal of Neuroscience*, *50*(3-4), 195-208.

Tang-Martinez, Z. (2001). The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation. *Behavioral Processes*, *53*(1-2), 21-40.

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V. J., Sawyer, S. K., Greenfield, D. L., ... & Napierala, H. (2013). Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. *Science*, *342*(6160), 871-874.

Tobias, J. A., Montgomerie, R., & Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Phil. Trans. R. Soc. B*, *367*(1600), 2274-2293.

Tomkins, L. M., Thomson, P. C., & McGreevy, P. D. (2010). First-stepping Test as a measure of motor laterality in dogs (*Canis familiaris*). *Journal of Veterinary Behavior: Clinical Applications and Research*, *5*(5), 247-255.

Toms, C. N., Echevarria, D. J., & Jouandot, D. J. (2010). A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology*, *23*(1).

Topál, J., Byrne, R. W., Miklósi, A., & Csányi, V. (2006). Reproducing human actions and action sequences: "Do as I Do!" in a dog. *Animal cognition*, *9*(4), 355-367.

Topál, J., Gergely, G., Erdőhegyi, Á., Csibra, G., & Miklósi, Á. (2009). Differential sensitivity to human communication in dogs, wolves, and human infants. *Science*, *325*(5945), 1269-1272.

Topál, J., Miklósi, Á., & Csányi, V. (1997). Dog-human relationship affects problem solving behavior in the dog. *Anthrozoös*, *10*(4), 214-224.

Trouillard, E., & Blois-Heulin, C. (2005). Manual laterality and task complexity in De Brazza's monkey (Cercopithecus neglectus). *Laterality: Asymmetries of Body, Brain, and Cognition*, *10*(1), 7-27.

Turcsán, B., Kubinyi, E., & Miklósi, Á. (2011). Trainability and boldness traits differ between dog breed clusters based on conventional breed categories and genetic relatedness. *Applied Animal Behavior Science*, *132*(1-2), 61-70.

Udell, M. A., & Wynne, C. D. (2008). A review of domestic dogs' (*Canis familiaris*) human-like behaviors: or why behavior analysts should stop worrying and love their dogs. *Journal of the experimental analysis of behavior*, *89*(2), 247-261.

Udell, M. A., & Wynne, C. D. (2010). Ontogeny and phylogeny: both are essential to human-sensitive behavior in the genus *Canis*. *Animal Behavior*, *79*(2), e9-e14.

Vallortigara, G., & Rogers, L. J. (2005). Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behavioral and brain sciences*, *28*(4), 575-588.

van Oers, K., Klunder, M., & Drent, P. J. (2005). Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. *Behavioral Ecology*, *16*(4), 716-723.

Van Schaik, C.P. (1996). Social evolution in primates: The role of ecological factors and male behavior. *Proceedings of the British Academy, 88*, 9–31.

vonHoldt, B.M., Shuldiner, E., Koch, I. J., Kartzinel, R. Y., Hogan, A., Brubaker, L., Wanser, S., ... & Udell, M. A. (2017). Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Science advances*, *3*(7), e1700398.

Waller, D.E., & Nadel, L.E. (2013). Handbook of Spatial Cognition. American Psychological Association, Washington, DC, USA.

Ward-Fear, G., Brown, G. P., Pearson, D. J., West, A., Rollins, L. A., & Shine, R. (2018). The ecological and life history correlates of boldness in free-ranging lizards. *Ecosphere*, *9*(3), e02125.

Watts, D. P. (1998). Coalitionary mate guarding by male chimpanzees at Ngogo, Kibale National Park, Uganda. *Behavioral Ecology and Sociobiology*, *44*(1), 43-55.

Wells, D. L. (2003). Lateralised behavior in the domestic dog, *Canis familiaris*. *Behavioral processes*, *61*(1-2), 27-35.

Wells, D. L., Hepper, P. G., Milligan, A. D., & Barnard, S. (2016). Comparing lateral bias in dogs and humans using the Kong™ ball test. *Applied Animal Behavior Science*, *176*, 70-76.

Wells, D. L., & Millsopp, S. (2009). Lateralized behavior in the domestic cat, *Felis silvestris catus*. *Animal Behavior*, *78*(2), 537-541.

Williams, G.C. (1996). Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought. Princeton University Press, Princeton, NJ, USA.

Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology & Evolution*, *9*(11), 442-446.

Wilsson, E., & Sundgren, P. E. (1997). The use of a behavior test for the selection of dogs for service and breeding, I: Method of testing and evaluating test results in the adult dog, demands on different kinds of service dogs, sex and breed differences. *Applied Animal Behavior Science*, *53*(4), 279-295.

Wolf, M., Van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, *447*(7144), 581.

Wrangham, R. W., & Smuts, B. B. (1980). Sex differences in the behavioral ecology of chimpanzees in the Gombe National Park, Tanzania. *Journal of reproduction and fertility. Supplement*, 13-31.

Wright, J. C., & Nesselrote, M. S. (1987). Classification of behavior problems in dogs: distributions of age, breed, sex and reproductive status. *Applied Animal Behavior Science*, *19*(1-2), 169-178.

Wynne, C. D., Udell, M. A., & Lord, K. A. (2008). Ontogeny's impacts on humanedog communication. *Age (weeks)*, *6*(8), 16.

Yetkin, Y. (2002). Physical properties of the cerebral hemispheres and paw preferences in mongrel cats: Sex-related differences. *International journal of neuroscience*, *112*(3), 239-262.

Zuckerman, M. (1994). An alternative five-factor model for personality. In: Halverson, C.F., Kohnstamm, G.A., Martin, R., (Eds.), *The Developing Structure of Temperament and Personality from Infancy to Adulthood* (pp. 53–68). L. Erlbaum Associates, Hillsdale, NJ, USA.

CHAPTER 10

CONCLUSIONS

This three-year project aimed to assess how much experiential effects can modulate aspects of behavior in dogs, in which the process of domestication has played a key role in the development and evolution of the species.

A different life experiences, such as a specific training (i.e., quality exposure level), not induce the development of a different dog-human bond, rising just spending daily life together (Scandurra et al., 2016). However, the training experience could be a consequence of the behaviors displayed by dogs educated to specific works, not related to the attachment system (e.g., keeping the ball in the mouth not with the purpose of playing, but as a coping mechanism). Accordingly, it seems that the ontogeny leads trained dogs to differentiate themselves from untrained dogs under behavioral features.

The quantity exposure level, instead, can modulate the human-dog communication. Indeed, dogs born and raised in kennel are unable to follow simpler and more complex human gestures (i.e., proximal and distal pointing) (D'Aniello et al., 2017), meaning that for the most dogs with a low degree of human socialization the gestures normally used in mixed social groups (concerning both humans and dogs) are less informative. Such results highlight how the experiences deeply affect the socio-cognitive skills in dogs.

When acoustic and visual communicative channel were provided by humans during contrasting condition (i.e., bimodal incongruent request), the dogs prefer to perform the action required by the gestural command. This suggest that, when dogs are equally accustomed to both channels, visual cues are preponderant (D'Aniello et al., 2016). However, dogs' responses are also dependent on contextual information, such that while the gestures of both the owner and a stranger are equally relevant to the dog, the verbal commands are more effective when is the owner to give them, making the vocal message more difficult to generalize (Scandurra et al., 2017). The data, not only support the observation that the gestural communication is preferred by dogs in intransitive actions (D'Aniello et al., 2016; Scandurra et al., 2017), but the same also happens during transitive actions (Scandurra et al., 2018). Moreover, in such a context, when unimodal requests are made, the dogs respond more slowly than when the request is bimodal, regardless of whether the message is contradictory or not. Therefore, despite the tendency to prefer gestural signals, verbal inputs are crucial to trigger a faster response (Scandurra et al., 2018). These studies lead to the conclusion that, notwithstanding the long domestication process in which dogs improved their communicative skills toward humans (Feddersen-Petersen, 2000; Pongrácz et al., 2010), they continue to remain strictly connected to their ancestral origins, preferring communicative exchanges based on the body language, rather than the verbal one.

In addition to visual and acoustic channels, dogs communicate and acquire information also through the olfactory channel (Siniscalchi et al., 2018). Particularly, behavioral and physiological changes have been observed in dogs exposed to different emotional chemosignals contained in human sweat (D'Aniello et al., 2018). The dogs showed an increase in cardiac activation and stress levels during the exposition to the chemosignals associated to the human fear, confirming the results of previous study (Siniscalchi et al., 2016). Furthermore, for the first time it was shown that dogs modify their social interest according to the different chemosignals, increasing the interest toward the stranger when exposed to the happiness chemosignals and towards the owner upon fear chemosignals. Whether and for which extension the olfactory system participated in the co-evolutionary history of the two species should be clarified in future studies. However, it is plausible to assume that these mechanisms might have mediated the dogs' acquisition of human emotional states during the domestication process, which are useful tools for a cooperative purpose.

To explore if male-female differences in dogs are a consequence of the domestication process or, conversely, they reflected the sex differences existing in nature, it has been searched for all the papers having sex differences for topic (or as a side argument) to make a comprehensive review regarding personality traits (i.e., aggressiveness, boldness and courage, sociability), cognitive processes (i.e., spatial cognition, lateralization) and perception (i.e., visual focusing, olfactory skills) of male-female dogs. Overall, it seems that both male and female dogs maintained the sex differences described in wild animals, excepted for intraspecific sociability, one of the most important trait for the domestication success. Indeed, a side effect of the domestication is the increased social tolerance (Forkman et al., 2007; Campler et al., 2009; Hughes and Mcdonald, 2013), described as a lowered territoriality and aggression toward conspecific.

Gácsi et al. (2009a) offered a "Synergistic hypothesis" suggesting that sensitivity to human gestural cues may have emerged at both the evolutionary and developmental levels. According to this hypothesis the selection as a result of domestication and the individual experience contributes together to the increase animal-human communication and cooperation (Gácsi et al., 2009b). However, it is very hard to establish the related weight of the genetic and the ontogenetic input in the acquisition processes of the evolutionary or the developmental skills. All the results presented in this doctoral dissertation converge in emphasizing the heavy role of the ontogenetic processes in acquiring socio-cognitive skills, cognitive processes and perception in dogs. Whether the genetic changes have had a minor role, should be ascertained by studying with a major effort the wild equivalent of the domestic dog, the wolf (*Canis lupus lupus*).

11.1 References

Campler, M., Jöngren, M., & Jensen, P. (2009). Fearfulness in red junglefowl and domesticated White Leghorn chickens. *Behavioral Processes*, *81*(1), 39-43.

D'Aniello, B., Alterisio, A., Scandurra, A., Petremolo, E., Iommelli, M. R., & Aria, M. (2017). What's the point? Golden and Labrador retrievers living in kennels do not understand human pointing gestures. *Animal cognition*, *20*(4), 777-787.

D'Aniello, B., Scandurra, A., Alterisio, A., Valsecchi, P., & Prato-Previde, E. (2016). The importance of gestural communication: a study of human–dog communication using incongruent information. *Animal cognition*, *19*(6), 1231-1235.

D'Aniello, B., Semin, G. R., Alterisio, A., Aria, M., & Scandurra, A. (2018). Interspecies transmission of emotional information via chemosignals: from humans to dogs (*Canis lupus familiaris*). *Animal cognition*, *21*(1), 67-78.

Feddersen-Petersen, D. U. (2000). Vocalization of European wolves (*Canis lupus lupus* L.) and various dog breeds (*Canis lupus* f. fam.). *Archives Animal Breeding*, *43*(4), 387-398.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, *92*(3), 340-374.

Gácsi, M., Gyoöri, B., Virányi, Z., Kubinyi, E., Range, F., Belényi, B., & Miklósi, Á. (2009a). Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills. *PLoS One*, *4*(8), e6584.

Gácsi, M., McGreevy, P., Kara, E., & Miklósi, Á. (2009b). Effects of selection for cooperation and attention in dogs. *Behavioral and Brain Functions*, *5*(1), 31.

Hughes, J., & Macdonald, D. W. (2013). A review of the interactions between free-roaming domestic dogs and wildlife. *Biological Conservation*, *157*, 341-351.

Pongrácz, P., Molnár, C., & Miklósi, Á. (2010). Barking in family dogs: an ethological approach. *The Veterinary Journal*, *183*(2), 141-147.

Scandurra, A., Alterisio, A., & D'Aniello, B. (2016). Behavioral effects of training on water rescue dogs in the Strange Situation Test. *Applied Animal Behavior Science*, *174*, 121-127.

Scandurra, A., Alterisio, A., Aria, M., Vernese, R., & D'Aniello, B. (2018). Should I fetch one or the other? A study on dogs on the object choice in the bimodal contrasting paradigm. *Animal cognition*, *21*(1), 119-126.

Scandurra, A., Alterisio, A., Di Cosmo, A., & D'Aniello, B. (2018). Behavioral and perceptual differences between sexes in dogs: an overview. *Animals*, *8*(9), 151.

Scandurra, A., Alterisio, A., Marinelli, L., Mongillo, P., Semin, G. R., & D'Aniello, B. (2017). Effectiveness of verbal and gestural signals and familiarity with signal-senders on the performance of working dogs. *Applied Animal Behavior Science*, *191*, 78-83.

Siniscalchi, M., d'Ingeo, S., Minunno, M., & Quaranta, A. (2018). Communication in dogs. Animals, 8(8).

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2016). The dog nose "KNOWS" fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli. *Behavioral brain research*, *304*, 34-41.