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Developmental and other predisposing factors contributing to behavioural disorders related to fear and anxiety in the domestic dog

Developmental and other predisposing factors contributing to behavioural disorders related to fear and anxiety in the domestic dog

by

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Abstract

Developmental factors are known to contribute to behavioural disorders related to fear and anxiety. Based upon the established association between restricted early life experiences and the development of inappropriate avoidance and fear-related aggressive behaviour, a series of experiments was designed to test whether a dog's capacity to remain in emotional homeostasis at 7 to 8 weeks of age can be increased by exposure to video images during the period of parasympathetic dominance between 3 and 5 weeks of age. First, it was demonstrated that puppies between 3 and 5 weeks of age do react to video images. Second, the reactions of puppies, exposed to video images for 30 minutes per day for 14 days between 3 and 5 weeks old, to test objects in both familiar and unfamiliar environments, were compared with those of control, unexposed puppies; the control puppies visited most of the objects significantly more frequently than did the exposed puppies. Third, another sample of puppies given the same treatments was tested at 7-8 weeks of age; the control puppies were significantly more fearful than the exposed, and also tended to visit the objects more frequently.

A new classification of one class of problem behaviour related to anxiety and fear, separation problems, was developed and validated using a retrospective study of clinical data. In the same data, no evidence was found that a restricted maternal environment predisposed puppies to the development of separation problems; indeed, puppies raised in domestic maternal environments, seemed to be predisposed to have separation problems if they were homed at 7 weeks, but not at or after 8 weeks of age. Exposure to busy urban environments on a regular basis post vaccination, seemed to protect against separation problems triggered by noxious events.

Dedication

To my Daddy and my dogs Sep and Hymke who I miss very much

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I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author.

SIGNED: .... DATE: November 2005

Contents

Part 2:

Appendices

List of Tables

Page

Chapter 6: Does exposing puppies to video images increase behavioural organisation and decrease fearful and avoidance behaviour?

List of Figures

Part I:

Chapter 1: Introduction

Modern society has placed an increasing pressure upon dogs to fulfil the role of social companion. They share a great part of our lives and are exposed to an enormous variety of stimuli to which they are expected to adjust as easily as we do (Bowen 2003). Many dogs, however, show an inability to cope when faced with challenging or even apparently benign situations in their environment. Such incapacity may be primarily due to genetic factors (Houpt & Willis 2001), aversive experiences (whether accidental or due to "cruelty") or inadequate socialisation (Appleby 1993, Appleby *et al* 2002). The welfare of these dogs is at risk. They cannot relax and enjoy life, feel threatened by "normal" events and are more susceptible to stress and diseases. They are less likely to make rewarding pets and are at a higher risk of being abandoned, re-homed or euthanased than those that experience adequate socialisation during early development (McCune *et al* 1995).

The annual review of canine cases (N=1264 from participating members) of The Association of Pet Behaviour Counsellors in the UK showed that the most commonly referred canine behaviour problems in 2000 were fear aggression towards people (25%), although this could partially reflect client perception of the need to seek help (Turner *et al* 2000). In addition, fears and phobias were observed in another 8% of the cases referred (including sound and visual fears and phobias).

Companion animal behaviour counsellor David Appleby, based in the UK, treated 773 dogs in 1992. Ten per cent (79) of them were diagnosed as displaying fear for people or environmental stimuli because of inadequate socialisation and habituation. These figures, however, are probably just the tip of the iceberg because many dogs display problem behaviour without their owners seeking help from a behaviour counsellor (Appleby 1993, Bradshaw *et al* 2002a).

Behaviour problems have multiple causes. The temperament and character of a dog are dependent on both its genetic background, including its breed, and the environment in which it has been reared (Scott & Fuller 1965; Nott 1992). Subsequent experience moulds and modifies the behaviour expressed (Nott 1992). During critical or sensitive periods a puppy is more responsive to its environment, and experiences appear to have a more lasting effect than those occurring in later life (Scott & Marston 1950, Scott & Fuller 1965, Bateson 1979, 1981, Serpell & Jagoe 1995, Overall 1997). Between 2.5 and 3 weeks, to some time between 12 and 14 weeks, is frequently cited in the literature as the sensitive period for socialisation (Freedman *et al* 1961, Scott and Fuller 1965, Fox 1978). Research appears to show that exposure to benign novelty during this period is essential to the development of sound temperament (Scott & Fuller 1965, Appleby 1993, Overall 1997). Additionally, extensive clinical experience (Appleby 1993, Jagoe 1993, Askew 1996, Serpell & Jagoe 1995, Overall 1997, Landsberg *et al* 1997, Appleby *et al* 2002) suggests that puppies that do not have the opportunity to experience particular kinds of stimuli during the socialisation period have an increased likelihood of developing a fear response to those stimuli, which may present either as inappropriate avoidance behaviour, fear or fear-related aggression (Campbell 1975; Appleby 1993, 1999, Serpell & Jagoe 1995).

The developing fear response is associated with a decline in the process of attachment. Theoretically attachment can be defined as the process *"that organizes the puppy's behaviour in relation to those individuals, canine or human with which it usually comes into contact during a critical or sensitive period"* (Scott 1992 page 74). In a social situation attachment can be mutual and involve two or more individuals, but the word can also be applied to a non-social situation in which an individual becomes attached to an inanimate stimulus (Scott 1992). In dogs the attachment process begins around 3 weeks of age; it soon reaches its maximal rate, which is maintained until approximately 8 weeks of age, after which it declines (Scott 1992). As in other altricial young (e.g. domestic cats, Deag *et al* 2000), behavioural interaction takes place immediately after birth between the bitch and the puppies (Fox 1978), during which behaviour of one individual elicits a response in the other. Initially these interactions are primarily initiated by the mother, but as the pups become more mature, and as the interactions are repeated, the sequence becomes more predictable, and the level of behavioural organisation increases, resulting in a behavioural interdependence between the mother and her offspring. As the mother is involved in most of the behavioural activities of the puppy, she will become a significant component in those stimulus configurations that support the offspring's normal maintenance behaviour. Changes in the dyadic interaction between mother and offspring commence when the puppies' sensory and motor capabilities develop. They become more orientated towards salient stimuli and events in their environment (Cairns 1972).

The attachment process is an internal one. The function of the environment is to provide a social or non-social stimulus to which the young animal can become attached (Scott 1992). An organizational process, such as the attachment process, is most easily modified at the moment in development when it is proceeding at maximum rate, and becomes increasingly difficult to influence as the system becomes well established (Scott 1992). The learning experiences the puppy receives to become skilled at organising its behaviour towards the variety of animate and inanimate stimuli in its environment, which are often termed "socialisation", determine the objects, persons and animals to which the individual learns to organize its behaviour towards (Scott 1962). The puppy may then become dependent upon these to maintain behavioural organization (often referred to as "attachment"). Conversely, stimuli with unfamiliar characteristics may elicit anxiety or fear. The level to which a stimulus acquires control over a response system of the individual will determine its dependency or attachment on that stimulus. Among other factors, this is influenced by the presence of the stimulus during the performance of diverse variations of the response system (Cairns 1966). The dependency on the stimulus can be transferred during development, but is not generalized to other similar stimuli. In contrast, the learned acquisition of positive or neutral associations, also an element of "socialisation" in the broad sense, with animate and inanimate stimuli can be generalized to similar objects.

The amount of exposure to stimuli, and the amount and quality of socialisation received during the sensitive period, to a large extent influence the level to which the puppy will be able to cope and adapt to changes in its environment in later life (Scott 1992). If a puppy lives in a restricted environment and has no experience outside of that environment, it may develop an exceptionally narrow basis of attachment, leading to behaviour abnormalities such as kennel dog syndrome (Scott 1992). In such dogs, only a very limited range of stimuli are available to support the organization of behaviour when confronted with new stimuli, and conversely a wide range of stimuli elicit a fear response. This implies, although in a less extreme form than in the "separation syndrome" described by Scott (1992), that a lack of exposure to a wide variety of stimuli during early life might also influence the probability of developing separation related problems, which are a common behaviour problem in dogs. For example, separation-related anxiety is diagnosed in 20-40% of the cases presented at behaviour clinics in the United States (Simpson 1997) and in Great Britain and may occur in up to a quarter of pet dogs in the UK (Bradshaw *et al* 2002a).

The established association between a lack of early life experiences and the development of inappropriate avoidance behaviour, fear and fear related aggression, and the possible association between lack of early life experiences and the development of separation related problems, form the basis for the goals of this project. The objective of Part I. is to find a practical method to decrease the likelihood of developing inappropriate avoidance behaviour, fear and fear related aggression, through increasing the exposure to stimuli for puppies that grow up in a restricted maternal kennel environment. The aim of Part II. is to investigate a possible relationship between the development of separation related problems and early life experiences in dogs, and to introduce and test a new model for the diagnosis and treatment of such problems.

Chapter 2: Literature review

2.1 Wolves, dogs and men

Although significant debate surrounded the identity of its closest relative among wolf subspecies (Clutton-Brock 1984), on the basis of both genetic and behavioural studies the biological ancestry of the dog (*Canis familiaris*) is now certainly a domesticated wolf (*Canis lupus*) (Clutton-Brock 1996, Lindsay 2000a). Molecular genetic data have strengthened the conclusion that dogs originated from wolves (Wayne & Vilá 2001, Savolainen *et al* 2002, Leonard *et al* 2002). A survey of several hundred dogs and grey wolves showed that they have only slightly different mitochondrial DNA control region sequences (Wayne & Vilá 2001). Dogs and wolves have allozyme alleles in common, have highly polymorphic microsatellite allles and mitochondrial DNA sequences comparable or equal to those found in grey wolves (Wayne & Vilá 2001). Archaeological evidence indicates that the dog was the first species of animal to be domesticated which occurred towards the end of the last Ice Age (Clutton-Brock 1996). The earliest find of a domesticated dog consists of a mandible in a paleolithic grave at Obercastle in Germany, dated at 14,000 years BC (Nobis 1979).

The species has undergone pronounced biological and behavioural changes as the result of domestication. The foremost factor in the process of domestication is suppression of the animal's "perceptual world". A high degree of alertness or sensitivity, combined with fast reactions to stress, are crucial for an animal in the wild. For domestication the opposite characteristics of docility, lack of fear and tolerance of stress are important requirements (Clutton-Brock 1996). Through deliberate and accidental selection man has modified the animal's perception of its environment by reduction of brain size, less acute sight and hearing, hormonal changes and the retention of juvenile characteristics and behaviour into adult life (Clutton-Brock 1996). This leads to a neotenisation of the wild prototype, a process in which maturity is developmentally delayed and growth rates altered

(Fox 1978). Domestic dogs appear in many respects to act like 4 to 6 months old wolf cubs. Domestication has also strongly increased the motivation to seek social contact with man, and has enhanced the ability of dogs to learn from man (Hare & Tomasselo 2005). Dogs readily form social bonds with humans, often preferring human contact over that with a conspecific when given a choice. Wolves generally only form such attachments with humans in the absence of adult conspecifics (Zimen 1987).

2.2 Present concept of socialisation

The contemporary concept of the sensitive period for socialisation in the dog is based on several early studies on the development of dog behaviour initiated by Scott and Fuller at the Roscoe B. Jackson Laboratory in the US, and the work of Melzack and colleagues, at the McGill University in Canada, and Fox and colleagues at Thudichum Psychiatric Research Laboratory in Illinois (Webster 1997).

In 1945 an extensive program of research into the relationship between heredity and social behaviour in dogs was started by Scott and his associates. Scott and Marston (1950) classified the social development of puppies into four natural periods based on definite and important changes in behaviour, which in most cases coincide with significant changes in social relationships. In their view there are two major factors that determine the periods of life critical in the development of social behaviour: the maturation of the nervous system, and times when social adjustment is made.

This and subsequent studies that refined the concept of critical or sensitive periods in the early development of the dog, divided it into the following stages.

2.2.1 The prenatal period

Historically this period has been largely overlooked in the ontogenesis of canid behaviour and has been described as a period when external forces cannot affect development (Serpell & Jagoe 1995) However, there is a growing body of evidence illustrating that prenatal influences can have long term effects on development (Weerth *et al* 2005). There are findings from studies in e.g. rodents and humans that suggest transplacental maternal influences may affect the behaviour of offspring (Owen *et al* 2005, Kaiser & Sachser 2005) which may have important implications for the management of a pregnant bitch (Jagoe 1993). Females that are subjected to stressful experiences during pregnancy tend to give birth to more emotional or reactive offspring later in life (Thompson *et al* 1962, DeFries, Weir & Hegmann 1967, Owen *et al* 2005, Kaiser & Sachser 2005). These changes in emotionality and reactivity are probably caused by direct effects of maternal corticosteroid hormones, and androgen influences from the sex and proximity of littermates in the womb (Fox 1978, Serpell & Jagoe 1995, Owen *et al* 2005, Kaiser & Sachser 2005), on the development of the foetus's subsequent physiological responsiveness to stress, rather than genetic influences (Denenberg & Morton 1962). These factors could alter the effect of exposure or deprivation during the sensitive period, but have not been investigated systematically (Appleby 1999).

2.2.2 Neonatal period (0-13 days)

Born at a relatively early stage of neurological development, neonatal puppies are, to a large extent, isolated from their environment. The mostly unmyelinated forebrain and spinal cord and consequent poor impulse transmission means that they have limited motor, sensory and investigative abilities (Scott & Marston 1950, Scott & Fuller 1965, Fox 1972, 1978, Nott 1992). However, it has been suggested that some external influences e.g. exposure to smells and exposure to handling and mild stress-inducing stimuli may have long-term effects on the development of social behaviour (Fox & Stelzner 1966, 1967, Serpell & Jagoe 1995), learning, emotionality, and general adaptability (Lindsay 2000a).

2.2.3 Transition period (13 to 20 days)

The transition period begins with the opening of the eyes at around 13 (+/-3) days and ends at approximately 18-20 days with the opening of the ear canal (Serpell & Jagoe 1995). Rapid brain development and sensory and motor development make walking possible and enable exploratory behaviour to develop (Scott & Marston 1950, Scott & Fuller 1965, Nott 1992). At the end of the transitional period tactile and thermal reactions are no longer dominant, and are supplanted by responses related to visual and auditory stimuli (Scott & Marston 1950). Olfactory stimuli remain important throughout. These changes mean that puppies experience a rapid increase in the amount of social stimulation that they must process, and enter into a period of adjustment.

At the end of the neonatal period the puppy displays the first reactions that indicate differentiation of social environment: e.g. awareness of and attention to an observer. By three weeks a puppy will yelp if it is in an unfamiliar environment, even if it is warm and well-fed (Scott & Fuller 1965, Fox 1971). Play fighting first appears near the end of this period and pups start displaying their first social signals, such as tail wagging (Serpell & Jagoe 1995).

In terms of learning and the effects of early experience, this period resembles the neonatal stage. There is a steady increase in response to both classical and operant conditioning, although rates of learning and the stability of conditioned responses do not reach adult levels until 4-5 weeks of age (Scott & Fuller 1965).

2.2.4 The socialisation period (3 to 9 weeks + 1)

This period is described as the period during which a puppy learns species identity, and will direct species-typical behaviour towards animals that match it. It is suggested that domestic dogs form a multiple species identity (McCune *et al* 1995) because experience during the socialisation period determines the nature of the persons, animals, places and objects to which it becomes accustomed (Scott 1962, Scott & Fuller 1965).

The beginning and early stage of the socialisation period correlates with the maturation and myelination of the spinal cord (Fox 1964), as a consequence of which sense organs become functional, the puppy becomes mobile, is aware of visual and auditory stimuli, and learns to recognise and differentiate between such stimuli (Scott & Marston 1950). Complex learning also becomes possible, although opportunities are limited until seven or eight weeks, by which time sensory perception appears to have reached adult ability (Scott & Fuller 1965).

The upper and lower boundaries of the socialisation period have been identified by experiments in which social contacts were observed and manipulated at different points and for different periods. The onset of the socialisation period is defined by the appearance of the auditory startle response (Scott & Fuller 1965). The upper boundary is now thought to be much less clear cut than originally suggested (Serpell & Jagoe 1995, Appleby 1999). In what is often regarded as the definitive study (Serpell & Jagoe 1995), Freedman *et al* (1961) concluded that *'2,5 to 9-13 weeks of age approximates a critical period for socialization'*. Others have proposed that the actual sensitive period is probably much shorter (Webster 1997). For example, McCune *et al* (1995) and Appleby (1999) suggest 8-10 weeks, which is when weaning takes place in natural conditions. There is consensus amongst some commentators that 10 weeks is the upper limit (Pfaffenberger & Scott 1976, Markwell & Thorne 1987). However, there are reports of canids socialised well beyond the age of ten weeks (Nieburh *et al* 1980) and evidence for an extended gradual decline in sensitivity can be found in a series of experiments on other species e.g. Immelmann & Suomi (1981) and Bateson (1981).

Scientists have investigated the effects on social behaviour of exposure to social stimuli by removing puppies from their mother and/or litter mates, and then exposing them only to humans or other species for various periods of time (Fox 1969, 1971, 1978), or by completely restricting social contacts to other conspecifics (Freedman *et al* 1961, Fox & Stelzner 1966, 1967, Pfaffenberger & Scott 1959). For example Fox (1969, 1971, 1978) fostered puppies individually into litters of four week old kittens. By twelve weeks of age, the cat-reared puppies preferred contact with cats over contact with other puppies that had not been fostered. Another experiment involved litters of puppies split into three groups. One group was hand reared from three days old and received no canine contact. The second group was given equal canine and human contact. The third group only experienced other puppies and their dam. When these three groups of puppies were brought together at 12 weeks and run through a series of tests and observations until 15 weeks, they showed a preference for puppies that had received similar rearing experience. The puppies raised in isolation from conspecifics showed an overall deficit in their relations with other puppies. They were non reactive and non-aggressive when first put together, but quickly became aggressive towards their peers (Fox & Stelzner 1967, Fox 1978).

The effects of social deprivation during the socialisation period have been widely studied (Scott & Marston 1950, Melzack & Scott 1957, Clark *et al* 1951, Melzack 1954, Melzack & Thompson 1956, Melzack & Scott 1957, Fuller & Clark 1966, Fuller 1967). Puppies raised in socially deprived and restrictive laboratory environments demonstrate extreme neophobic responses and appear hyperactive. They also show decreased social activity, exploratory behaviour and learning ability when removed from familiar environments or stimuli (Melzack 1954, Melzack & Thompson 1956, Melzack & Scott 1957, Wright 1983). Scott & Fuller (1965) reported an experiment in which puppies reared in small individual pens did not adopt active escape responses, assumed strange postures when approached, and engaged in fear biting. Other surveys appear to show that the abnormal behaviours most likely to arise from failure to develop species recognition and familiarity with benign environmental stimuli are fear-based, and take the form of either avoidance of novel stimuli, or fear-based aggression (Campbell 1975, Appleby 1993, 1999, Magnus & Appleby 1995, Serpell & Jagoe 1995, Appleby *et al* 2002).

Surprisingly little is known about how much time is needed to socialise a dog (Hubrecht 1995). Experiments looking at the early encounters necessary for socialisation to occur vary considerably in the amount of exposure necessary to achieve adequate socialisation. There are indications that socialisation to humans can be achieved through relatively short exposures, compared to the amount of time that the pups have access to the dam. Forty minutes per week per litter or even less were reported as effective by Scott & Fuller (1965). Wolfe (1990) described a programme that achieved 'adequate socialisation' of laboratory beagles with less than five minutes of human social contact per pup per week. Subsequently, Hubrecht (1995) reported that an extra 2.5 minutes per day of human contact, in addition to normal routine cleaning and feeding activities, with pups aged 5-11 weeks produced animals more likely to approach humans when tested 6-11 months later. However, these studies were conducted in a laboratory environment; which is very predictable and restricted in terms of stimulation and routine and involved Beagles, which, when compared with many other breeds, have been bred to adapt physically and temperamentally to laboratory environments (McCune *et al* 1995).

2.2.5 Juvenile period (9 + 1 weeks – until sexual maturity)

This period from weaning until sexual maturity is primarily one of growth, and the development of skills using motor patterns that appeared in earlier periods. In free living animals this period would involve learning to hunt and to become self supporting. Regression of previously established acceptance of stimuli can occur during this period (Appleby 1999). For example, dogs that are well socialised at three months will regress and become fearful again if periodic social reinforcement is withdrawn until the age of 6-8 months (Woolpy & Ginsberg 1967, Woolpy, 1968, Fox 1971, 1978). Once properly socialised throughout the entire period of approximately 6 months, adult wolves appear to remain socialised despite long periods of isolation from human contact. They maintain their friendliness and generalise it to all humans who act appropriately to them (Woolpy & Ginsberg 1967). However, there are large differences in the development of social behaviour between dogs and wolves (Frank & Frank 1981, Coppinger & Coppinger 2001) that are suggested to result from the selection in domestic dogs for prolongation of juvenile behaviour and morphological neotenized characteristics (Frank & Frank 1981, Coppinger & Coppinger 2001). Coppinger & Coppinger (2001) report that socialisation to humans in wolves has to take place before the age of nineteen days, otherwise it is too late. Depending on the breed, in dogs it might be too late if socialisation to humans has not taken place before eight, nine or maybe ten weeks. Frank & Frank (1981), who compared the social development of four Alaskan Malamutes *(C. familiaris)* with four Eastern timber wolves (*C. lupus lycaon),* described that regardless of the amount of daily contact with the wolf pups, their socialization to humans was tenuous and shifted from around six to eight weeks of age from passive acceptance to wariness and from the onset they showed an explicit preference for canine social partners. The Alaskan Malamute puppies, by contrast, displayed the opposite social preferences. As soon as they developed the necessary locomotor ability, they approached the experimenters, and at around 4 weeks of age were much more socially independent of the (foster) mother and showed effusive greeting behaviour to humans. It may be relevant that anecdotal evidence exists (Serpell & Jagoe 1995) that young wolves, and many young dogs, seem to experience a second, sudden phase of heightened sensitivity to fear-arousing stimuli at around 4-6 months of age (Mech 1970, Fox 1971).

2.3 Imprinting

The development of a social preference of a young animal for its parent or for another individual has been a prominent subject in the study of animal behaviour (Bolhuis 1999). At the moment in time when the pioneering work was conducted on the socialisation phase of dogs, the concepts of the "critical period" and "imprinting" were widely accepted among ethologists and the latter was used synonymous with the term "primary socialisation". Essentially filial imprinting is a learning process in young animals, typically precocial birds, involved in the formation of an attachment to and preference for the parent, parent-surrogate or siblings (Bolhuis 1999). Over a period of time of exposure to the object it forms an attachment and the young animal increasingly restricts its social interactions to that object (Hadden 2002). A second system involved in the development of filial behaviour has been called a predisposition which refers to a perceptual preference that develops spontaneously in young animals without having experience with the particular stimulus (Bolhuis 1999).

Filial imprinting is thought to consist of a sequential series of events, beginning with searching behaviour as soon as the necessary sensory and motor systems are capable. The animal will then learn to recognize the object to which, by means of the internal template, it is predisposed to respond strongly (Bolhuis & Honey 1998), after which approach behaviour is stimulated and searching inhibited. All stimuli received within a short time of each other are generalized together to form a composite picture of the object. Subsequently, the familiar object stimulates approach and the animal will display affiliative social behaviour to it. Unfamiliar objects will start to trigger withdrawal behaviour. However, if an unfamiliar object is presented repeatedly, the animal may become habituated to it, but it is unlikely to display affiliative behaviour (Bateson 1979).

The great importance of the process of primary socialization was first recognized by Konrad Lorenz, who studied filial imprinting in waterfowl. He called the process of forming a primary social relationship "Prägung" which has been translated as "imprinting". This translation might be a bit unfortunate. The word also means "impress" which seems to be a better translation, as the young birds seem to be highly impressed by a limited experience early in life (Scott & Fuller 1965).

The main technique for testing the existence of the process of imprinting and socialization has been to foster young animals on to another species. If the fostered animal transferred its social attachment to the new species it was concluded that socialisation had taken place. To test for the critical period for the process the next step was to try this at different ages (Scott & Fuller 1965). The most common way in which imprinting is measured is the animal's tendency to approach the training object it was imprinted on, compared to a novel object, when given a choice between them (Hadden 2002).

Experiments into imprinting and socialisation which have used the technique of fostering young with unrelated parents have generally produced consistent results in a wide range of species, including insects, birds, and mammals such as dogs, wolves (Scott & Fuller 1965) and chimpanzees (Kellogg & Kellogg 1933 cited in Scott & Fuller 1965, Hayes 1951 cited in Scott and Fuller 1965), sheep and guinea pigs (Gray 1958 cited in Scott & Fuller 1965). These studies have led to the conclusion that forming an emotional attachment to members of the parent species is largely independent of outside circumstances. Whether rewarded, punished or treated indifferently, the young animal of the right age will form an attachment to whatever is present in the environment at that time.

2.4 Critical and sensitive periods

The essential mechanism of imprinting is an internal process acting on information from the external environment (Scott & Fuller 1965, Bateson 1979). The onset and completion is biologically defined, making the animal susceptible to the crucial experience or its absence for a limited time (Lindsay 2000a). Highly social animals show a critical period for socialization early in development. The behavioural mechanisms which limit the period differ from species to species and can not be predicted in advance. However, a developing fear reaction is a common mechanism (Scott & Fuller 1965).

Imprinting was originally thought to be a component of an inflexible cycle of development, either in a fixed time "window", or in a more flexible "window" which was time-limited but also depended on the quality and quantity of stimulation received (Bateson 1979, Hadden 2002). During this "window" particular events in the environment have a strong and unalterable effect on subsequent behaviour; encountered either before or after the critical period, the same events have little or no effect. However, altricial young, such as puppies, form social attachments during a longer period of time due to their slower development. For that reason the term 'sensitive period' is now favoured by most authorities (Serpell & Jagoe 1995). Generally, the concept of the "critical period" has now been replaced in behavioural development with that of the "sensitive period". It is described as a stage when the environment exercises more influence on later behaviour than at it does at other times. The developmental trajectory has not been affected irreversibly, but it may be more difficult to redirect it once the sensitive period has passed (Bateson 1979). It may possibly be that the ability to form social attachments remains, but the willingness to learn is blocked, *e.g.* by fear (Bateson 1979).

Bateson (1981) has developed a "competitive exclusion" model to account for the blocking of subsequent learning. It consists of two components, a recognition system and an executive system which produces the affiliative behaviour. Once the sensitive period begins, sets of stimuli which the young animal encounters first, and/or produce a best-fit with the initial template, are learned by the recognition system and at the same time build connections with the executive system. Other sets of stimuli can be incorporated by the recognition system but will usually fail to produce sufficient connections to the executive system. It is an assumption of the model that the number of potential connections between the two systems is limited.

2.5 Comments on the current concept of socialization

2.5.1 Present practice of puppy rearing

Although early studies still remain influential (e.g. Serpell & Jagoe 1995, Hubrecht 1995), more recently it has been suggested that some practices based on them have little or no effect e.g. attendance at puppy socialisation courses and early homing of puppies (Seksel *et al* 1999, Slabbert & Rasa 1993), or can even be detrimental to the puppies' welfare (Slabbert & Rasa 1993). Slabbert and Rasa (1993) concluded that separation from the dam at six weeks does not improve socialisation with humans, when compared to identical exposure to humans but remaining with the dam until 12 weeks. However, in their study socialisation was specific to the handlers involved in the research. The purpose of removing puppies from the dam as early as possible is to ensure the maximum opportunity for exposure to a broad range of novel stimuli, as promoted by the Guide Dogs for the Blind Association (Freeman 1991). Slabbert and Rasa did not test this, since both groups of puppies were housed and tested in identical circumstances until 12 weeks. They were able to conclude that there was a greater risk to health and of mortality in the group separated from the dam at six weeks. This has been shown in other experiments and may be associated with inadequate parasympathetic arousal (Fox 1978). Slabbert and Rasa did not consider the possibility that for the rehomed puppy significant periods of exposure to human owners may be a sufficient substitute for ongoing maternal presence. This was not fully tested in the 2 hour per day exposure the puppies were provided within their experimental design (Appleby 1999).

Seksel *et al* (1999) did not find that puppy socialisation classes resulted in significant improvement in confidence or socialisation with people in puppies with a minimum group mean age of 9.5 weeks. However, they did not control for the experience the five groups tested received away from the classes, which may have swamped any effect of the classes themselves. Alternatively this finding may indicate that experiences prior to 9 weeks of age have the most substantial effect (Appleby 1999).

2.5.2 Comments on the experimental design of early research

Several authors have asserted that the results of the research into the socialisation process in dogs are questionable (Lehrman 1970). Criticism has been particularly directed at experimental designs, small sample sizes and small ranges of breeds (Webster 1997, Overall 1997). For example, in Freedman *et al*'s (1961) experiment, during which litters of puppies were kept with their littermates and dam but isolated from humans except for one week of exposure to human handling and testing at either two, three, five, seven or nine weeks (etc.) would, indeed, have been more informative if additional groups of puppies had been tested at 10, 11 and 12 weeks, with control groups similar to those for puppies tested at 14 weeks (Appleby 1999). Bateson (1981) points out (Jagoe 1993) that many results of the experiments into the timing and effects of the socialisation period are confounded by the fact that the puppies which are exposed to a stimulus earlier in development, are also exposed for longer periods of time. Alternatively, if the age of testing is kept constant, the period from the end of exposure to testing is not. Even where the length of time between exposure and testing is kept constant, the age of exposure is then confounded with the age of testing.

Additionally, little is known about the correlation between the prevalence of behaviour problems in the canine population and the effects of early experience, both during the sensitive period and also during the juvenile period (Serpell & Jagoe 1995). Appleby *et al* (2002) tested the hypothesis that dogs referred to a pet behaviour counsellor exhibiting avoidance, or aggressive behaviour related to fear, should present a history of limited early experience more frequently than dogs exhibiting types of problem behaviour unrelated to fear. They demonstrated, by comparisons with dogs living in a non domestic maternal environment or not having been exposed to a busy urban environment post vaccination: "*that domestic maternal environments and urban environments post-vaccination are associated with a reduced probability that dogs will later display both avoidance behaviours and aggression to unfamiliar people. As anticipated, this effect is modified by the age at which the puppies are homed. For most behavioural signs, the longer a puppy remained in a particular environment the greater was the association between that environment and its later behaviour* (Appleby *et al* p 11-12)". Such retrospective studies are, however, limited by the extent to which dog owners can report the details of the environment in which their dog has been raised. To get more insight into the effects that early experiences have on the development and expression of inappropriate avoidance behaviour, fear and fear-aggression, observations need to be made of the amount of experience puppies receive in the maternal environment and in the home environment, combining precise measurement of passive and active exposure to novel stimuli, and the age of homing

It appears that from the experiments carried out we have learned a great deal about how to produce abnormal behaviour through early and extreme manipulation of an organism's environment, but considerably less is known about the behaviours that immature animals exhibit in natural settings on their way to becoming adults. A more thorough understanding of the impact of early experience could be gained by designing experiments examining the range of behaviours that occur naturally, the range of conditions that affect them, and the influence they have on later behaviour, instead of designing experiments involving unusual treatments or extreme deprivations (Simmel & Baker 1980).

2.6 Time for a new, more appropriate, theoretical framework?

In addition to the comments made above, it is important to bear in mind that the theoretical framework that guides research into the ways in which social preferences are formed is largely based on experiments using domestic species of birds, such as waterfowl and zebra finches (Lorenz 1935, Bateson 1979, 1981). Filial imprinting in birds is therefore the most complete model on which to base the socialisation of dogs to people (Bradshaw unpublished). There are some similarities between the "imprinting" process in birds and socialisation in dogs but there are also some important differences, some of which it is speculated may be a consequence of domestication (Bradshaw unpublished). The focus of the literature on dogs and cat behaviour has been how they learn to behave in a friendly and appropriate way towards people. It seems to involve simultaneous imprinting to several species and is not restricted to the recognition of close kin exclusively. For that reason the competitive exclusion model cannot apply qualitatively and may even not apply quantitatively, because there has been no evidence so far that dogs which are well socialised to people are less skilled to interact socially with other dogs or vice versa (Rooney *et al* 2000, Bradshaw unpublished). Therefore, before drawing any conclusions about how useful the current conception of filial imprinting in birds may be for the socialisation of puppies it is worth thoroughly re-examining the socialisation of dogs (Bradshaw unpublished).

"It is certainly the case that a great deal more needs to be learned about the mechanisms underlying the socialisation of both dogs and cats, an especially important task because so many behavioural problems seem to have their origins in inadequate socialisation. It is possible that the processes underlying the analogous processes in birds will prove useful in guiding research, but it is also possible that, in the words of Dorothy Parker (1893–1967), American writer and wit "You can't teach an old dogma new tricks", and a new, more appropriate theoretical framework will eventually emerge (Bradshaw, unpublished manuscript p. 5)".

Chapter 3: Sensitive periods in the development of behavioural organization in the dog and the role of emotional homeostasis

(This chapter is based on a paper presented at the International Veterinary Behaviour Meeting 2003 in Caloundra, Australia).

3.1 Introduction

In the view of Scott and Marston (1950) the two most important factors that determine the periods of life critical to the development of social behaviour are maturation of the nervous system and times when social adjustment is made. Therefore, their classification of the developmental periods of puppies was based on the observation of definite and important changes in the behaviour of puppies, which in most cases coincide with significant changes in social relationships.

In this chapter an alternative view is presented on the classification of developmental periods in puppies and a different explanation for the development of inappropriate avoidance and fearful behaviour. This is achieved by examining how behavioural organisation is influenced by the emotional development of the dog, which suggests that the three to five week period of development forms the foundation for the whole of the sensitive period.

3.2 Emotions

Current thinking suggests that animals experience primary emotions such as fear, euphoria and anxiety, comprising of a cohesive set of behavioural, physiological and cognitive integrated responses to environmental experiences (Spruijt 2001, Paul *et al* 2005). They have an organizing effect on other brain structures, and induce an internal state, or "affect", which may be indirectly recognized by observing behaviour patterns and by the presence of internal organizing signals such as hormones (Spruijt 2001, Paul *et al* 2005). Brain structures involved in the selection of responses integrate cognitive cue and context-related information with the affective state of the animal (Fraser & Duncan 1998, Spruijt 2001, Paul *et al* 2005). The cognitive element refers to the information processing mechanism by which the individual acts on information from the environment e.g. through sensory processing or associative learning, the affective element in its strict sense is often described as being similar to 'mood' states in humans (Paul *et al* 2005). The term "emotion" will be used in this text to refer to the process linking information processing (e.g. appraisal of stimuli) via affective states to action tendencies (Frijda 1988, Paul *et al* 2005).

Emotions are not hedonically neutral but are experienced as either positive or negative (Frijda 1988, Fraser & Duncan 1998) and may occur because of a match or mismatch between events and interests (Frijda 1988, Fraser & Duncan 1998). When an individual experiences a negative emotion, physical and behavioural reactions to regain emotional homeostasis are likely to be activated. If such reactions are inadequate or inappropriate, stress reactions may be induced, and welfare compromised (Paul *et al* 2005).

3.3 Behavioural organisation, emotional homeostasis and maintenance sets

Behavioural organisation allows an organism to achieve emotional homeostasis, which is defined as neurophysiological stability, in a varying environment, equipping it with an independent capacity to cope and adapt (Vincent 1986). By neurophysiological stability it is meant that no stimuli that are observed are perceived as threatening, i.e. causing a negative emotional state and activation of the sympathetic autonomic nervous system.

It is proposed that the dog's capacity to remain in emotional homeostasis develops throughout the sensitive period for behavioural organisation, as part of the processes whereby mental representations of stimuli are formed and linked to associations and responses. Some of those associated with parasympathetic activity, the means whereby emotional homeostasis is achieved, become part of a "maintenance set" of animate and inanimate objects. Emotional dependence upon these objects or stimuli associated with them is formed and maintained by exposure (Scott 1963, Cairns 1966, Bateson 1981, Pageat 1998) and, for social stimuli, may not depend upon but may be enhanced by the presumed primary reinforcers, such as suckling (Harlow & Zimmerman 1959, Igel & Calvin 1960, Pageat 1998) and physical contact (Cairns & Johnson 1965).

Whether a stimulus becomes part of a maintenance set and the extent to which dependency upon it develops is determined by cue salience, duration of exposure, context (Cairns 1966), the stimulation the object provides (Cairns 1966, Gubernick 1981, Gross 1996) and the extent to which a maintenance set has developed and enabled behavioural organisation (Scott 1968). During the sensitive period for behavioural organization the process is rapid and easily influenced, but is initially regulated by stages of sensory and neurophysiological development during which higher levels of neural organisation build upon more primitive mechanisms (Fox 1971). Therefore disturbance at an earlier stage of neural development will have negative consequences for subsequent development (Fox 1971).

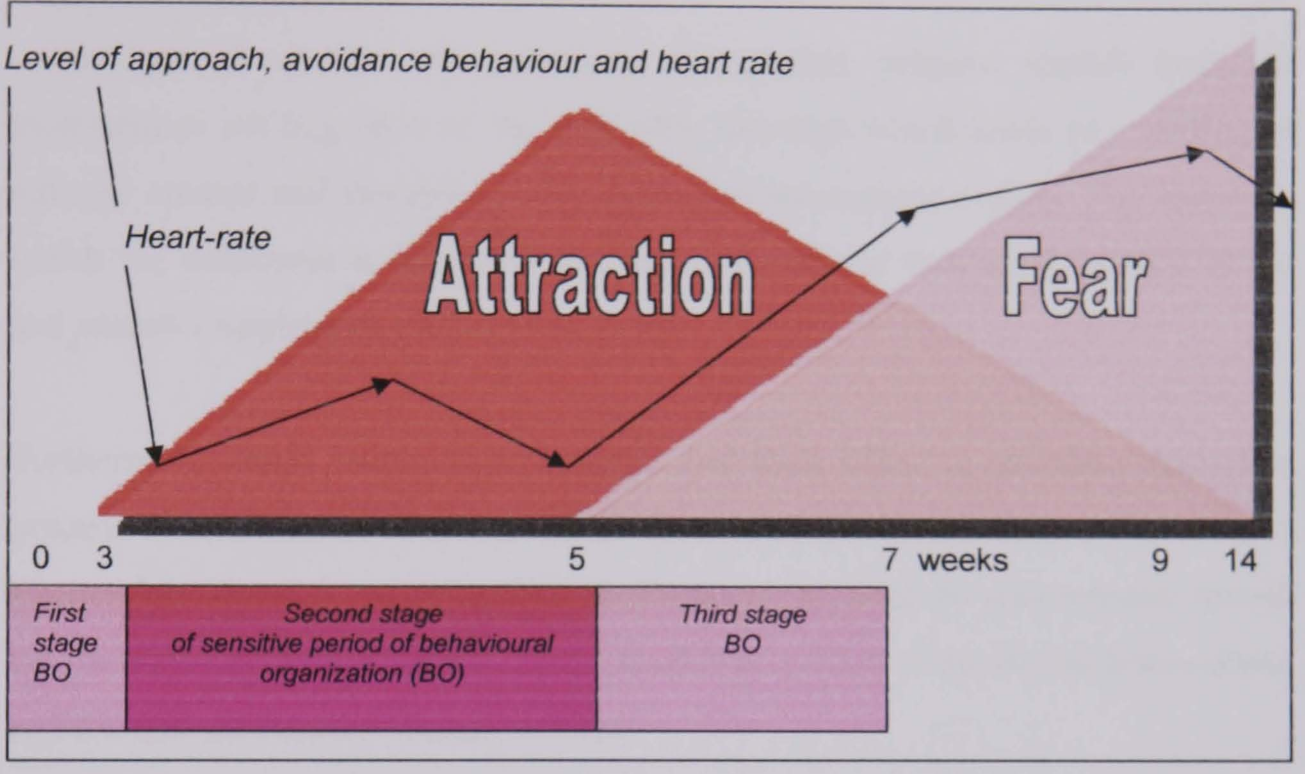
3.3.1 Heart rate development, approach/avoidance behaviour and emotional homeostasis

In the first 16 weeks of life periods of decreased and raised heart rate occur (Scott 1958). Heart rate is a sensitive indicator of both bodily activity and various types of emotions (Scott 1958, Scott & Fuller 1965). During the first two weeks of life the heart rate of puppies is very high, shows a firm dip between three and five weeks, raising to normal level from five weeks to around seven weeks of age after which the heart rate declines towards the adult level (Scott 1958). These general changes in heart rate seem to be independent of breed (Scott 1958. Scott & Fuller 1965) and correlate with parasympathetic (three to five weeks) and sympathetic dominance (week five to a peak at seven/eight weeks followed by gradual decline) respectively, and are manifested in changes in approach-avoidance behaviour (Freedman *et al* 1961).

Ontogenetically, the parasympathetic approach-process develops before the sympathetic-withdrawal process (Schneirla 1965). The phenomenon of excitation and inhibition underlying the approach-withdrawal processes ensure that during the period of excitation, through seeking perceptual homeostasis, the animal will seek stimulation. This ensures that the developing organism will receive optimal stimulation and experience, which is essential for forming normal social relationships and the later development of appropriate approach and avoidance behaviour to novel stimuli, as the avoidance phase is dependent upon what the individual learns during the approach period. Lack of experiences during the approach phase leads to restrictions on subsequent socio-environmental interactions and subsequent development of fear responses. As a result of the imbalance between approach and avoidance processes, and insufficient development of inhibition, a chronic state of arousal arises (Fox 1971).

Lindsay (2000a) interprets the three to five week dip in heart rate as an outcome of the integration of neural connections and the development of emotional responses to social and non-social stimuli. After the raising of the heart rate from normal level from 5 weeks to around 7 weeks, the end of the period in changes in heart rate is at 7 weeks. This coincides approximately with the time of an adult EEG (Fox 1964). It is supposed that this is the period when complete cortical connections with the hypothalamus are established. Scott (1958) concludes that the period from 3 to 7 weeks is an especially sensitive period for the development of emotional reactions.

Figure 1: Schematic depiction of parallels in changes in approach-avoidance behaviour towards novel objects and changes in heart rate. The arrows represent the heart rate, the triangles approach and avoidance behaviour. The Y-axes represent the frequency of heart-rate and level of performance of approach and avoidance behaviour. Based on Freedman *et al* (1961) and Scott (1958).



3.4 Disruption of homeostasis

Disruption of behavioural organisation, and thereby disruption of emotional homeostasis, can cause frustration, anxiety and/or fear. Reduction in parasympathetic autonomic system activity results in activation of regulatory mechanisms with the aim of re-establishing emotional homeostasis. If the challenge to these mechanisms is too great the organism will experience a sense of loss of control, reducing its capacity to cope and adapt (Appleby & Pluijmakers 2003).

Disruption of homeostasis can be caused by:

- Innate/prepared threatening stimuli: animals may react fearfully towards a stimulus because of its physical characteristics (e.g. intensity, duration, suddenness, speed).

- Conditioned threatening stimuli: the stimulus is associated with a threatening event as a result of learning.
- Novel stimuli: as the level of behavioural organization increases, the presence of novel stimuli and stimuli that do not perform to expectation may lead to a negative emotional state.
- The loss of animate and inanimate objects that generate stimuli from the maintenance set (e.g. loss of the dam after homing) which leads to a feeling of reduced control and disruption of responses to subsequent events. The extent to which the behaviour is disrupted will increase with the combined salience of the lost stimuli (Appleby & Pluijmakers 2003).

Furthermore, these factors can combine and their effect accumulate through a process of sensitisation. For example, exposure to novel stimuli or unconditioned threatening stimuli in an unfamiliar environment in which no maintenance stimuli are present, might increase the experienced level of loss of emotional homeostasis and the concomitant emotional reaction.

3.5 Development of behavioural organisation

3.5.1 Initial phase: approximately 0 to 3 weeks of age

Behavioural organisation in the ontogenetic initial phase is largely reflexive and concerned with survival (Scott & Marston 1950). Only rapid changes in physical stimulation, such as sudden loss of support (hunger, cold, lack of contact, pain), cause distress (Scott & Marston 1950; Kagan 1970). There is no wariness of novelty (Smith 1979) and limited capacity for conditioning (Fox 1971). The initial phase of behavioural organisation and normal maintenance set development results in a puppy becoming emotionally dependent upon its mother and to a lesser extent on its littermates and nest-site. This is inevitable because of the availability and salience of the stimuli, sensory and cognitive development (Fox 1978, Scott 1992) and absence of opportunities to attach to other stimuli, due to limited drive and mobility. Initial behavioural organisation, manifested as dependency on the maternal figure, is not simply an affectional bond but a way of maintaining homeostasis of the autonomic nervous system (Cairns 1966, Bourdin 1999, McFarland 1999).

3.5.2 Second phase: approximately 3 to 5 weeks of age

The second phase of development reduces dependence upon the very narrow and salient maintenance set already established, by increasing the number and variety of stimuli for behavioural organisation, after perceptual and locomotor abilities develop and reflexive behaviour declines. Stability developed in the first phase establishes the confidence to explore other stimuli and develop parasympathetic responses through further learning (Bradshaw *et al* 2002a). There are two mechanisms involved in the seeking system and development of behavioural organisation at this stage: perceptual learning and response selection (Smith 1979). Perceptual learning involves recognition of new stimuli and variations in familiar stimuli (Carlson 1998) but this is only useful in conjunction with other forms of associative learning. This learning involves the acquisition of cue properties for stimuli that elicit the organization of behaviour (Carlson 1998, Cairns 1966). Exploration is driven by the seeking components of the brain (Panksepp 1998), which were without intrinsic cognitive content in the initial phase of development but now exhibit spontaneous learning.

Seeking (Panksepp 1998) is aroused by:

- Regulatory imbalances that drive consummatory reflexes, leading to general arousal and motor output of forward locomotion.
- External stimuli. These can be subdivided into biologically relevant stimuli which are unconditional and are relevant for survival, and biologically irrelevant cues. The former have a strong innate interaction with the system. The latter have weak interactions with the system prior to conditioning because during the evolution of the species in question they were not reliable indicators of environmental events that promoted survival. The motor output consists of exploration, approach-sniffing behaviour, investigation and species-typical foraging.

- Cues associated with incentives. The seeking system interacts with higher brain circuits that mediate the ability to anticipate rewards. Animals exhibit expectancies in response to cues which have been previously associated with arousal of this system, and display anticipatory approach towards them (Panksepp 1998). Consummatory behaviour results in disarousal of the system (Panksepp 1998).

Behavioural organisation is also developed through exposure to different contexts, and novel and challenging stimuli (Fox 1978, Serpell & Jagoe 1995) that disrupt it and result in the learning of responses that maintain emotional homeostasis. Expectation of these outcomes increases the sense of control and reduces emotionality.

3.5.3 Third phase: approximately 5 to 7 weeks

The third phase is reached when a broad maintenance set is established and behavioural organisation to the known environment is achieved. Subsequent change is more likely to upset than to benefit the system (Scott 1992). However, the composition of stimuli in maintenance sets is variable because their effect can diminish, extinguish or be superseded by more salient or more available stimuli (Cairns 1966).

An expectation of aversive consequences based on learned associations is an important cause of fear (Smith 1979). Cumulative experience will increasingly become a determinant of fearful and non-fearful responses during the rest of life. Stimuli which are moderately difficult to assimilate – for example, those that are somewhat unpredictable - will generally bring about exploratory responses, so long as other contextual factors are reassuring and that the animal has a preference to investigate novel stimuli or locations over those that are familiar (Smith 1979).

The ability of the individual to maintain contingent behaviour sequences or control is (probably) very important to maintain emotional homeostasis (Smith 1979). The individual may readily learn specific fears of, for example, unfamiliar stimuli, and retain and generalise them, because of their initial discrepancy in stimulus characteristics, and/or noncontingent or unpredictable sequencing of behaviour (Smith 1979).

3.6 The sensitive period of behavioural organisation: An alternative view to the present concept of socialisation and habituation

The previous model, reviewed in Chapter 2 ,of the development of fear responses to novel stimuli in dogs (Scott & Marston 1950, Freedman *et al* 1961, Scott & Fuller 1965) and subsequent research based on it (Fox 1971), clearly showed that behavioural development in the dog takes place during identifiable phases. Isolation experiments to determine the timing of the development of behavioural problems due to a lack of socialisation have limited use, and may even be slightly misleading because they are based on observation of behavioural change. In fact, these changes follow a period of development of the relevant brain structures, their integration and most importantly a period of behavioural organisation that makes subsequent response to novel stimuli possible (Fox 1964, Fox 1971). In other words, before an animal is in a position to identify a stimulus or event as being "novel", it must have formed a cognitive representation of the world in which it lives, making it possible to form expectations (Gray 1971, Williams *et al* 1997).

In the second stage, (between three and five weeks of age) it is hypothesised that approach and investigative behaviour is directed equally to novel and familiar objects, but most attention is paid to rapidly changing stimuli, e.g. movement and sounds. As the formation of maintenance sets becomes more sophisticated, greater attention is paid to moderately discrepant stimuli that evoke investigative behaviour, and very discrepant stimuli that evoke fear. Conversely, attention to familiar stimuli declines. A comparable development of investigative behaviour in stages has been described in children by Kagan (1970). Discrepant events in the environment will lead to arousal and attempts to compare with previous events, and to find a suitable coping response (Kagan 1970). Successful assimilation will lead to behavioural organisation and reduced attention.

During this second stage, recognition and recall memory develops (Smith 1979). Searching behaviour for missing social or non-social objects starts to appear (Kagan 1970) and latency of approach to novel objects starts to increase. Evaluation and attempted assimilation of context, rather than arousal by stimuli in isolation also starts to occur (Smith 1979). Therefore, introduction of a new stimulus in a familiar context may cause a positive emotion, whereas introduction of the same stimulus in an unfamiliar context may cause a negative emotion (Smith 1979).

3.7 Conclusion

Changes in behaviour and increases in emotionality both result from the maturation and integration of structures in the brain. The increase in bi-directional interchange of information between the various areas of the brain, but especially between the hippocampus and neocortex, make more detailed information processing possible. Once parasympathetic dominance has declined and the maintenance set has been formed, unfamiliar stimuli encountered may cause sympathetic arousal. The characteristics of these stimuli and the characteristics of the maintenance set will determine the extent of sympathetic arousal, and the particular behaviour displayed. The presence of an effective maintenance set also increases the individual's confidence to explore and broaden that maintenance set over the ensuing weeks. It follows that a failure to develop an adequate maintenance set during the period of parasympathetic dominance between three to five weeks and beyond should have a detrimental effect on the development of subsequent behaviour, and by implication on welfare, and will increase the probability that behavioural disorders will develop.

Chapter 4: Video images as a means of environmental enrichment

(This chapter is based on a paper presented at the Australian Veterinary Association Conference 2005, Gold Coast, Australia and the European Society of Veterinary Clinical Ethology 2005, Marseille, France.)

4.1 Introduction

As a result of studies of deprivation and normal development it is generally accepted that many aspects of perceptual, cognitive and social-emotional development are greatly dependent on experience (Nelson 1999). Exposure to benign novelty during a sensitive period of canine behavioural development has been shown to have a more profound and lasting effect than those that occur in later life (Scott & Marston 1950; Scott & Fuller 1965; Serpell & Jagoe 1995), and appears to be essential to the development of sound temperament and optimal welfare (Nott 1992; Serpell & Jagoe 1995). Limited experience in the maternal environment and absence of regular exposure to busy urban environments are significant predisposing factors for the development of inappropriate avoidance behaviour and some forms of aggression (Appleby *et al* 2002). Raising puppies in domestic environments and exposing them to busy urban environments before the end of the socialisation period is indicated as the current best procedure for avoiding problem behaviours related to fearfulness and aggression towards people (Appleby *et al* 2002).

Current perceptions of the process of "socialisation" in the domestic dog largely stem from the ethological concepts of imprinting and critical periods. Appleby *et al* (2002) have shown that the development of inappropriate avoidance and fear-related aggressive behaviour, as a consequence of inadequate socialisation, takes place over several weeks or even months, arguing against any "critical period".

In the model of the sensitive period of behavioural organisation, described in Chapter 3, it is proposed that during the "socialisation period" the capacity for dogs to integrate their early experiences relies upon a sensitive period at the beginning of the socialisation phase, i.e. between three and five weeks of age, and that experience between three to five weeks could form the foundation of the socialisation period. The model suggests that a dog's capacity to remain in emotional homeostasis develops throughout the sensitive period, as a result of the process whereby mental representations of stimuli are formed and associated with emotional responses. Some of those associated with parasympathetic activity, the means whereby emotional homeostasis is achieved, become part of a maintenance set of animate and inanimate objects. Once parasympathetic dominance between three and five weeks has declined and the maintenance set has been formed, unfamiliar stimuli encountered may cause sympathetic arousal.

In the this and the following chapters, I have set out to test whether exposure to video images during the three to five week period can be used to introduce puppies to a large variety of stimuli. The applied aim is to investigate whether this is a practical means to decrease the discrepancy between the stimuli perceived in the maternal environment and the stimuli a dog is exposed to in modern society after homing. It is hypothesised that, if the three to five week period forms the foundation of the sensitive period of behavioural organisation and results in the formation of cognitive representations associated with parasympathetic activity of the autonomic nervous system, this will result in decreased fearfulness when the dog is exposed to an unfamiliar environment at an older age. The first step in this process consists of testing whether puppies react to a television screen displaying video images.

4.2 Predisposing factors for the development of behavioural problems related to fear

Several factors may predispose a puppy to the development of behavioural problems related to fear. Research by Fox (1967), during which pups were reared in partial social and sensory isolation between four and five weeks of age, illustrates that without adequate stimulation for a prolonged period, isolation-reared animals are unable to adapt when suddenly confronted with the enriched environment and stimulation that constitutes a "normal environment". A state of acute reticular arousal occurs, that is characterized by a heightened sensitivity to visual stimulation, together with severe behavioural arousal. In the view of Melzack & Burns (1963) the lack of prior experiences by these animals results in a failure to filter out irrelevant information, leading to extreme arousal which interferes with the mechanisms that normally act in the selection of cues for adaptive response.

In the case of the short time isolation-reared puppies used by Fox, rapid behavioural adaptation occurred after the test period. However, if isolation is prolonged until the period of integration, during which the CNS matures and sensory-motor mechanisms are organized (from one to three months of age), social and perceptual deficits leading to permanent behavioural abnormalities have been reported (Fox 1967). The adaptive behavioural processes lose their plasticity, become rigid and are surpassed by fear and avoidance responses to novel stimuli (Fox 1967).

Fuller and Clark (1966) conducted a study of long-term social isolation with pups singly housed in illuminated cages. In contrast to Melzack & Burns (1963), they stated that behavioural disturbance does not result from a perceptual deficit induced by experiential deprivation, but from blocking of approach and tactile responses by anticipatory defensive aversive reactions to unfamiliar stimuli. Recovery in the "normal" environment is then extended, and because of the chronic state of arousal, complete adaptation may be impossible. Freedman *et al*

(1961) showed that from five weeks on, puppies start to develop avoidance behaviour towards novel stimuli.

4.3 Environmental enrichment

Although kennel or kennel-type environments are not generally considered stimulating enough to equip a puppy with the capacity to cope and adapt in a varying environment in later life, breeders' housing often consists of barren pens (Hubrecht 1995). Many institutions housing dogs (e.g. shelters, laboratories) now recognise the importance of environmental enrichment for optimal welfare and adequate psychological well-being in kennelled dogs (Prescott et al. 2004, Wells 2004). The provision of social contact with dogs and humans is considered absolutely necessary. The introduction of a stimulating inanimate environment through the introduction of appropriate toys which are rotated regularly, and the introduction of scents and cage furniture can augment the level of stimulation provided by the environment (Wells 2004). Anecdotal information suggests that these techniques are increasingly applied in breeders' establishments but data on the frequency and level to which they contribute to increasing the dogs' capacity to maintain emotional homeostasis in a changing environment in later life are unknown.

In the author's opinion, even puppies that are raised in a domestic maternal environment will still experience an enormous discrepancy between the quality and quantity of stimuli they are exposed to in that environment, and the diversity of stimuli they have to adapt to during the rest of their life. Additionally, breeders and puppy owners are often reluctant to leave their premises with the puppy before the vaccination program is completed, which in the Netherlands is on average around 12 weeks of age, because of the risk of infection.

4.3.1 Can video images be used as environmental enrichment?

Video stimulation can be considered as a form of environmental enrichment (Platt 1997, Clarke & Jones 2000) but has also been used to study the effects of visual stimuli on behaviour, for example on domestic chickens *(Gallus gallus)* (Evans & Marler 1991, Jones *et al* 1996). It is for example known that chickens show appropriate anti-predator responses to video playback of ground and aerial predators (Evans & Marler 1991, Evans *et al* 1993) and that they show feeding and dust-bathing behaviour when exposed to video images of feeding (Keeling & Hurnik 1993) and dust-bathing conspecifics (Lundberg & Keeling 1997).

Visual stimulation in the form of videotapes has been successfully used to enrich the environment of captive monkeys, whose housing conditions are generally considered impoverished in comparison to their natural settings, (Platt & Novak 2000). Exposure to abstract video images of a computer screen saver appear to cause a reduction of fear in the context of an open-field test (Clarke & Jones 2000). Furthermore, regular exposure of chicks to a complex video image of a computer screen saver during the first week of life has been shown to decrease the chick's fear when tested in an unfamiliar environment (Clarke & Jones 1999). This raises the possibility that video images could be used to overcome the practical difficulties associated with introducing many varied stimuli to puppies in their maternal environment. However, there are fundamental differences between the canine and human visual and auditory systems. It is, for example, suggested by Miller & Murphy (1995) that the reason why most dogs do not spend much time watching television is because the pictures may appear as rapidly flickering images, as the refresh rate of television is about 50-60 Hz. Flicker fusion, being the frequency at which rapidly flickering light fuses into a constantly illuminated light, may be 70 to 80 Hz for dogs (Miller & Murphy 1995).

4.4 Vision and audition in the dog

4.4.1 Neurophysiological development

From two weeks of age a period in the dog's development starts in which the behaviour patterns associated with neonatal existence disappear and are replaced by those typical for puppyhood and adult life. The opening of the eyes takes place at around 13 (+3) days and the opening of the ear canals and the first appearance of an auditory 'startle' response to loud noises at approximately 18 to 20 days (Serpell & Jagoe 1995).

The most striking increase in the development of dendrites in the visual and auditory regions of the cortex of the dog occurs between three and five weeks of age. From six weeks on changes are slower and involve cessation of neuronal growth and final organization of dendrites (Fox 1967, 1971). Both visual and auditory evoked potentials are relatively mature at four to five weeks (Fox 1967, 1971). The onset of a relatively mature EEG occurs between three and four weeks when the puppy is neurologically mature (Fox 1967, 1971).

4.4.2 Vision

A number of functional components are involved in vision, such as: the perception of light and motion, the visual perspective, visual field of view, depth perception, visual acuity and the perception of colour and form. A dog's retina holds considerably more rods than cones, which makes the dog's vision better suited to differentiate light and dark and perceiving movement than seeing colour and detail. Studies done to investigate the colour vision of dogs have produced conflicting results. Whether dogs routinely depend on a rudimentary form of colour distinction, or rely mainly on differences in brightness (Stone 1921) remains an area of controversy (Lindsay 2000a). Highly controlled vision studies carried out by Neitz *et al* (1989) and Jacobs *et al* (1993), however, demonstrated that dogs do possess dichromatic colour vision and that colour provides a useful source of environmental information (Neitz *et al* 1989).

Dogs are probably more aware of the world around them than humans are because of the larger visual field. The visual field of view in dogs is approximately 240 to 250 degrees, which is 60 to 70 degrees greater than the normal human's field of view (Miller & Murphy 1995). McGreevy *et al* (2003) found that there is a correlation between skull dimensions and eye radius which suggest that the visual field varies between breeds. Another remarkable discovery was that the distribution of ganglion cells in the retina varies greatly between breeds (Greyhound, Siberian Husky, Australian Cattle Dog, Stafford Shire Bull Terrier and Pug) from a horizontally aligned visual streak of fairly even density across the retina, as in the Wolf, to a strong area centralis with virtually no streak, in, for example, the Pug (McGreevy *et al* 2003). The visual streak and central area play an important role in enhancing visual acuity, binocular vision and horizontal scanning (Peichl 1992). McGreevy's *et al* (2003) findings suggest that dogs of dissimilar skull shapes may see the world in a different way. For example, dogs bred with a short face and more frontally placed eyes could have a larger ability to focus on human faces because of their retinal ganglion cell distribution (McGreevy *et al* 2003).

Binocular vision depends on a field of ocular overlap between the right and left eye. As a consequence of the placement of the eyes and the muzzle blocking a full frontal view, most dogs have an approximately 40 to 60 degrees overlap between both eyes, which gives them binocular capabilities which are good but inferior to humans (Lindsay 2000a). Binocular vision is also an important aspect of depth perception, which in the dog is limited to some extent by the lack of full binocular vision, restricted to a narrow field of vision directly in front of the snout (Lindsay 2000a). However, by making head movements sensory input can be obtained about objects moving towards each other at different speeds, offering information about relative distance and depth between them (Miller & Murphy 1995). Other information about depth can be provided by foreground/background contrast, clarity of contour, relative size/scale of objects, linear perspective and overlapping and vertical location in the visual field (Miller & Murphy 1995).

Lindsay (2000a) reports an early study done by Karn and Munn (1932) (cited in Lindsay 2000a) which suggests that a dog's ability to form clear object images at a distance and under close-up conditions is very limited, indicating that their range of effective vision is very narrow. These findings conflict to some extent with other findings (Pavlov, 1927 cited in Lindsay 2000a). Notwithstanding their possible difficulties in discriminating stationary shapes and patterns, Miller and Murphy (1995) reported a study in which 14 police dogs could identity moving objects at 810 to 900 meters but could only identify the same objects when stationary at 585 m or less.

4.4.3 Audition

The dog's range of hearing is superior to human audition in many respects. They can, for example, easily hear beyond the human range of audibility (20,000 Hz). The estimation of the upper range of hearing in dogs varies between authors from 26,000 Hz (Fuller & DuBuis, 1962, cited in Lindsay 2000a) to 65,000 Hz (Houpt 1991). Fox and Bekoff (1975) estimated the dog's range of hearing to be between 15 and 60,000 Hz. A variable capacity between individuals to localize the origin of a sound has been shown by Ashmead *et al*, (1986) to be evident in puppies as early as 16 days of age.

It is logical that the hearing abilities between breeds should be different, for example between the smallest and largest breeds, as the surface area of the eardrum that affects frequency response is proportionate to body size (Bradshaw 1992). However, in a study in which the hearing abilities of Chihuahuas, Dachshunds, Poodles and St Bernards were compared (Heffner, 1983) the audiograms were very similar (Bradshaw 1992). Nonetheless, there might be differences between the hearing abilities of dogs of different breeds because of the differences in sizes of the ears and particularly the pinna (Bradshaw 1992).

4.5 Experiment I

The first experiment was designed to explore whether puppies orientate towards and observe a television screen displaying video images and sounds of inanimate and animate stimuli. The relative importance of visual and audio cues was assessed by presenting each in isolation of the other.

4.5.1 Materials and methods

Tests were conducted on 48 puppies, 23 males and 25 females, belonging to the breeds: Maltese Terrier (N= 18), Boomer (small to medium sized crossbred dog with usually a mostly white coat) (N= 27) and Jack Russell (N= 3). The puppies used in this experiment were aged between 26 days to 39 days and therefore their sensory systems would be sufficiently mature to at least detect the visual and auditory stimuli originating from the video playback. They were tested in groups of three littermates in a room that was divided into two parts by a plastic barrier to form the test arena (size: 2.40 m x 2.40 m). A television and video player were installed in the corner of the arena. A video camera was mounted on the ceiling of the room to record the behaviour of the puppies. The puppies were observed via a video monitor in a separate room. Prior to and after the experiment the puppies were housed in their group pens, with their littermates and their mother, within the facility. The puppies were carried into the testing room using a basket and placed on a marker indicated on the floor facing the television screen, after which the videotape was started. The videotape was 7.16 minutes long, and contained 50% animate (e.g. people, dogs) and 50% inanimate (e.g. traffic, vacuum cleaner) stimuli which alternated throughout. The images were played in colour and the sound was played at a level that was relative to the sound level the stimuli would produce in a normal situation. Each group of puppies was exposed to one of four experimental settings:

- treatment 1: video images with sound
- treatment 2: video images only
- treatment 3: sound only
- treatment 4: television and video on but blank screen (no images, no sound), to control for the effect of a heat source and mechanical sounds.

One minute after starting the videotape three observers, each of whom had been allocated one puppy to monitor, directly recorded over the subsequent 7.15 minutes the number of times the puppy orientated its head in the direction of the screen from any distance, and the number of physical (e.g. pawing, play bow.) and vocal reactions (e.g. barking, whining) towards the screen (for ethogram see Table 4.1), and whether a social or non-social stimulus was displayed on the screen at that moment.

Table 4.1 Behavioural variables measured during experiment 1

Category	Variable	Description
Orientation	Head movement	Dog moves his head from a position not directed at the television screen to a position to observe the screen, while sitting, lying or standing still.
	Running/walking	Dog runs/walks in the direction of the television to observe the television screen
Physical	Play bow	Dog places the front part of its body in a lying position with its back end in the air in the direction of the television screen
	Pawing	The dog raises one front paw to the horizontal towards the television screen or touches the television screen and then places it back on the ground
	Scratching/digging	Dog scratches with front paw(s) at the television screen or floor immediately around the television
Vocal	Barking	Dog barks in the direction of the television screen
	Whining	Dog makes one long whine in the direction of the television screen
	Whimpering	Dog makes a high pitched whimpering vocalisation in the direction of the television screen

4.5.2 Results

Since the puppies within a group were unlikely to have behaved independently of one another, means for each group were calculated before differences between treatments were tested using non-parametric statistics (K-W ANOVA).

Comparing the four different treatments, significantly fewer total orientations were elicited by the blank screen (treatment 4) than any of treatments 1-3 (Non-parametric test: Kruskal-Wallis Chi-square=10.5, d.f.=3, P=0.015) (Table 4.2).

Comparing the three treatments with the tape playing with the type of elicited reaction the sound only (treatment 3) elicited, as expected, mainly non-specific orientations, which are orientations to the screen when there was no stimulus present or the type of stimulus eliciting the reaction could not be identified.(K-W Chi-square=10.5, d.f.=2, P=0.005). Physical and vocal reactions were generally infrequent, so were combined for further analysis

Regarding the reactions to different types of stimuli, no significant difference was found for reactions towards social, non-social and non-specific stimuli between the three treatments (Non-parametric test: K-W Chi-square=2.85, d.f.=2, P=0.42). The ratio between orientations to social and non-social stimuli was approximately 1:1 throughout (one-sample t=1.10, d.f.=35, P=0.28) and was similar for treatments 1-3 (F=0.77, df=2, P=0.49). Sound and vision combined (treatment 1) elicited slightly more orientations to social stimuli than treatments 2 and 3 (K-W Chi-square=3.85, d.f.=2, P=0.15). Ratios between physical and vocal reactions to social and non-social stimuli could not be calculated due to zero frequencies, but, excluding the blank screen treatment, slightly more physical and vocal reactions were directed to social stimuli (Non-parametric test: Wilcoxon matched-pairs signed ranks test, Z=1.69 , N=12 , P=0.09).

Table 4.2 Median freauencies for frequency of orientations, and physical/vocal reactions in total towards, towards non-specific, social and non-social stimuli for treatments 1-4.

Measure	Sound and vision	Vision only	Sound only	Blank screen
Orientations (total)	57.0	38.0	55.5	21.5
Orientations (non-specific)	0.0	0.0	8.0	21.5
Orientations (social)	26.5	19.0	20.5	0.0
Orientations (non-social)	26.00	23.0	22.0	0.0
Physical and vocal reactions (total)	5.0	3.0	1.5	1.5
Physical and vocal reactions (non-specific)	0.5	0.0	0.0	1.5
Physical and vocal reactions (social)	3.0	3.0	0.0	0.0
Physical and vocal reactions (non-social)	2.0	0.0	0.0	0.0

4.6 Discussion

This first experiment showed that video images during the three to five week period can be used as a form of environmental enrichment. When exposed to a television screen displaying video images and sounds of inanimate and animate stimuli, the puppies orientated towards the television screen and displayed vocal or physical reactions towards it, some of which (e.g. play bow, pawing at the screen, barking at the screen) could possibly be interpreted as attempts to initiate social interaction.

This is in line with the work of Fox (1966) and Scott (1966). Fox (1966) described that from the onset of the socialisation period, at three weeks of age, dogs possess a CNS with perceptual and motor development sufficient to enable full interaction with the environment (Fox 1966). As soon as the eyes open, which is on average at 13 days of age, reflexes concerned with eye function appear in reaction to light and darkness. Scott (1957) suggests that the capacity to perceive images. is not fully developed until four or five weeks. The onset of hearing is estimated to be on average about 19.5 days (Scott 1957). There is, however, individual variability and differences between breeds in the development of these functions (Scott 1957). Both visual and auditory evoked cortical potentials, and visual and auditory orientation behaviour, are relatively mature at four to five weeks (Fox 1967, 1971).

The results support the hypothesis, that the sensory systems of puppies aged between 26 days to 39 days sensory systems are sufficiently mature to detect the visual and auditory stimuli originating from the video, and thus video playback could be used as a tool for environmental enrichment.. This strengthens the hypothesis that the three to five week period might form the basis for the formation of a maintenance set during sensitive period of behavioural organisation, as the three to five week period is a period of parasympathetic dominance during which maintenance stimuli are suggested to be formed most easily. In addition, between three to five weeks of age puppies uninhibitedly approach novel stimuli (Freedman *et al* 1961) after which an avoidance response, and from 7 weeks on a fear reaction, can be elicited by unfamiliar stimuli which have not previously been associated with a parasympathetic response of the Autonomic Nervous System.

Visual images accompanied by sound appeared to be the optimum to elicit orientation towards the screen. There was no significant difference in behaviour compared to the sound-alone treatment and vision-alone treatment. Sound-alone elicited both non-specific and specific orientations. Video images-alone and video images with sound elicited most physical and vocal reactions.

It has been suggested by some authors that most dogs do not watch television because the pictures have little significance to them, due to the fundamental differences between the canine and human visual system (Miller & Murphy 1995, Beaver 1999), for example, differences in flicker fusion frequency and colour vision. This experiment, however, illustrates that puppies between 26 to 39 days do watch television. They orientate and show physical and vocal reactions to a television screen displaying video images only, and show only slightly less orientations or physical and vocal reactions to video images only when compared to a television screen displaying both images and sound. Although some authors state (e.g. Beaver 1999) that the dog's attention is mainly drawn to the screen by the sounds (Beaver 1999) these results suggest that attention is also drawn to the screen through the visual perception of the video images.

The small differences between the numbers of orientations elicited by treatment treatment 1 (video images with sound) and treatment 2 (video images only) are in line with an experiment reported by Clarke and Jones (2001) on domestic chickens. They found that video images of feeding chick elicited approach regardless of whether the associated soundtrack was played or not. The combination of visual and auditory component failed to exert a significant additive effects and the visual signals were suggested to be responsible for eliciting approach (Clarke & Jones 2001). These results are inconsistent with other propositions that the potency of a stimulus increases through the combination of auditory and visual cues. For example, a test cockerel displays more alarm calling when exposed to video images and sound of a hen, than when either of the stimuli are presented independently (Evans & Marler 1991). However, the most salient reaction, namely physical and vocal reactions, were elicited by treatment one, displaying the most salient stimulus combination namely, images and their accompanying sounds. A possible explanation for these contradictions between findings might be differences in experimental set up e.g. the stimuli used. The test situation or age of testing, might have influenced the type and level of motivation to react to the stimuli. Different species may vary in their sensitivity to visual and sound characteristics of stimuli (McFarland 1999). However, in this experiment no significant differences between stimuli consisting of sound only, vision only or a combination of sound and vision were found.

The lower amount of orientations elicited by treatment 2 (video images only), compared to treatment 3 (sound-only) and 1 (images and sound), might possibly be explained by the fact that the puppies were tested in groups of three littermates. This might have reduced the salience of the video images only treatment during, for example, social interaction and play. In addition, the puppies could move around freely in the test area. A visual stimulus might be easier to ignore when investigating other parts of the test area, compared to the auditory stimuli which were intermittent, and might each cause a reaction to the screen regardless of the position and activity of the puppy.

Not measured was the role of sound in redirecting the attention to the television screen when they displayed other activities than observing the screen (e.g. play, exploring the environment) which might be substantial.

As expected, the puppies of this age did not orientate significantly differently to social and non-social stimuli presented as video images. Next to the process of socialisation, there is evidence that there exists an analogous process of primary 'localisation' in which a young animal starts to react and becomes physiologically attached to a particular environment (Scott 1957). Both processes can be readily distorted by a lack of early experiences during the critical period (Scott 1980). The development of social and site attachment (localisation) can take place with visual stimulation alone, but it occurs much more rapidly if active interaction and tactile stimulation with the objects is possible (Scott 1981). A lack of difference in reaction to social and non-social stimuli in puppies from three weeks on therefore is in line with the work of Scott (1957).

It has been hypothesised in Chapter 3 that puppies between three to five weeks, initially direct their attention equally to familiar and unfamiliar animate and inanimate stimuli. Through exposure to stimuli their maintenance set becomes more sophisticated, and attention will then be directed more to novel stimuli or slightly discrepant stimuli and attention to familiar stimuli will decrease (Chapter 3; Pluijmakers *et al* 2003). These puppies were housed in kennels with their littermates and dam. They had received exposure to, and handling from, humans during cleaning and feeding procedures and physical examinations. This did not result in significant differences in orientations to the screen displaying social stimuli (e.g. dogs, people) compared to non-social stimuli, which suggests that even with the exposure to stimuli (e.g. humans and dogs) they had received next to the video exposure, they reacted in a similar way to familiar and unfamiliar stimuli.

The total number of reactions elicited towards the screen in general, compared to the frequency of reactions displayed during the different treatments, may also have been influenced by the fact that the puppies were tested in a social setting and given the opportunity to engage in other activities, e.g. play. Schapiro and Bloomsmith (1995) exposed singly housed rhesus monkeys to video images of primates engaged in normal activities and found that they showed little interest in the videotapes. In another experiment, Bloomsmith *et al* (1990) displayed video images of a varying content to captive chimpanzees that were housed individually or segregated from their group for the study. These subjects watched the tape for 42% of the time and displayed a preference to watch different types of video images e.g. they preferred to watch images depicting agonistic behaviour most and videotapes of other species not as much.

A difference in the amount of reactions to the screen in a social setting and individual setting is also suggested by Platt and Novak (1997). Seven out of the nine rhesus monkeys in their research were socially housed and remained in their social setting when tested. The subjects in their study on average watched the video images for 25% of a given test session, which is substantially lower than the 42% measured by Bloomsmith *et al* (1990). Surprisingly they found that females watched the video tapes considerably more than males and did not habituate to them whereas males habituated to them across 20 days of presentation (Platt & Novak 1997).

4.7 Conclusion

Environmental enrichment to increase the behavioural repertoire and to decrease the potential for the development of inappropriate avoidance behaviour, fear and fear aggression in dogs, is normally achieved by the introduction of objects and/or social stimuli to the maternal kennel environment or by raising puppies in a domestic environment (Appleby *et al* 2002). In experiments done with other species, video images appeared to be a successful way of providing environmental enrichment, although differences in the effectiveness of eliciting reactions to the television screen seem to be influenced by the social setting they are displayed in, the type of images used, and the gender of the test subjects.

Since in this experiment the video images were shown to elicit an orientation response or vocal and physical reaction from the puppies, they might possibly provide an easy way to introduce a wide variety of domestic stimuli to puppies. However, from this experiment no conclusions can be drawn other than that the images used were perceived, and that sound and images displayed together was the most effective treatment to elicit orientations. Whether the puppies generalise the stimuli seen on the television screen to the 'real' stimuli, and whether individual exposure to the video images would be more effective has still to be explored. The former is the aim of the next experiment. The variation in effectiveness in eliciting reaction in an individual or social setting would be an interesting topic for further research. Individual exposure will be very time consuming for the breeders to apply and therefore, from a practical point of view, be a less desirable option to provide puppies with environmental enrichment using video images.

Chapter 5: Does exposure to video images between 3 to 5 weeks of age result in subsequent changes in exploratory behaviour?

(This chapter is based on a paper presented at the Australian Veterinary Association conference 2005, Gold Coast, Australia and the European Society of Veterinary Clinical Ethology 2005, Marseille, France.)

5.1 Introduction

From the experiment described in Chapter 4, it was concluded that puppies between 26 and 35 days of age pay attention to a television screen displaying video images. This, however, does not demonstrate that the puppies learn to recognize the stimuli observed on the TV screen, or generalize these to stimuli they encounter during daily life, or form expectations about the actions of the stimuli. A second experiment was conducted to measure reactions to real objects, including those that had been presented to them as video images.

The purpose of this experiment was to explore whether exposure to video images between three to five weeks of age might result in perceptual learning and if the objects displayed on the video images become familiar to the puppies. This was measured by comparing the exploratory behaviour of puppies that were exposed to video images with the exploratory behaviour of puppies that had not been exposed to them, in both familiar and unfamiliar environments.

5. 2 Learning to recognize visual stimuli

The primary function of learning is to develop behaviour that is adapted to a constantly changing environment. It provides the individual with the capability to display the appropriate behaviour in the appropriate situation (Carlson 1998, Goldstone 1998). Perceptual learning, which involves relatively long-lasting changes to an individual's perceptual system is caused by the environment the individual is exposed to, and improves the capability to respond to that environment (Goldstone 1998). It also develops the subject's ability to recognize and discriminate between stimuli. Reinforcement is not necessary for perceptual learning to occur. Simple exposure to stimuli leads to substantial learning about the properties and relationships of stimuli (Lieberman 1993). After perceptual and motor abilities have developed during the first three weeks of life, reflexive behaviour declines and behaviour becomes organised through the learning of cue properties of stimuli and the selection of responses towards the stimuli, during the second stage of the sensitive period of behavioural organisation (three to five weeks). So that the puppy can learn to organise its behaviour towards the large variety of stimuli in its constantly changing environment, it is necessary that the cognitive representations formed from stimuli exposed to earlier in their life are compared with the stimuli perceived, and thus for perceptual and associative learning to take place.

5. 3 Novel stimuli and exploration

Attending to novel stimuli is essential for responses to be appropriate in a constantly changing environment (Powell *et al* 2004). In their cognitive map theory O'Keefe and Nadel (1978) describe novelty as a stimulus or place that does not have representation in the cognitive mapping of stimuli previously perceived. When the hippocampus signals a mismatch or lack of information about the current environment, exploratory behaviour may be initiated that facilitates the collection of information about the unfamiliar stimuli and related parts of the environment, enabling the animal to collect more information (Crusio & Van Abeelen 1986). However, reaction to novel stimuli changes during behavioural development and exposure to novel stimuli does become increasingly aversive as the animal becomes older (Freedman *et al* 1961, Fox 1978).

In the model of the sensitive period of behavioural organisation (Chapter 3), it is suggested that during the three to five week phase of the sensitive period exploratory behaviour is initially directed to novel and familiar objects equally. As the formation of the maintenance set becomes more sophisticated, and maturation and integration of various structures in the brain gradually increase the detection of novelty, through comparison between new stimuli and stored representations, greater attention is given to novel or moderately discrepant stimuli that evoke investigative behaviour. Conversely, attention to familiar stimuli declines. In this chapter, I investigate whether exposure to video images between three to five weeks results in the formation of cognitive representations and the predicted increase of exploratory behaviour directed towards novel stimuli compared to stimuli the puppies could familiarise with through exposure to video images.

Although "exploration" is a widely used concept in animal behaviour research, definitions vary widely (Russell 1973) and a large diversity of apparatus and tests (e.g. elevated plus maze, open field test), procedures (e.g. single and multiple trail testing) and measures (e.g. locomotion, preference measures, latency to approach) are used to evaluate exploration (Russell 1973). In addition, there is substantial controversy about what comprises exploration (Table 5.1), interpretation of the measures used and the motivations underlying the behaviour (Russell 1973).

There is a lack of agreement in the literature about the relationship between exploration and anxiety or fear, and the underlying motivation for animals to explore their environment. The two most widely-supported theories, the "Two Factor Theory" (Montgomery 1955) and the "Halliday-Lester Theory" (Halliday 1966, Lester 1967), concur that there is an inverse relationship between exploratory behaviour and anxiety or fear (Russell 1973) and that high levels of fear decrease exploration (Russell 1973, Goddard & Beilharz 1986). However, the

Halliday-Lester theory proposes that low to moderate levels of fear and anxiety actually facilitate exploration. The level of exploration is presumed to be determined by the fear aroused by novelty only; low levels of fear resulting in approach behaviour and high levels in avoidance. An individual that encounters a new stimulus or is placed in an unfamiliar environment explores with the aim to obtain information to decrease its uncertainty. According to the Two Factor Theory, novel stimuli can elicit both curiosity and anxiety or fear, and exploration is the outcome of the opposite tendencies to approach and avoid. A higher level of the 'exploratory drive' results in approach behaviour and a higher level of fear/anxiety in avoidance behaviour

Part of the appeal of the Halliday-Lester theory stems from its capacity to explain exploration from a biological point of view, the function of exploratory behaviour being to gather information to decrease anxiety or fear, and low levels of fear motivating exploration. However, although information-gathering is the primary function, it is unlikely that all exploratory behaviour is motivated by fear. For example, the motivation could change during development; puppies between three and five weeks of age explore new stimuli without showing signs of fear (Freedman *et al* 1961), or external factors could be involved, such as the presence of maintenance stimuli in the environment. Since the hypothesis to be tested in this chapter is based on the assumption that exposure to video images will result in the formation of cognitive representations (O' Keefe & Nadel 1978), a rather broad and pragmatic meaning of exploration is used, aimed at measuring the capacity of the puppies to generalise the video images seen to the real stimuli, without the attribution of a motivation (curiosity or fear) to the displayed behaviour other than gathering information. Therefore exploration is described as approach and/or active investigative behaviour evoked by a novel or partly novel situation that permits the collection of information through increasing the salience of the stimulus input. Although, at this stage limited to the measurement of behavioural acts, this definition is in line with the view of Crusio and Van Abeelen (1986), who defined exploration as: 'exploration is evoked by novel stimuli and consists of behavioural acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment.'

It has been suggested that exposure to novelty in a familiar environment is much less aversive than in an unfamiliar environment (Powell *et al* 2004, Zimmerman *et al* 2000) and is therefore better suited to the study of cognitive (e.g. learning and memory) mechanisms controlling exploration (Zimmerman *et al* 2000), compared to activity in a forced open field test, where activity is supposed to reflect emotionality rather than exploration. In this experiment the puppies are tested in both a familiar and unfamiliar environment, but without making assumptions about their emotional states, rather to research whether differences in relative novelty between the two environments results in differences in the amount of exploratory behaviour displayed.

Table 5.1 Some examples of commonly used definitions of exploration and exploratory behaviour.

Heymer (1976)	Exploratory behaviour: Behaviour which produces the species typical orientation in time and through space necessary for effective learning.
Archer and Birke (1983)	Exploration in the broad sense refers to all activities concerned with gathering information about the environment which normally occur under conditions of stimulus change and is accompanied by physiological changes concerned with energy mobilisation.
Berlyne (1960)	Specific exploration: exploration directed at a specific source of stimulation. Diverse exploration: responses directed towards a variety of stimuli to satisfy a need for interaction with environmental stimuli. Extrinsic exploration: behaviour caused by a specific requirement and directed at a specific goal e.g. searching for a way of escaping from a dangerous stimulus. Intrinsic exploration: investigation of stimuli as a result of interest in these stimuli.
Crusio and Van Abeelen (1986)	Behavioural acts and postures evoked by novel stimuli that allow the gathering of information about new objects and unfamiliar parts of the environment.
O'Kleefe & Nadel (1978)	Exploration is emitted in response to external (unpredicted) stimulation, its function being the collection of information about that stimulation, pursuant to the construction of cognitive maps.

5.4 Experiment 2

5.4.1 Materials and methods

The study was conducted with puppies that had been raised and were housed in a kennel environment (For a detailed description see Appendix 1). Nine litters of puppies (8 litters of Boomers [a small to middle size mongrel dog with long, mostly white coloured fur]; 1 litter of Jack Russell Terriers; n= 29) from the age of 3 to 5 weeks, in groups consisting of the whole litter, were placed in a hall (10 m x 15 m) in which the test arena (3 m x 5 m) was sited. In this location they were exposed to a television screen with video/audio images consisting of inanimate and animate stimuli that dogs usually encounter in domestic and busy urban environments, for 30 minutes each day over a two-week period. The puppies had not been exposed to video images before this experiment, and were a different sample from that described in the previous Chapter. For a detailed description of the content of the video images see Appendix 2. Nine control litters (3 litters of Maltese dogs; 6 litters of Boomers; n= 34) were exposed to a blank television screen in the same arena for the same periods of time, but were otherwise maintained in the same conditions as the test litters.

The dogs were tested individually at the age of 36 days (one control litter of 5 Boomer puppies was tested at 35 days) in a familiar environment, and a few hours later in an unfamiliar environment. Both environments contained four objects. In the familiar environment, which was the test area (3 m x 5 m) where the puppies had been exposed to the television, two of the added objects, a ball and a bicycle wheel, were an approximation of images recorded on the videotape (namely another larger ball and the wheel being a part of a bicycle) that had been played to the puppies exposed to video. The other two objects, a paper bag and a duck sculpture made out of stone, were unfamiliar objects to all puppies (Figure 5.1).

In the unfamiliar test environment, the shop belonging to the premises (3 m x 2.40 m) all possibly distracting objects were removed before the test objects where placed in the room. The objects unfamiliar to all puppies were a toy crane and a toy shoe (Figure 5.1); the objects that had been represented on the tape were a vacuum cleaner and a bicycle pump. The puppies were each placed in the familiar and unfamiliar environment for 5 minutes and videotaped. The total number of visits to the objects in each environment was recorded from the videotape.

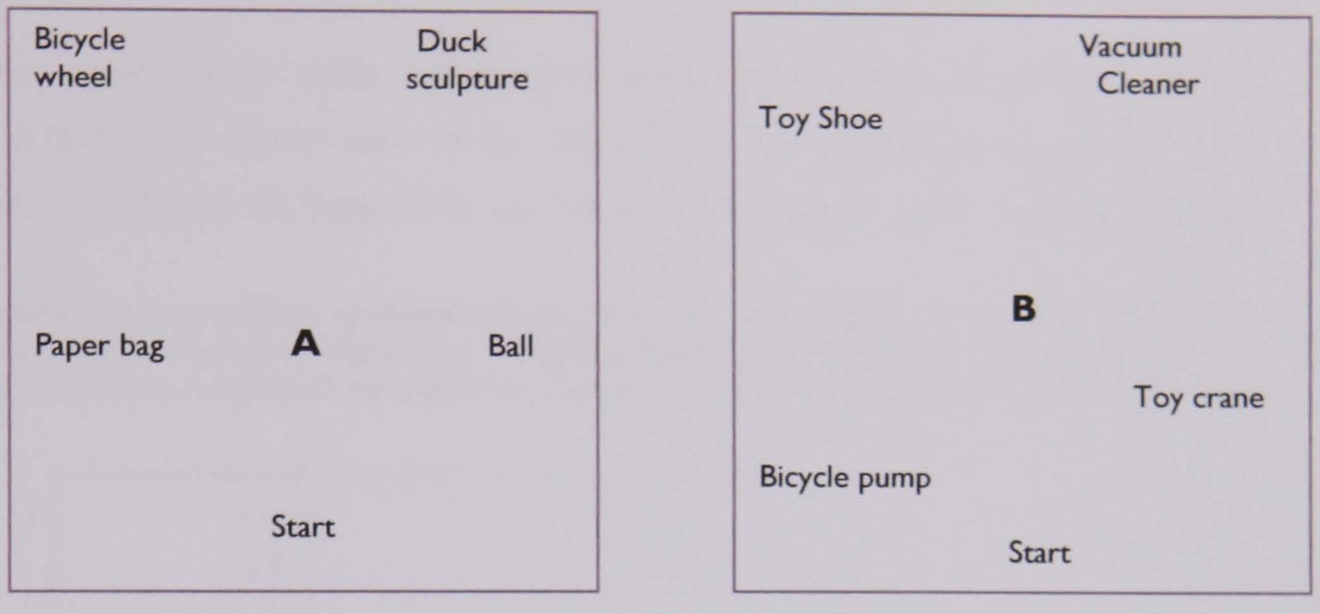
It was hypothesized:

1. That puppies that had not been exposed to the video images would make more visits to the objects in both the familiar and unfamiliar environments. Their threshold for exploration was expected to be higher because of the lack of experience of novelty between three and five weeks of age, resulting in a higher level of novelty of the situation, compared to the puppies that had been exposed to video images and were in a position to form cognitive representations of the two out of the four displayed stimuli, in the familiar and unfamiliar test situation.

2. The amount of exploratory behaviour displayed by both the exposed and control group, would be higher in the unfamiliar environment compared to the familiar environment. The increased level of novelty resulting from exposure to the unfamiliar environment should cause a higher motivation to gather information about the environment.

3. Puppies that had been exposed to the video images should show a preference for visiting unfamiliar objects, compared to the control group, because of the novelty of the unfamiliar objects in both the familiar and unfamiliar environment. It is suggested in the model of the sensitive period of behavioural organisation (described in Chapter 2) that during the three to five week period attention will initially be given equally to familiar and unfamiliar stimuli but will gradually shift to an increase of attention to unfamiliar stimuli, as recognition of stimuli and discrimination of stimuli increases as a result of perceptual and associative learning.

Figure 5.1 Schematic depiction of the positions of the test objects in the familiar (A) and unfamiliar (B) environment.



5.4.2 Measures

At the beginning of the session, each puppy was placed at the starting spot marked on the floor of the area. Around each object a circle of 30 cm was drawn. Each entrance into these circles with, as a minimum, one front paw, was scored as an occurrence of exploratory behaviour.

5.4.3 Statistical analysis

To measure the main effect of pre-exposure to the video images in both environments, Univariate Analysis of Variance (ANOVA) tests were used. ANOVA tests with litter as a nested factor were used to compare the scores between the groups in the familiar and unfamiliar environment.

5.5 Results

5.5.1 Visits to objects in familiar and unfamiliar environment combined

The control group made significantly more visits to the objects ($F_{(1,21)}$=12.5, P=0.002), and visited more of the objects than the exposed group ($F_{(1,21)}$=5.46, P=0.03) (Figure 5.2,Table 5.2a: see Table 5.2b for nested ANOVA model used).

Figure 5.2 Total number of objects visited, per group, to all objects, for familiar and unfamiliar environments combined. Heavy lines = medians, boxes indicate 25th and 75th percentiles, light horizontal lines = minimum and maximum. Control group: N=42, Exposed group: N=29.

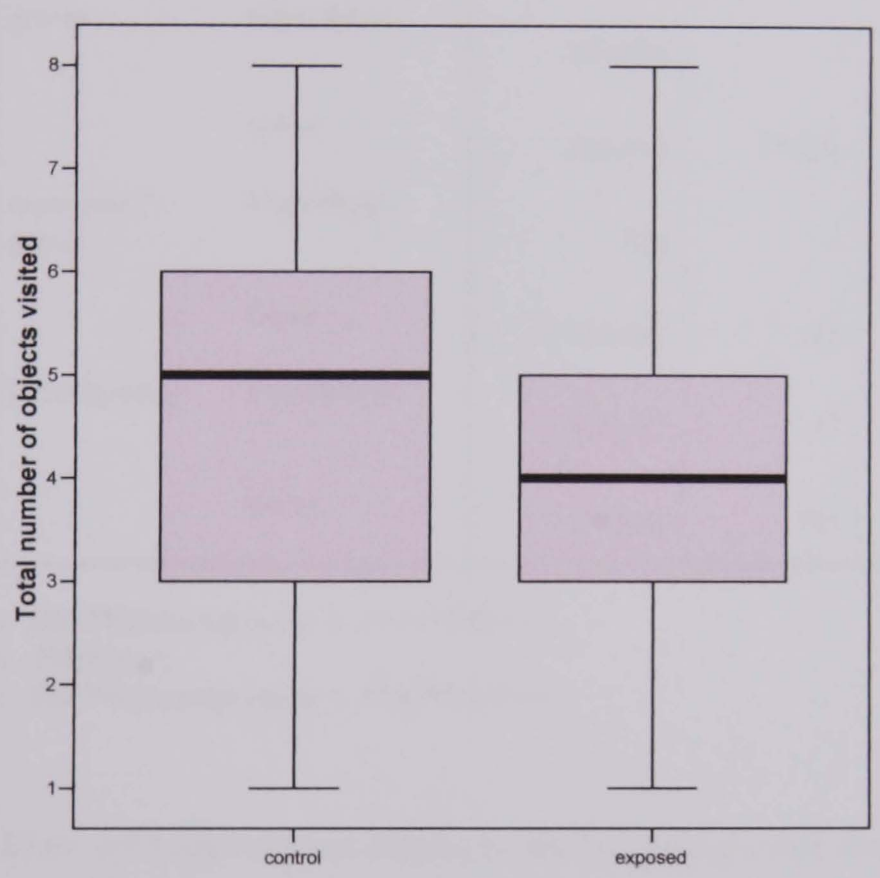


Table 5.2a Mean frequencies and Standard error per litter group of objects visited, and total visits to all objects (including repeat visits), for familiar and unfamiliar environments combined.

Measure	**Control** (N= 42)	**Exposed** (N=29)
Mean number of objects visited Standard error	4.89 +/- 0.28	3.80 +/-0.33
Mean number of visits to all objects Standard error	7.16 +/-0.43	4.71 +/-0.5

Table 5.2b Example of ANOVA model used

Dependent Variable: Total visits to objects represented/not represented on tape (*tape_not*)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4399.967	1	4399.967	294.423	.000
	Error	293.489	19.639	14.944(a)		
tape_not	Hypothesis	128.646	1	128.646	13.816	.000
	Error	1126.641	121	9.311(b)		
group	Hypothesis	187.492	1	187.492	12.549	.002
	Error	293.665	19.656	14.940(c)		
tape_not * group	Hypothesis	.054	1	.054	.006	.939
	Error	1126.641	121	9.311(b)		
Litter(group)	Hypothesis	266.171	17	15.657	1.682	.055
	Error	1126.641	121	9.311(b)		

a .888 MS(litter(group)) + .112 MS(Error)
b MS(Error)
c .887 MS(litter(group)) + .113 MS(Error)

When split into objects displayed on the tape and not displayed on videotape, both of the groups visited the objects not on the tape more often ($F_{(1,20)}$=13.8, P<0.001) (Figure 5.3, Tables 5.2b, 5.3). Since the control pups should not have been able to distinguish between the objects on the basis of experience, this suggests that these objects were (accidentally) intrinsically more attractive. This difference appears to have obscured any difference between exposed and control group in their responses to the objects included or not included on the videotape.

Figure 5.3 Total visits to all objects (including repeat visits), for objects represented and not represented on the videotape, in familiar and unfamiliar environments combined. Key: as Fig 5.1, single point is outlier, more than two interquartile ranges from the median. Control group: N= 42. Exposed group: N=29.

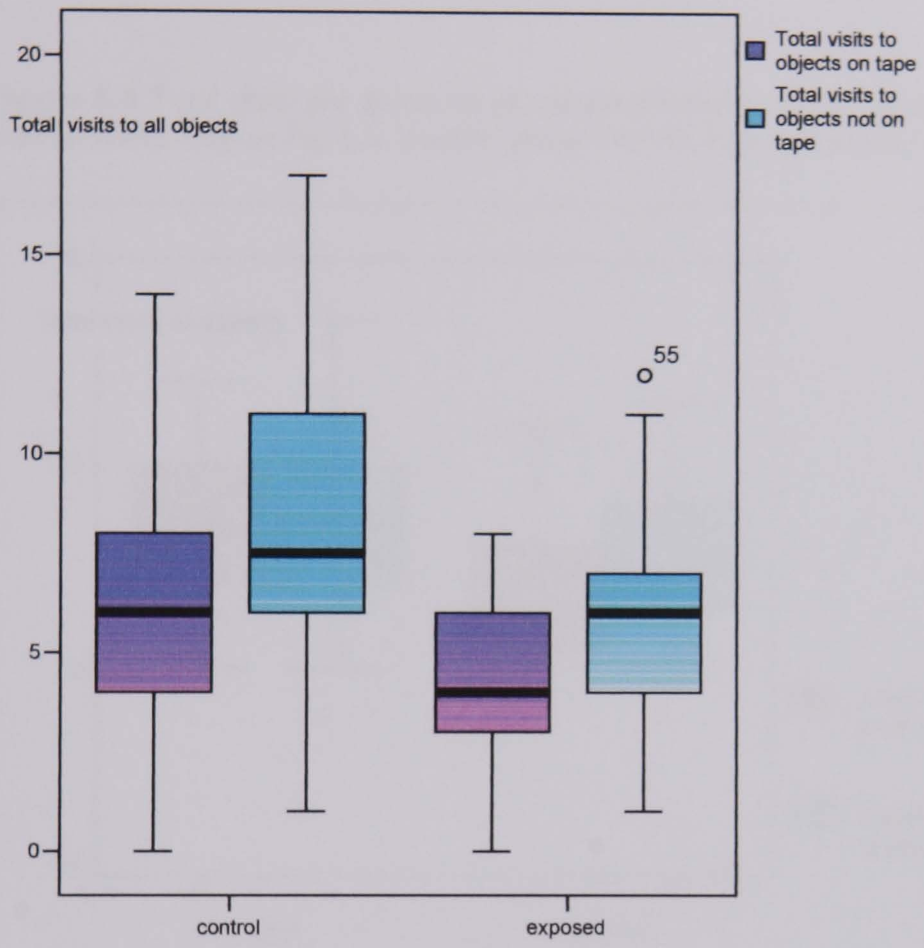


Table 5.3 Mean total visits to all objects (including repeat visits) and standard errors, for objects represented and not represented on the videotape, in familiar and unfamiliar environments combined.

Type of object	**Control** (N= 42)	**Exposed** (N=29)
On tape (mean) Standard error	6.17 +/- 0.49	3.76 +/-0.58
Not on tape (mean) Standard error	8.15 +/-0.49	5.66 +/-0.58

5.5.2 Effects of familiar and unfamiliar environment

The total number of inspections of objects was higher in the unfamiliar environment ($F_{(1,21)}$=2.57, P=0.11), but this was almost entirely due to the exposed group (Figure 5.4; Table 5.4). The control group made more inspections of objects in both environments and significantly more inspections in the familiar environment than the exposed puppies (familiar environment, F(1,22)=14.6, P=0.001; unfamiliar, F(1,20)=2.28, P=0.15) (Figure 5.4).

Figure 5.4 Total visits per group to all objects (including repeat visits) in familiar and unfamiliar environments. Key: as Fig. 5.2. Control group: N= 42. Exposed group: N= 29.

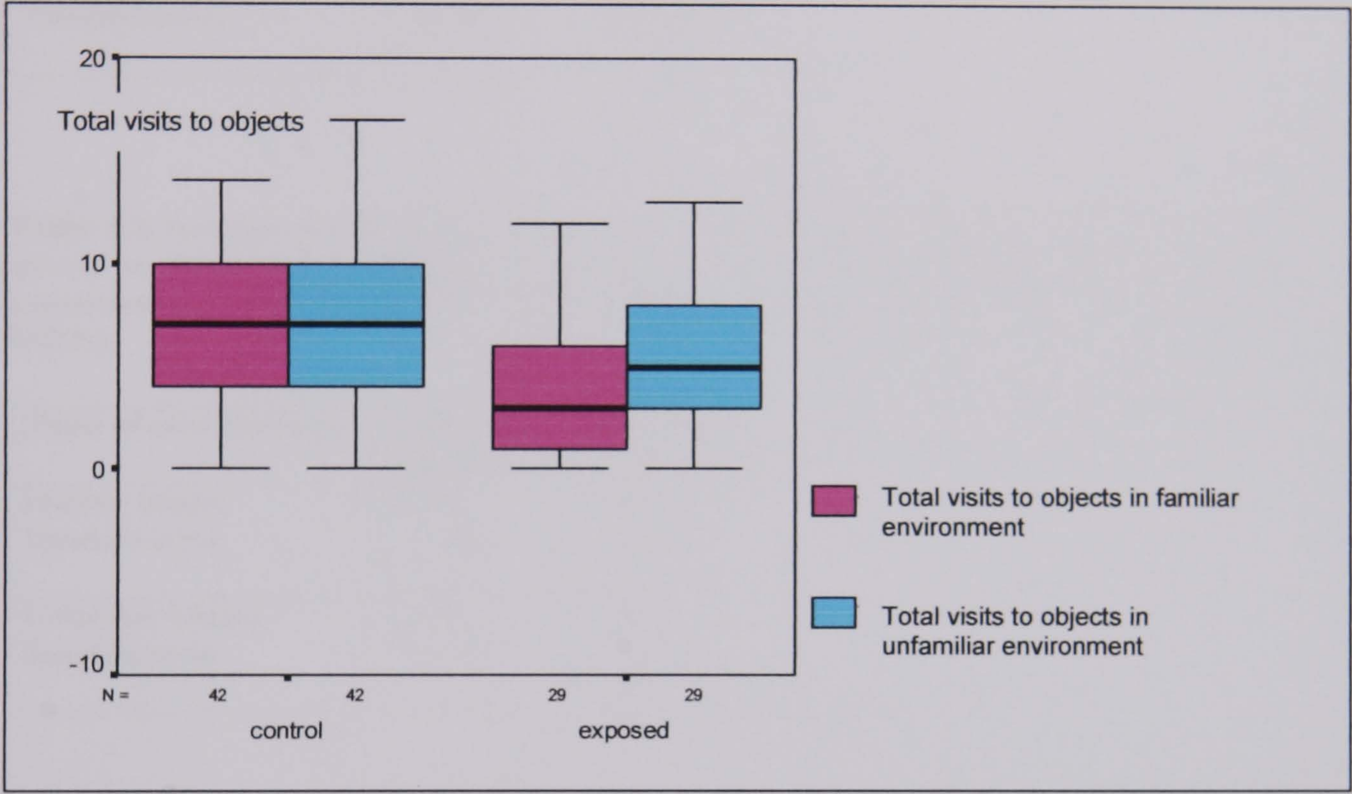


Table 5.4 Mean total visits to all objects (including repeat visits) and Standard errors, in familiar and unfamiliar environments. F-ratio for environment$_{(1,21)}$=2.57, P=0.11; F-ratio for interaction between treatment group and environment$_{(1,21)}$=2.69, P=0.10).

Type of environment	**Control** N=42	**Exposed** N=29
Familiar (mean) Standard error	7.17 +/-0.59	3.69 +/-0.7
Unfamiliar (mean) Standard error	7.15 +/-0.59	5.73 +/-0.7

Table 5.5 Mean number of objects visited and standard errors, in familiar and unfamiliar environments, out of a maximum of 4 objects per environment. F-ratio between treatments for familiar environment$_{(1,21)}$=4.36, P=0.05; F-ratio for unfamiliar environment$_{(1,20)}$=1.59, P=0.22

Type of environment	Control (N= 42)	Exposed (N=29)
Familiar (mean) Standard error	2.47 +/-0.2	1.82 +/-0.23
Unfamiliar (mean) Standard error	2.42 +/-0.19	1.98 +0.22

Table 5.6 Average rate of visiting objects (total visits/number of objects visited) and standard errors, in familiar and unfamiliar environments. F-ratio between treatments for familiar environment$_{(1,20)}$=11.9, P=0.002; F-ratio between treatments for unfamiliar environment$_{(1,21)}$=0.10, P=0.75)

Type of environment	Control (N=42)	Exposed (N=29)
Familiar (mean) Standard error	2.95 +/-0.14	2.00 +/-0.18
Unfamiliar (mean) Standard error	3.17 +/-0.24	3.03 +/-0.29

In the familiar environment, the exposed group both visited fewer of the available objects, and visited them less frequently, than did the control group (Table 5.5, 5.6). Neither of these differences was statistically significant in the unfamiliar environment.

The only type of object that was inspected at a high rate by the exposed group was the two objects in the unfamiliar environment that had not been represented on the videotape (Figure 5.5, Table 5.7). However, the interpretation of this difference is complicated by the apparent intrinsic attractiveness of some of the objects not represented on the videotape (see above).

Figure 5.5 Mean total visits to all objects (including repeat visits), for objects represented and not represented on the videotape, in familiar and unfamiliar environments separately. Key: as Fig. 5.2. Control group: N= 42, Exposed group: N= 29. See Table 5.7 for corresponding means and Standard Errors.

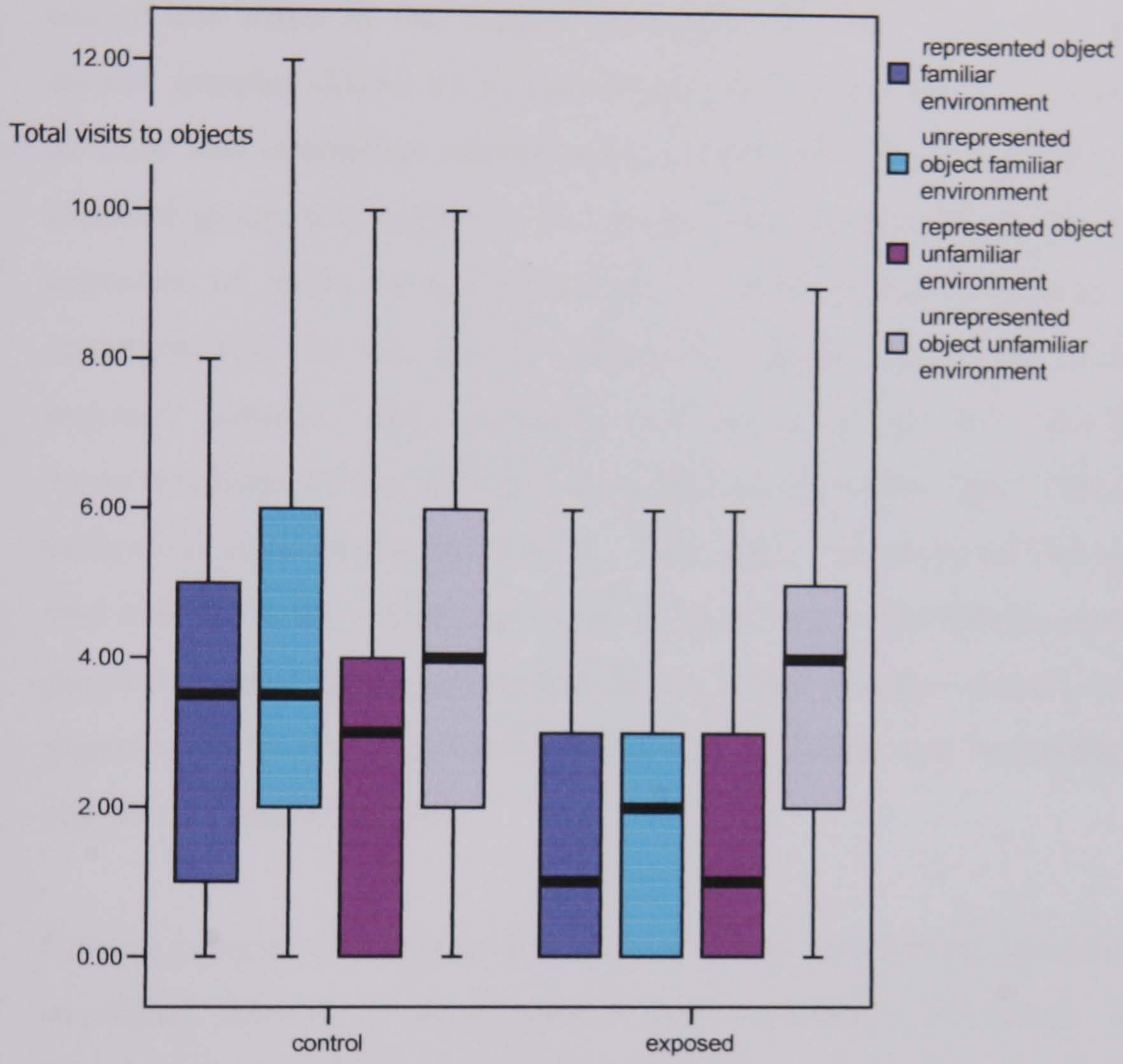


Table 5.7: Mean total visits to all objects (including repeat visits) and standard errors, for objects represented and not represented on the videotape, in familiar and unfamiliar environments separately. Control group: N= 42 ,Exposed group: N= 29.

	Measure	**Control** (N= 42)	**Exposed** (N=29)
Represented objects in familiar environment	Mean Standard error	3.29 +/-0.37	1.9, +/-0.36
Unrepresented objects in familiar environment	Mean Standard error	3.76 +/-0.41	1.9 +/-0.34,
Represented objects in unfamiliar environment	Mean Standard error	2.76 +/-0.38	2.0, +/-0.36
Unrepresented objects in unfamiliar environment	Mean Standard error	4.26 +/-0.43	3.8 +/-0.45

5.6 Discussion

These experiments yielded three main results. First, there was an effect of pre-exposure to video images, because the exposed pups were generally less interested in new objects (Figure 5.2). They inspected fewer objects and inspected each object less often in the familiar environment (Table 5.5, 5.6). Secondly, the control puppies displayed a high amount of exploratory behaviour in both the familiar and unfamiliar environment, whereas the exploratory behaviour of the exposed group was higher in the unfamiliar environment (Table 5.4). Thirdly, exposure to video images appeared to result in the formation of cognitive representations of the specific stimuli the puppy had been exposed to. The exposed puppies were generally less interested in the stimuli that were approximations of the stimuli represented on the video tape (Table 5.3). In the unfamiliar environment most of the exploratory behaviour of the exposed group was directed to the unfamiliar stimuli (Figure 5.5). In the familiar environment the puppies seemed to be surrounded by sufficient familiar stimuli, increasing the experienced level of familiarity with the environment and decreasing the need to explore unfamiliar stimuli.

Several experiments suggest that exposure to an enriched environment results in a decreased interest in novel stimuli and exploratory behaviour. For example Mackay and Wood-Gush (1980), found that beef calves from a socially-restricted housing environment showed a higher exploratory behaviour and showed a greater tendency to approach novel objects. They investigated more areas of the novel environment than calves from a loose-housing system that were equally likely to approach or withdraw from the novel stimulus. Goddard and Beilharz (1983) found a significant positive relationship between the effect of inadequate experience during puppy walking and an increase in olfactory exploration in Guide Dogs for the Blind dogs when tested between 6 and 12 months of age. Rats raised in social isolation for the first 45 days, are more active than socially reared rats and make a different type of contact with novel objects over a long period of time. Rats isolated prior to 45 days showed permanent differences in behaviour, whereas rats isolated after 45 days did not differ from socially-reared rats (Einon 1980).

These findings suggest that exposure to representations of social and non-social stimuli in early life results in a lower motivation to explore novel stimuli or environments. One could hypothesise that this is due to the animals having observed more variation in their environment, and because they are used to changeable conditions, have come to accept novel stimuli more casually (Corey 1978). The results of the experiments conducted in this project add depth to the literature in this area. They suggest that the motivation to display exploratory behaviour results from a signalled lack of information about the stimuli present. The control group displayed significantly more exploratory behaviour in the both environments in which four novel stimuli where placed instead of only two for the exposed puppies.

The motivation to display exploratory behaviour is not only influenced by the degree of contrast between past experiences and present perception of the stimuli (Fox 1971, Barnett & Cowan 1976, Corey 1978), but also by the relative novelty of the object, which is determined by the context in which the novel stimulus is presented (Fox 1971, Barnett & Cowan 1976, Corey 1978, Powell *et al* 2004). The control group displayed a higher rate of exploration in both environments which was s significantly higher in the familiar environment, compared to the exposed puppies (Table 5.2, 5.4). The differences in exploratory behaviour between the two groups were not statistically significant in the unfamiliar environment (Table 5.4), because of the increase in exploratory behaviour in the pre-exposed group to the unfamiliar stimuli, but the mean level of total exploration of the exposed group in the unfamiliar and familiar environment was still lower compared to the control (Table 5.4, Figure 5.5). This illustrates that exposure to video images influences the level of novelty-induced exploratory behaviour, through a combination of learning about the characteristics of the stimuli and the process of familiarisation with the environment, resulting in a lower level of novelty-induced exploratory behaviour towards unfamiliar stimuli in some but not all situations. In the familiar environment the maintenance set of the exposed puppies was sufficient not to evoke exploratory behaviour. In the unfamiliar environment there was a sufficient removal of maintenance stimuli to evoke exploratory behaviour directed most at unfamiliar objects.

It is generally accepted that, as a result of an associative conditioning process, individuals become dependent upon stimuli they have been exposed to, since they provide information/guidance for the maintenance of organised behaviour (Cairns 1966). Removal from a familiar context, or introduction of novel stimuli to a familiar context, can both cause disruption of behavioural organisation (Cairns 1966). The height of the dependency is determined by the length of association with an object in a given context and the relative cue weight of the stimulus compared to the other stimuli (Scott 1963, Cairns 1966). An individual can become dependent on almost any stimulus, animate or inanimate to which that individual has been maintained in a proximate relationship, for maintaining organised behaviour, and emotional homeostasis (Scott 1963). Although the process of familiarisation with a stimulus and developing dependency on it can be facilitated by several conditions, as for example physical contact, no other event, environmental or social, is as essential as proximity for the learning to take place (Cairns 1963, Scott 1963). The number of 'interactions' with a stimulus, and not the quality of the 'interaction', is the direct determinant of the strength of the dependency for maintaining behavioural organisation (Cairns 1966). The results of this experiment underline the statement that exposure to stimuli is sufficient for them to become maintenance stimuli, and add that in dogs between three and five weeks of age this can be achieved by exposure to video images only.

Environmental enrichment at an early stage of development induces morphological and biochemical alternations in the cortex and hippocampal formation (Greenough 1975, Fiala & Greenough 1978, Kempermann *et al* 1997). The enrichment-dependent plasticity of the brain is mediated by the possibilities for informal learning, which are influenced by the stimulus complexity of the environment (Zimmerman *et al* 2000). The exposure to the video images appears to have resulted in perceptual learning, which involves *"relatively long-lasting changes to an organism's perceptual system that improve its ability to respond to its environment"* (Goldstone 1998 p. 586). Exposure to video images resulted in learning about the characteristics of stimuli and the formation of neural models of the stimuli seen on the television screen. The puppies that had been pre-exposed to the video images were generally less interested in novel objects and in the unfamiliar environment directed significantly more exploratory behaviour to the stimuli not represented on the videotape (Table 5.3, 5.4, 5.5, 5.6 and Figure 5.3,5.4, 5.5). This suggests that the puppies successfully transferred the video images observed on the television screen into real stimuli and developed the capability to differentiate between familiar and novel stimuli in the real world.

The exposed group displayed most exploratory behaviour towards the unfamiliar objects in the familiar and unfamiliar environment (Figure 5.5). Corey (1978) states that the approach tendency to initially novel stimuli and the concomitant investigatory behaviour decreases with repeated exposure to the stimulus, and if the stimulus has little intrinsic value it will eventually fail to elicit a reaction. The results of this experiment are in line with this statement and are also consistent with the work done by Solokov (1960). He reported a group of 'orientating' responses, the most important being the EEG arousal response, which are elicited by novel stimuli in any sensory modality, that habituate with repetition of exposure to the stimulus. The specificity of habituation leads to the formation of the hypothesis that the brain forms a 'neural model', which when exposed to the stimulus is compared with the actual stimulus. This makes it possible to signal familiarity or novelty as result of the combined action of 3 types of neurons: (i) afferent neurons: which always respond to an appropriate stimulus; (ii) extrapolatory neurons: responding when the stimulus has been presented repeatedly and (iii) novelty or comparator neurons: which signal 'novelty' if the comparison of the afferent and extrapolatory neurons produces a mismatch (Solokov 1960, Gray 1987, Vinogradova 1995). By directly recording the firing patterns of individual nerve cells during repeated presentation of an originally novel stimulus, 'novelty' neurons or 'comparator' neurons were found in the largest concentrations in the hippocampus and also in the visual cortex, the reticular formation and the caudate nucleus. Afferent neurons were found in the sensory cortex and sensory nuclei of the thalamus, and exploratory neurons only in the hippocampus (Gray 1987). This provides a biological basis for the observed differences in exploratory behaviour directed towards familiar and unfamiliar stimuli and supports the hypothesis that exposure to video images results in the formation of cognitive representations which influence the organisation of behaviour in the real world.

A developmental change in attention paid to different types of stimuli in children is described by Kagan (1970). In the first few weeks children only pay attention to rapidly changing stimuli (moving, talking faces). In the next months, however, the longest attention is given to stimuli that are moderately discrepant from established representations, and less attention is given to familiar stimuli. Kagan's explanation is that a discrepant stimulus in the environment causes alerting and attention, as the infant attempts to assimilate the discrepant stimulus to find a suitable coping response. If the assimilation is successful this results in a loss of attention. Failure to assimilate may cause avoidance or crying (Smith 1979). This pattern seems to be comparable with the development of an avoidance response to novel stimuli from 5 weeks on in dogs (Freedman *et al* 1961) where a failure to assimilate a novel or discrepant stimulus causes a lack of information about suitable way of organising its behaviour towards the stimulus, which may be expressed as avoidance behaviour or a fear reaction.

The results seem to support the prediction made in the model of the sensitive period of behavioural organisation, which proposes that perceptual learning takes place through exposure to the stimuli only and results in a preference to explore novel stimuli at the age of five weeks compared to familiar stimuli. The exposed puppies displayed the lowest amount of exploratory behaviour in the unfamiliar environment which was relatively more familiar because of the presence of two maintenance stimuli; and the highest level of exploratory behaviour in the unfamiliar environment towards the unfamiliar stimuli (Figure 5.5.). In the familiar environment most exploratory behaviour was directed towards the unfamiliar stimuli (Figure 5.5). This illustrates that cognitive representations of the video images were formed. Although the novel stimuli were more attractive to both the control and exposed group, the specificity of the direction of the exploratory behaviour of the exposed group in the familiar and unfamiliar environment and lack of differences in specificity in exploratory behaviour by the control group, is supportive to the assumption that exposure to video images results in the formation of cognitive representations of the stimuli perceived that are generalised to the real environment.

5.7 Conclusion

It can be concluded that systematic exposure to video images between three and five weeks of age can be used to increase a puppy's knowledge of the world.and results in the formation of maintenance stimuli .From the measures taken during this experiment no conclusions can be drawn about the extent to which the formation of the cognitive representations resulting from the exposure to the video images developed into maintenance stimuli, which are associated with parasympathetic activity of the ANS and increase the capacity of the individual to maintain emotional homeostasis in a changing environment. This is the aim of the experiment described in Chapter 6. However, it can be concluded that the presence of maintenance stimuli influences the way behaviour towards unfamiliar stimuli is organised

Although the period of exposure to video images was chosen to coincide with a natural period of parasympathetic dominance in the puppies, the amount of daily exposure (30 minutes) was selected arbitrarily. Based on the experimental set up used, no conclusions can be drawn about the exact amount and type of exposure necessary to achieve an effect. Additional experiments will be necessary to refine the findings of this project, and it will be desirable to research what the effect of exposure at different ages would be, to refine the amount and type of stimulation necessary, and to research the long lasting effect on the emotional development of the dog.

Chapter 6: Does exposing puppies to video images increase behavioural organisation and decrease fearful and avoidance behaviour?

(This chapter is based on a paper presented at the Australian Veterinary Association conference 2005, Gold Coast, Australia and the European Society of Veterinary Clinical Ethology 2005, Marseille, France)

6.1 Introduction

The experiment described in Chapter 5 suggests that exposure to video images results in the formation of cognitive representations. However, to decrease the potential for the development of fear and inappropriate avoidance behaviour, and to increase the capacity to maintain emotional homeostasis, it is essential that the stimuli become associated with activity in the parasympathetic system, and ideally for this to generalise to the real stimuli, as a more sophisticated maintenance set should lead to an increased capacity to maintain emotional homeostasis when exposed to unfamiliar stimuli and/or environments (Chapter 3; Pluijmakers *et al* 2003).

Disruption of emotional homeostasis, resulting from a decreased parasympathetic activity and increased sympathetic activity, is more generally referred to as stress (Chrousos & Gold 1992) which is reflected in the physiological, behavioural and psychological state of an individual when confronted with, from the individual's point of view, a potentially threatening situation (Chrousos & Gold 1992). More specifically, a stress response caused by the anticipation of a threatening event is referred to as the animal being anxious. A stress response caused by the actual exposure to a threatening stimulus is referred to as fear (O'Farrell 1992).

Fear is thus regarded as specific stress response resulting in a negative emotional state where an individual responds to a specific stimulus, to protect it from an actual or potentially dangerous situation (McFarland 1981, Gray 1987). According to Gray (1978), fear eliciting stimuli can be categorized into: intense stimuli, novel stimuli, stimuli associated with evolutionary dangers, stimuli associated with aversive social interaction with conspecifics, and conditioned fear stimuli.

An individual's fear response to a stimulus or environment can be influenced by factors such as genetics, age, breed, gender, type of stimulus, context, previous experiences, and the individual's assessment of the controllability and predictability of the situation (Boissy 1995). Although fear of novel stimuli is one of the most frequently tested fear responses (King *et al* 2003), and some influential work has been done on the use of behavioural stress parameters and physical measures in dogs by Beerda *et al* (1997a, 1997b, 1998), there seems to be no general agreement about how fear can be recognized and measured in other species (Roy & Chappilon 2004, Van Reenen *et al* 2005) or in domestic dogs (King *et al* 2003). A possible cause for this lack of agreement may result from the many factors that influence the fear response, the variety of tests used, and the application of measures that can be interpreted in a variety of ways. The situation is further complicated because the relationship between activity, exploration and fearfulness is complex and sometimes contradictory (Goddard & Beilharz 1983). The relationship between fear and exploration is expected to be n-shaped (Russell 1973). Factors like stimulus novelty increase both fear and exploration (Goddard & Beilharz 1983) but high levels of fear are normally found to inhibit exploration (Russell 1973). In dogs both high and low levels of activity have been associated with fear (Murphree & Dykman 1965; Scott & Fuller 1965, Melzack 1969, Goddard & Beilharz 1984). Although activity and exploration are different in concept they have often been measured using similar variables (Goddard & Beilharz 1984).

Fear reactions to novel stimuli or environments have been reported in several domesticated species (e.g. chickens: Jones & Carmichael 1999, rats: Kabbaj & Akil 2001, dogs: Freedman *et al* 1961, Pagani *et al* 1991). They create a conflict between the motivation to explore the unfamiliar stimuli/environment and an unconditional fear of novelty (Roy & Chapillon 2004). Dogs, when introduced to an unfamiliar environment or exposed to a novel object, have been found to have an augmented sympathetic activation (Pagani *et al* 1991) and HPA activity (King *et al* 2003).

To research animals' reactions to novel environments and objects, different types of tests (e.g. open field test, elevated plus maze) and various measures are used (Augustsson & Meyerson 2004), such as behavioural responses (e.g. flight and avoidance reactions), and physiological measures such as heart rate and concentrations of catecholamines and cortisol (Boissy 1995, King *et al* 2003). Behavioural parameters that reflect exploration, such as locomotion, latency to explore a certain area, and time spent at a certain location (Augustsson & Meyerson 2004), are also used to assess emotionality, based on the idea that a non-emotive or non-anxious animal will explore any novel situation (Roy & Chapillon 2004). However, the interpretation of exploration measures in the context of the emotional state they reflect, is difficult. It is, for example, suggested that activity may reflect confidence in a non-emotive animal, but in another animal might be an attempt to escape from the environment, motivated by fear or anxiety (Roy & Chapillon 2004). Because of the difficulties already described with interpreting what emotional state exploration reflects, in the previous experiment exploration was interpreted functionally, as gathering information.

The occurrence of displacement activities is more generally associated with decision making processes (Maestripieri *et al* 1991). More specifically they are linked with a state of conflict in the animal (Landsberg *et al* 2003, Maestripieri *et al* 1991), e.g. when two conflicting motivational tendencies are elicited at the same time (Maestripieri *et al* 1991), and as such have an emotional complement in the form of, for example, anxiety or uncertainty (Maestripieri *et al* 1991). They are regarded as a powerful parameter to measure emotional states, like anxiety, because of their consistent association with activation of the autonomic nervous system induced by conflict situations. Quantitative data have produced evidence that stressful situations elicit more displacement activities in primates compared to non-stressful situations. As such they are regarded as a powerful non-invasive observational parameter to quantify emotional reactions to social and non-social stressors (Maestripieri *et al* 1992).

The aim of the experiment described in this chapter was to explore whether exposing puppies to video images makes it easier for them to maintain emotional homeostasis and behavioural organization when encountering familiar stimuli in an unfamiliar environment. Although there is disagreement concerning the way in which emotional reactions such as fear and anxiety should be measured (Boissy & Bouissou 1995). In the following experiment behavioural measures associated with stress (e.g. vocalisation, body postures), most of which are derived from the work of Beerda *et al* (1997a, 1997b, 1998), and displacement activities (e.g. scratching, yawning) which are likely to occur in stressful situations (Maestripieri *et al* 1992) were scored, in addition to measures reflecting exploration, such as the frequency of objects visited, the latency to approach the first object and time spent exploring objects.

In the previous experiment it was shown that the presence of maintenance stimuli influences the amount of exploratory behaviour displayed, the availability of lesser maintenance stimuli resulting in an increase in exploratory behaviour. In relation to the experiment conducted in this chapter it was hypothesized that compared to the unexposed control group, puppies exposed to the video images would be more likely to maintain emotional homeostasis and behavioural organisation as it is suggested in the model of the sensitive period of behavioural organisation (Chapter 3) that stimuli the dog is exposed to between three and five weeks of age become associated with parasympathetic activity of the ANS. It was predicted that the presence of more maintenance stimuli in an otherwise novel environment should result in their body postures being those associated with relaxation instead of fear, and a lower frequency of the display of displacement activities. Significant differences in frequency of visits to objects were not expected, as the previous experiment has shown that in an unfamiliar environment the frequency of objects visited between the control and exposed group is comparable. It was further hypothesised that the control puppies would spend more time exploring objects and would approach the first object sooner than the exposed group because of the higher level of unfamiliarity with the stimuli and environment.

6.2 Experiment 3

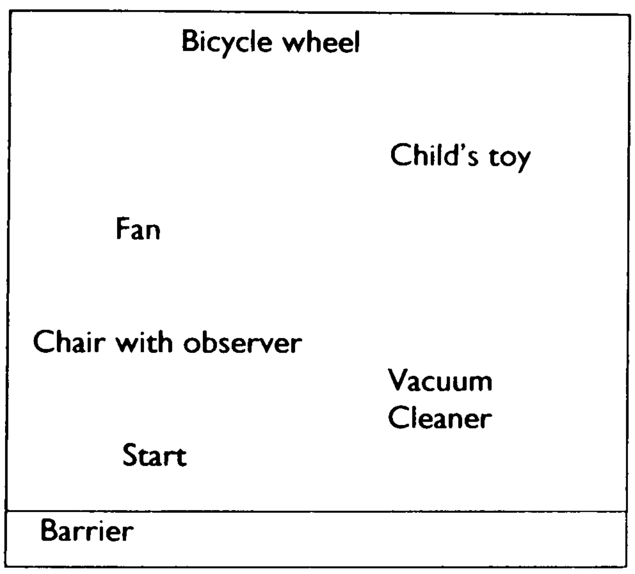
6.2.1 Materials and methods

The study was conducted with puppies that were housed in a kennel environment. Six litters of puppies (2 litters of Cavalier King Charles Spaniels, 1 litter of Jack Russell Terriers, 1 litter of Beagles and 2 litters of Jack Russell Terrier x Beagle cross: n = 28) were used. Half of each litter was assigned to treatment and half to control. From the age of 3 to 5 weeks littermate groups (n = 15) were placed in a room (5 m x 2.5 m) and exposed to a television screen showing video/audio images, depicting inanimate and animate stimuli that dogs usually encounter in domestic and busy urban environments, for 30 minutes each day over a two week period. The other halves of the litters (n = 13) were exposed to a blank television screen whilst the TV and video were switched on, to control for the effects of handling and exposure to the video equipment itself. They were otherwise maintained under the same conditions as the exposed puppies. The puppies were tested individually, between 51 and 61 days of age. The puppies had not been exposed to video images before this experiment, and were a different sample from that described in the previous Chapter.

The tests were conducted in an unfamiliar environment, being a room in the breeder's establishment (6 m x 6.5 m) which the puppies had not visited before. Noises from the kennel, including barking or whining of other dogs, could be perceived. At one side of the room a low barrier was placed to make the test arena smaller and to block the door that gave access to the rest of the kennel where the littermates were present. Two novel objects (a child's toy and a fan) were placed in the arena, and also two objects (wheel and vacuum cleaner) comparable but not identical to those in the video images (Figure 6.1). Only inanimate objects were used as test objects, since exposure to these stimuli could be controlled totally in the kennel environment, in contrast to exposure to social stimuli such as people.

To follow the puppies' development of fear responses when placed in the home environment questionnaires were distributed to each of the puppy owners after homing (See Appendix 3). Unfortunately insufficient questionnaires were returned to analyse.

Figure 6.1 Schematic depiction of the positions of the test objects in the unfamiliar room (6m x 6.5m).



6.2.2 General measures of behaviour

At the beginning of the session, the puppy was placed at the starting point marked on the floor of the area and filmed for two minutes. Behavioural responses that have previously been proposed to be associated with stress, such as ear position, tail position, tail movement, body position, type of locomotion and vocalization (Beerda *et al* 1997a) were time sampled from the video tape every ten seconds (Table 6.1). The display of activities associated with sympathetic arousal, so-called behavioural stress parameters (Beerda *et al* 1997a, 1997b) or displacement activities (Maestrippieri *et al* 1992) were scored as they occurred (Table 6.2). This non-invasive observational technique was chosen as it is less likely to influence the results when compared to the sampling techniques necessary for physiological methods, such as cortisol levels or heart rate (Beerda *et al* 1997b).

Table 6.1 Behaviour patterns ("Behaviours") time sampled every 10 seconds

Ear position *(Scored as presence/absence of four states).*	1. Maximally back: ears pulled back on the head or downwards 2. Partly back: ears are partly backward/downwards 3. Neutral position, not flat on the neck or back, normal ear position according to the breed 4. High: ears are pushed forward and/or turned towards another
Tail position *(Scored as presence/absence of four states).*	1. Maximally low: the tail is tucked between the legs 2. Half low: the tail is lower than neutral but not tucked between the legs 3. Neutral: the tail follows the line of the back of the dog and does not emerge above the back 4. High: the tail is held above the back
Tail movement *(Scored as presence/absence of three states).*	1. Fast: fast, repetitive, movement of total tail or tip of the tail 2. Normal: slow movement of the total tail 3. Motionless: no movement
Body posture *(Scored as presence/absence of three states).*	1. Normal: the dog walks normally with straight fore and hind legs 2. Crouched: the dog walks with flexed fore and/or hind legs with lowered head but still in line with the back 3. Maximally crouched: the dog walks with flexed fore and hind legs and head lowered below the line of the back
Locomotion *(Scored as presence/absence of four states).*	1. Lying 2. Standing 3. Running 4. Walking
Vocalisation *(Scored as presence/absence of three states).*	1. No vocalisation 2. Whimpering/whining: the dog makes a high pitched whimpering or whining vocalisation 3. Barking: one or repeated barks

Table 6.2 Behaviours scored as frequency of occurrence

Digging at the floor	Scratching at the floor in a way that is similar to when dogs are digging a hole
Jumping up	Jumping up at the wall with the front paws
Hiding	Hiding under the chair of the observer
Climbing	Attempts to climb over the barrier
Scratching	Scratching with front paws directed at an object or person
Licking	Licking an object or the floor with the tongue
Autogrooming	Maintenance behaviours directed to the dog's own body e.g. scratching, licking, biting
Body shaking	The dog shakes its head or whole body
Paw lifting	One fore paw is lifted slightly, without forward locomotion
Tongue out	The tip of the tongue is extended for a moment
Snout licking	The tongue is extended and moved along the upper lip(s)
Yawning	Dog slowly opens its mouth to yawn
Urinating	Dog passes urine
Defecating	Dog passes faeces

6.2.2.1 Exploratory analysis of behaviour patterns recorded

Since it was unlikely that all of the behaviour patterns recorded would be independent of one another, preliminary statistical analysis was carried out in order to reduce the number of variables to be tested for the effects of exposure to the video. Since only 26 dogs had been tested, the number of variables recorded was similar to the sample size, so the data was unsuitable for multivariate analysis. Therefore, histograms were plotted for each variable to visualise the number of puppies performing them, and at what frequencies. Correlation matrices were calculated for groups of variables that were likely to be dependent on one another because they were mutually exclusive, e.g. ear positions. The composite variables that were constructed (see Results) were approximately normally distributed and were analysed by partially nested ANOVA for the effects of exposure to the video, gender, and breed type (see Table 6.5 in the Results for the model used). Variables that could not be combined together were not normally distributed and were analysed for the effects of exposure to the video by Mann-Whitney U-Tests.

6.2.3 Behaviour directed at objects

Every approach within two puppy-lengths' proximity to an object, that appeared to be intended to increase the stimulus input (having a closer look), with or without making physical contact or manipulating the object, was scored as a visit to an object. Latency to approach the first object and time spent exploring objects was measured in seconds. Time exploring objects included observation of a stimulus when visiting it or actively making contact with the object (See Table 6.3).

6.2.3.1 Transformation of measures of contact with the objects

The following measures were examined for normality using histograms: latency to contact the first object (seconds), total time exploring objects (seconds), number of objects visited, number of visits to all the objects, and number of visits to each of the four objects. Latency was log10-transformed to improve normality, and time exploring and number of visits to all objects were square-root transformed, prior to analysis using the same ANOVA model as for the behaviour patterns. Number of objects visited, though on a five-point scale, was also analysed by ANOVA, for uniformity: this data was approximately normally distributed and its fit was not improved by transformation. Numbers of visits to the individual objects did not even approximate to normal and so were analysed by non-parametric tests.

Table 6.3 Behaviours scored as "time exploring object'. If different behaviours were displayed simultaneously they were scored as one sequence of exploratory behaviour.

Behaviour	Description
Observing	Approaching into close proximity (two puppies' length) of the object and observing it without making physical contact in a sitting, standing or lying position
Sniffing	The dog sniffs at the object
Chewing	The dog chews at the object
Licking	The dog licks the object
Manipulating	The dog makes physical contact with the object with its front paw(s)
Climbing	The dog climbs, sits against or lies on top of (a part) of the object

6.3 Results

6.3.1 Behaviour patterns

The behaviour of the puppies in the arena was examined for indicators of stress, comparing the video-exposed group with the control group.

6.3.1.1 Ear positions

Several of the four ear positions recorded were negatively correlated with one another (Table 6.4), as expected since they were mutually exclusive states. Ears high was only seen in Jack Russells or their crosses, and was therefore likely to be breed-specific. The frequencies of the other three positions were combined together, weighting Ears back maximum x 3, and Ears partially back x 2, and Ears neutral x 1, generating a scale from 1 (Ears always neutral) to 3 (Ears always maximally back). This Ear position score was significantly different between the puppies exposed to the video and the control group (Figure 6.2) but not between male and female puppies or between breed groups (Table 6.5). On average, puppies from the Control group held their ears in a position between partially and maximally back, whereas the exposed puppies held their ears between neutral and partly back (Table 6.6)

Figure 6.2 Boxplot representing score for ear position (1= neutral, 3= maximally back) of the exposed and control group. Heavy lines = medians, boxes indicate 25th and 75th percentiles, light horizontal lines = minimum and maximum. Single points represent individual scores more than two interquartile ranges from the median. See Table 6.6 for corresponding means and standard errors.

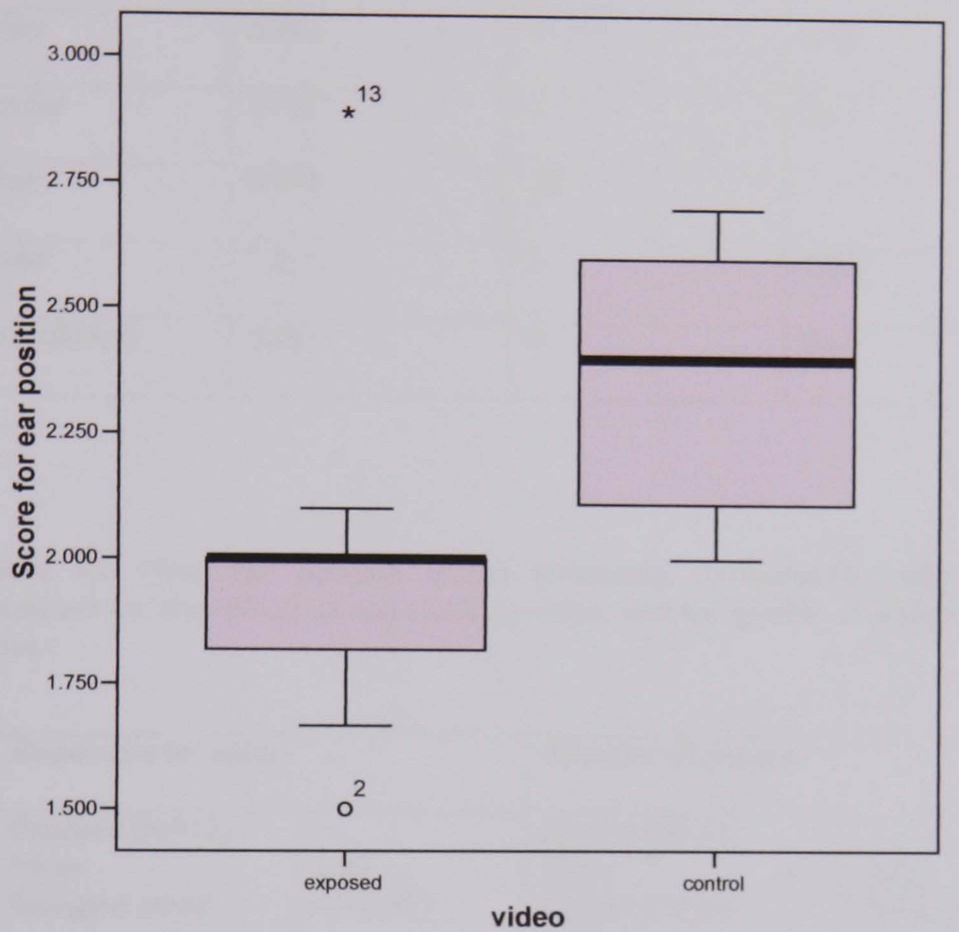


Table 6.4 Spearman rank correlation coefficients between ear positions. N=26, * P<0.1, ** P<0.01, *** P<0.001.

	Ears partially back	**Ears neutral**	**Ears high**
Ears back maximum	-0.763***	-0.351*	+0.076
Ears partially back		+0.009	+0.164
Ears neutral			-0.263

Table 6.5 ANOVA table for Ear position score. The residual Error Mean Square was used to calculate F-ratios for the effects of Video, Gender and Litter; for Breed, Litter was used as the error term, since each litter could belong to only one breed (i.e. a nested term).

Factor	Mean Square	d.f.	F	P
Video	0.902	1	9.56	0.006
Gender	0.050	1	0.53	0.47
Error	0.094	18		
Breed	0.058	3	1.06	0.58
Litter(Breed)	0.061	2	0.65	0.54

Table 6.6 Mean Ear position scores (1=neutral, 3=maximally back) +/- standard errors, calculated for the effects of exposure to video, and for gender of puppy. See Table 6.5 for F-ratios.

Exposure to video		**Gender of puppy**	
Exposed (N=13) Mean Standard error	 1.98 +/- 0.95	Female (N=11) Mean Standard error	 2.12 +/- 0.12
Control (N=13) Mean Standard error	 2.36 +/- 0.89	Male (N=15) Mean Standard error	 2.23 +/-0.08

6.3.1.2 Tail position

Tail positions, like ear positions, were mutually exclusive states and intercorrelated (Table 6.7), and so their frequencies were combined into a composite score, weighting maximally low by x4, half low x3, neutral x2 and up by x1. On average, puppies exposed to the video had slightly higher tail positions (lower scores, Table 6.9, Figure 6.3) but this was not statistically different using the same ANOVA model as for Ear position score, nor were there significant effects of gender, or breed group (Tables 6.8, 6.9).

Figure 6.3 Boxplot off effects of exposure to video on Tail position (4= maximally down, 1= up).

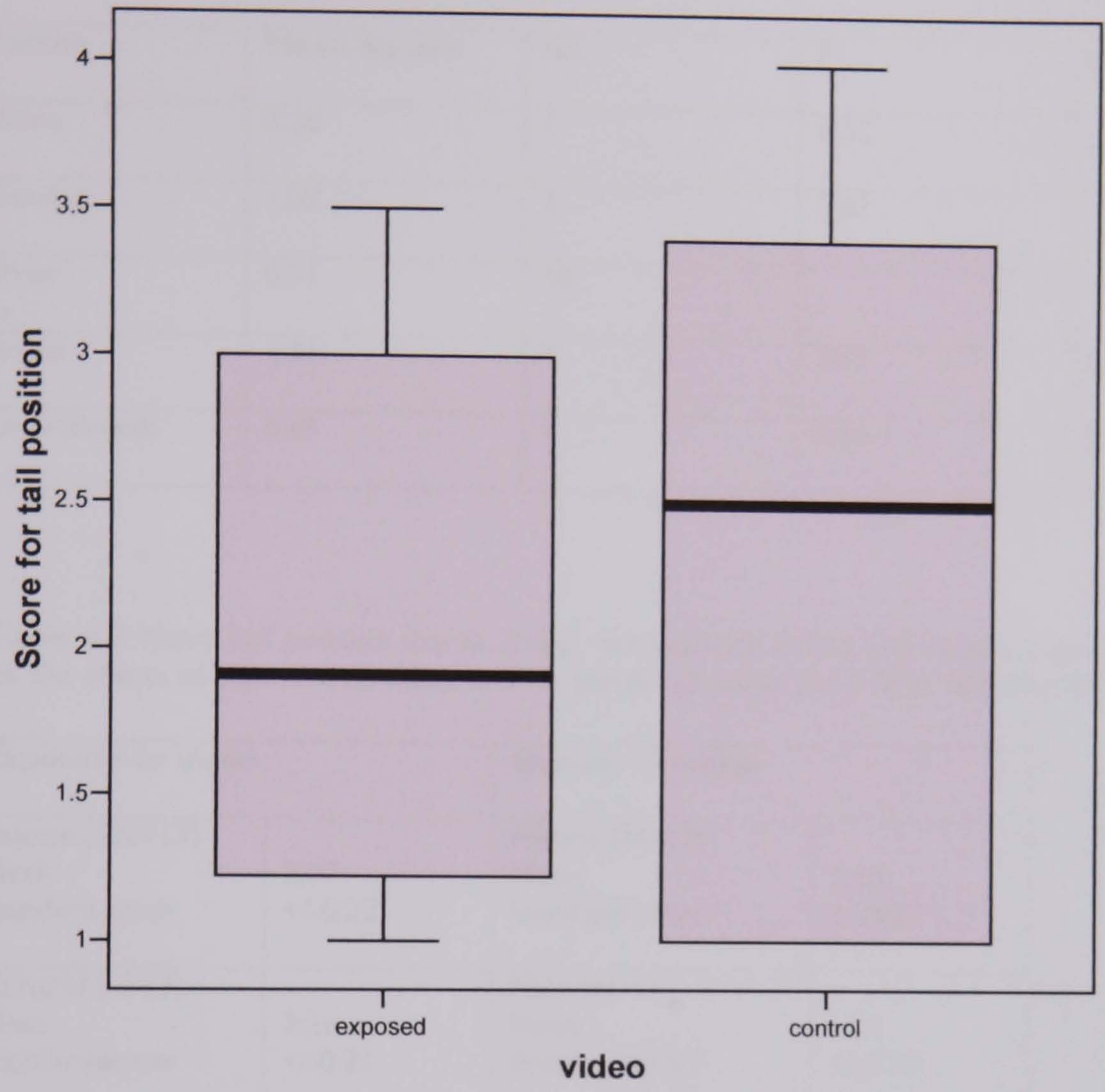


Table 6.7 Spearman rank correlation coefficients between tail positions. N=26, * P<0.5, ** P<0.01, *** P<0.001.

	Tail low maximum	Tail half low	Tail neutral	Tail high
Tail low maximum		0.302	-.159	-0.405*
Tail half low	0.302		0.339	-0.654**
Tail neutral	-0.159	0.339		-0.342
Tail high	-.405*	-0.654**	-0.342	

Table 6.8 ANOVA table for Tail position score. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	0.18	1	0.35	0.56
Gender	1.03	1	2.02	0.17
Error	0.51	18		
Breed	4.31	3	9.67	0.17
Litter(Breed)	0.45	2	0.89	0.43

Table 6.9 Mean Tail position scores (1=up, 4=maximally down) and standard error, calculated for the effects of exposure to video, and for gender of puppy (both NS). See text for F-ratios.

Exposure to video		Gender of puppy	
Exposed (N=13) Mean Standard error	 2.07 +/-0.22	Female (N=11) Mean Standard error	 2.40 +/-0.27
Control (n=13) Mean Standard error	 2.26 +/-0.21	Male (N=15) Mean Standard error	 1.91 +/-0.20

6.3.1.3 Tail movement

The three states for tail movement were mutually exclusive and hence negatively correlated with one another (Table 6.10), but there did not appear to be a logical way of combining them together, since each might indicate a different motivational state. Tail moves fast was approximately normally distributed and uncorrelated with Tail positions (Table 6.4) and so was selected as the outcome variable, converted to a proportion of observations. On average, puppies exposed to the video were significantly less likely to have their tails moving fast than the control group (Table 6.11, 6,12, Figure 6.4), but males and females, and breeds, were similar .

Figure 6.4 Boxplot representing effect of exposure to video images for behavioural measure: Tail moves fast (proportion).

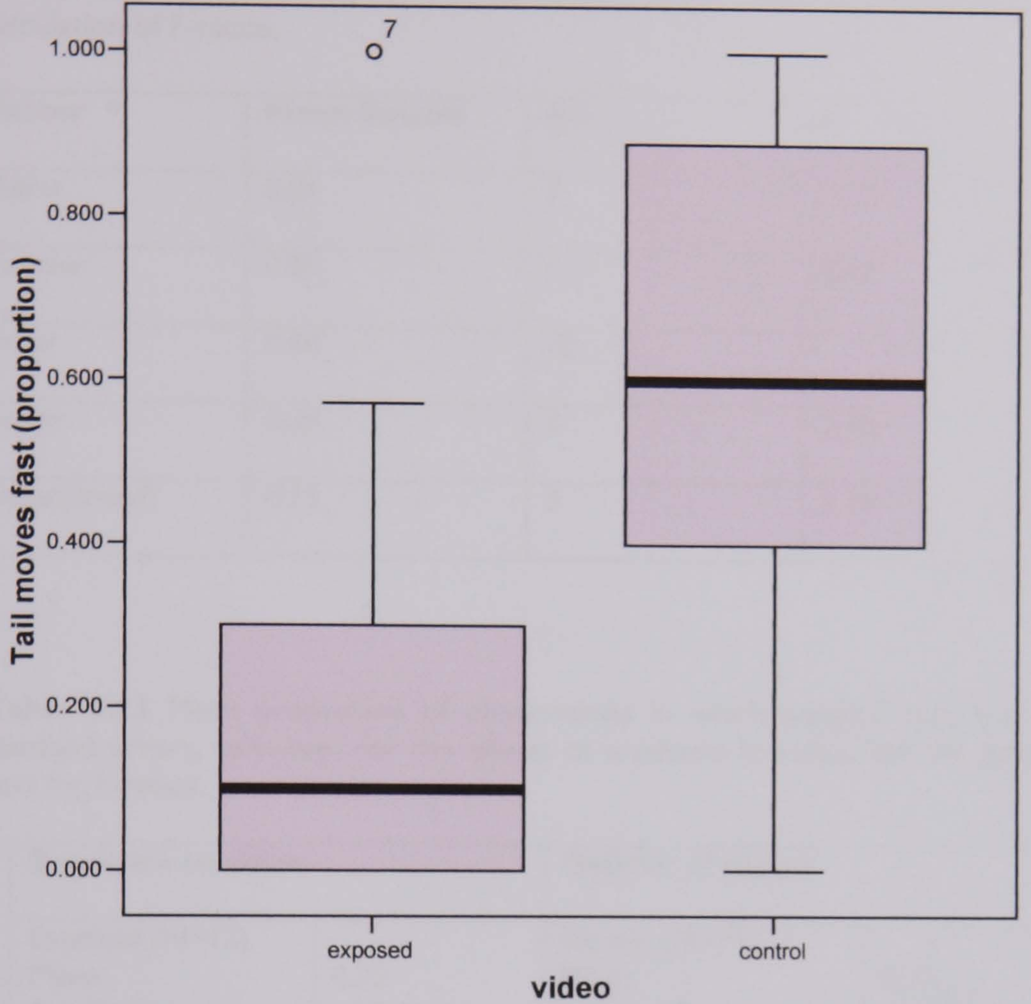


Table 6.10 Spearman rank correlation coefficients between Tail movements. N=26, * P<0.5, ** P<0.01, *** P<0.001.

	Tail moves fast	**Tail moves normal**	**Tail motionless**
Tail moves fast		-0.528**	-0.683**
Tail moves normal	-0.528**		-0.104
Tail motionless	-0.683**	-0.104	

Table 6.11 ANOVA table for Tail movement score. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	0.81	1	19.8	<0.001
Gender	0.01	1	0.24	0.63
Error	0.04	18		
Breed	0.34	3	2.34	0.33
Litter(Breed)	0.13	2	3.16	0.07

Table 6.12 Mean proportion of observations in which puppies' tails were moving fast and standard errors, calculated for the effects of exposure to video, and for gender of puppy. See text for F-ratios.

Exposure to video		Gender of puppy	
Exposed (N=13) Mean Standard error	 0.22 +/-0.06	Female (N=11) Mean Standard error	 0.37 +/-0.08
Control (N=13) Mean Standard error	 0.58 +/-0.06	Male (N=15) Mean Standard error	 0.43 +/-0.06

6.3.1.4 Body position

The three states for body position were mutually exclusive and inter-correlated (Table 6.13) and their frequencies were combined together into a scale using the weightings 1=normal, 2=crouch, 3=maximum crouch. Puppies exposed to the video were significantly less likely to have a maximally crouched body position (Tables 6.14, 6.15, Figure 6.5), but males and females, and breeds, were similar. In general the puppies from the exposed group displayed a body position varying between normal and crouched, and the control group between crouched and maximally crouched (Figure 6.5).

Table 6.13 Spearman rank correlation coefficients between Body positions. N=26, * P<0.5, ** P<0.01, *** P<0.001.

	Body crouch maximum	**Body crouch**	**Body normal**
Body crouch maximum		-.473*	-0.497**
Body crouch	-0.473*		0.026
Body normal	-0.497**	-0.026	

Figure 6.5 Boxplot of effects of exposure to video images on Score for body position (3=maximally crouch, 1= normal).

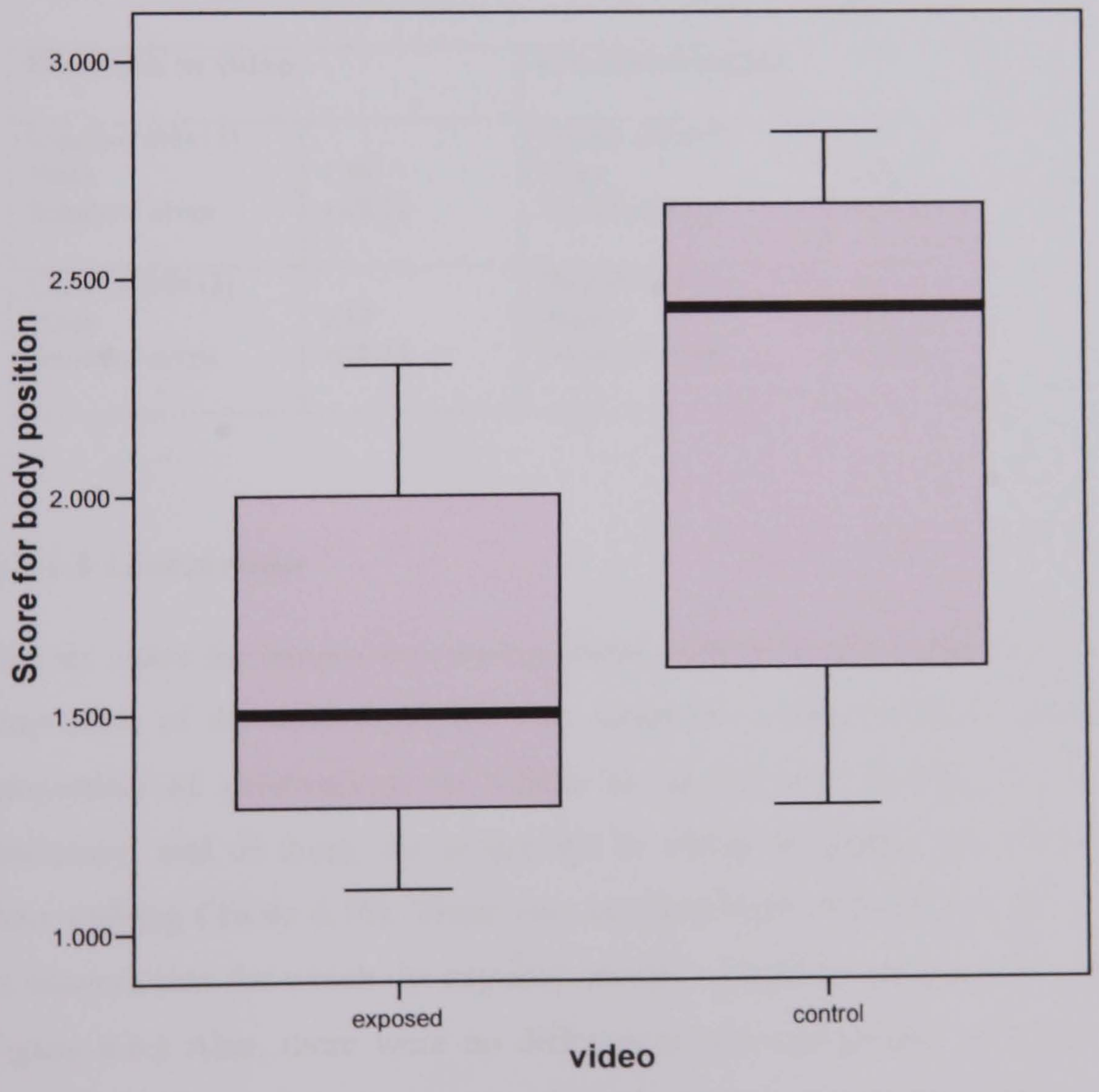


Table 6.14 ANOVA table for body position score. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	1.45	1	7.51	0.01
Gender	0.27	1	1.42	0.25
Error	0.19	18		
Breed	0.54	3	2.08	0.38
Litter(Breed)	0.25	2	1.30	0.30

Table 6.15 Mean proportion of observations of body position crouched and standard errors calculated for the effects of exposure to video, and for gender of puppy. See text for F-ratios.

Exposure to video		Gender of puppy	
Exposed (N=13) Mean Standard error	 1.70 +/-0.13	Female (N=11) Mean Standard error	 2.06 +/-0.17
Control (N=13) Mean Standard error	 2.17 +/-0.13	Male (N=15) Mean Standard error	 1.81 +/-0.12

6.3.1.5 Locomotion

The six states for locomotion were mutually exclusive and hence inter-correlated. Inspection of the data suggested two composite scores could be extracted, the proportion of observations in which the puppy was moving as opposed to stationary, and of these, the proportion in which the puppy was running rather than walking (Table 6.16). There was no significant difference in the proportion of observations for which the exposed and the control moved around, (Table 6.17, Figure 6.6.) Also, there were no differences between gender or breeds (Table 6.17, 6.18; Figure 6.6). As a proportion of all observations where the puppy was moving, the exposed puppies ran significantly less than the control group (Table 6.19, 6.20; Figure 6.7).

Figure 6.6 Boxplot illustrating effect of exposure to video images on the proportion of observations in which the puppies were moving around. For corresponding means and standard errors, see Table 6.18.

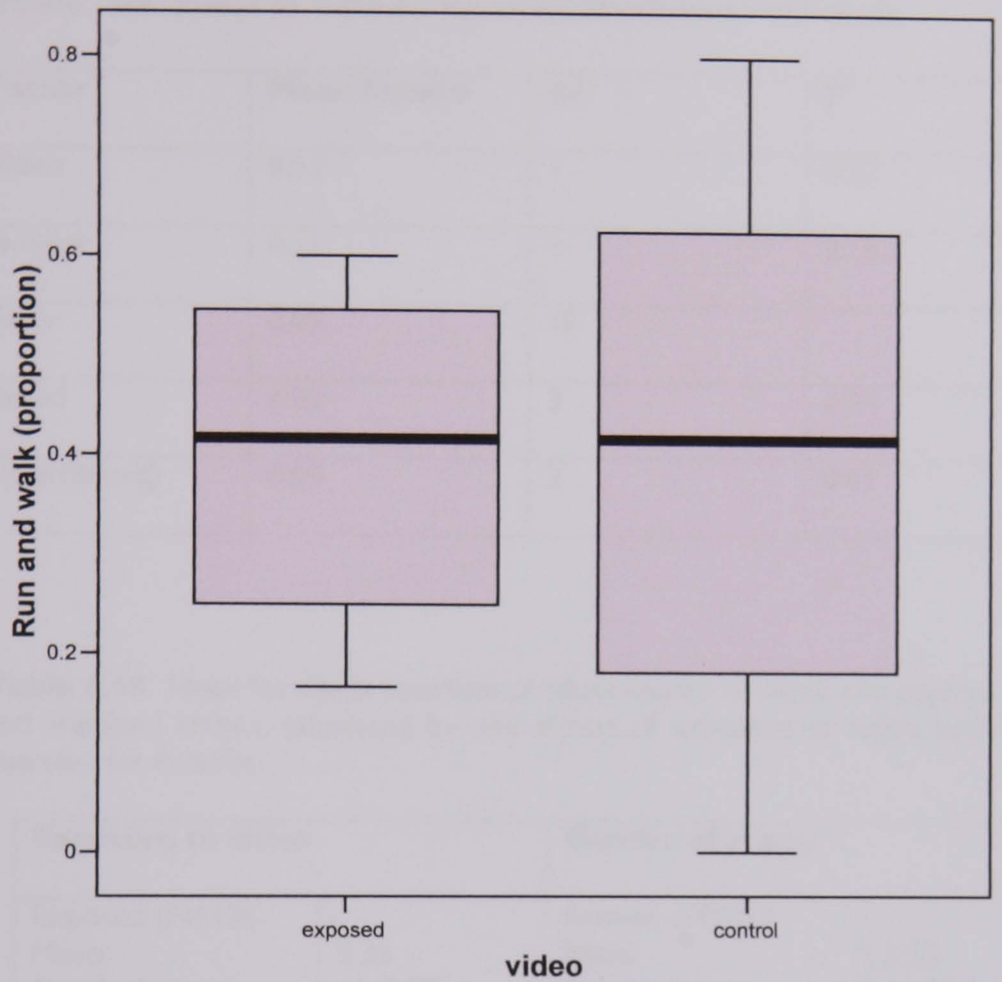


Table 6.16 Spearman rank correlation coefficients between Locomotion. N=26, * P<0.5, ** P<0.01, *** P<0.001.

	Locomotion sit	**Locomotion stand**	**Locomotion st/cl**	**Locomotion run**	**Locomotion walk**	**Locomotion lie**
Locomotion sit		-0.565**	-0.243	-0.360	-0.339	-0.482*
Locomotion stand	-0.565**		0.258	0.133	0.067	-0.559**
Locomotion st/cl	-0.243	0.258		-0.327	-0.167	-0.326
Locomotion run	-0.360	0.133	-0.327		-.161	-0.018
Locomotion walk	-0.339	0.067	-0.167	-0.161		-0.229
Locomotion lie	0.482*	-0.559**	-0.326	-0.018	-0.299	

Table 6.17 ANOVA table for the proportion of observations in which the puppies were moving around. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	0.13	1	0.27	0.61
Gender	0.11	1	2.14	0.16
Error	0.05	18		
Breed	0.02	3	0.74	0.66
Litter(Breed)	0.03	2	0.61	0.56

Table 6.18 Mean for the proportion of observations in which the puppies were moving around and standard errors, calculated for the effects of exposure to video, and for gender of puppy. See text for F-ratios.

Exposure to video		**Gender of puppy**	
Exposed (N=13) Mean Standard error	 0.35 +/- 0.07	Female (N=11) Mean Standard error	 0.30 +/-0.09
Control (N=13) Mean Standard error	 0.40 +/-0.07	Male (N=13) Mean Standard error	 0.46 +/-0.06

Figure 6.7 Boxplot illustrating the effect of exposure to video images on the proportion of the locomotion run [run/(run + walk)].

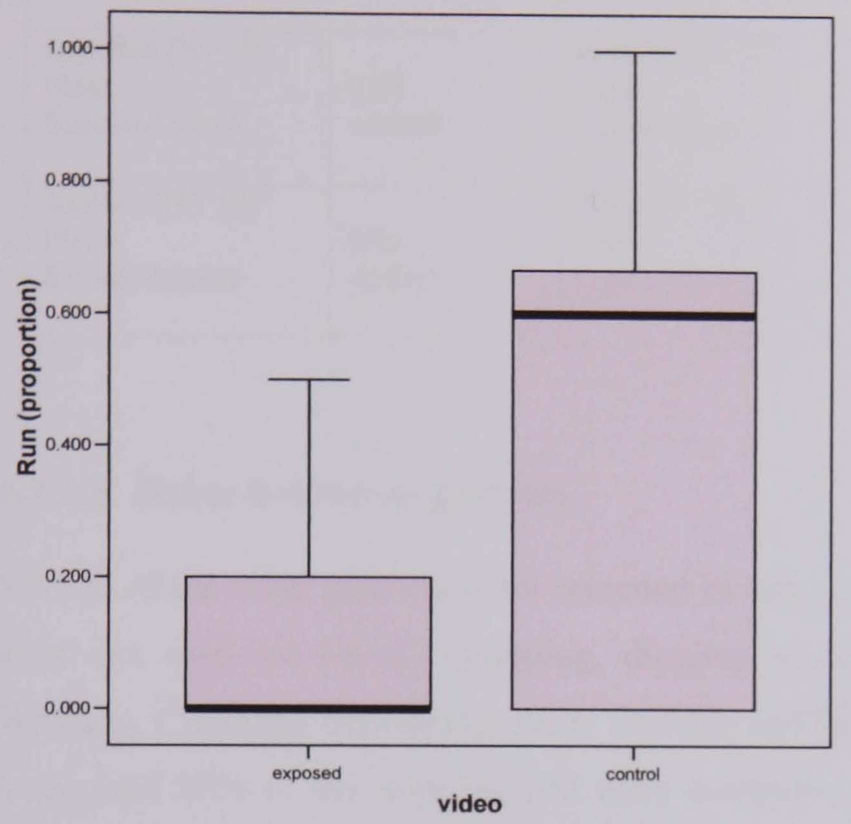


Table 6.19 ANOVA table for the proportion of locomotion that was Run. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	**Mean Square**	**d.f.**	**F**	**P**
Video	0.74	1	8.33	0.01
Gender	0.17	1	1.90	0.19
Error	0.09	18		
Breed	0.01	3	0.21	0.88
Litter(Breed)	0.07	2	0.73	0.49

Table 6.20 Mean for the locomotion Run and standard errors, calculated for the effects of exposure to video, and for gender of puppy. See text for F-ratios.

Exposure to video		Gender of puppy	
Exposed (N=13) Mean Standard error	 0.08 +/-0.09	Female (N=11) Mean Standard error	 0.15 +/-0.15
Control N= 13) Mean Standard error	 0.42 +/-0.42	Male (N=15) Mean Standard error	 0.35 +/-0.35

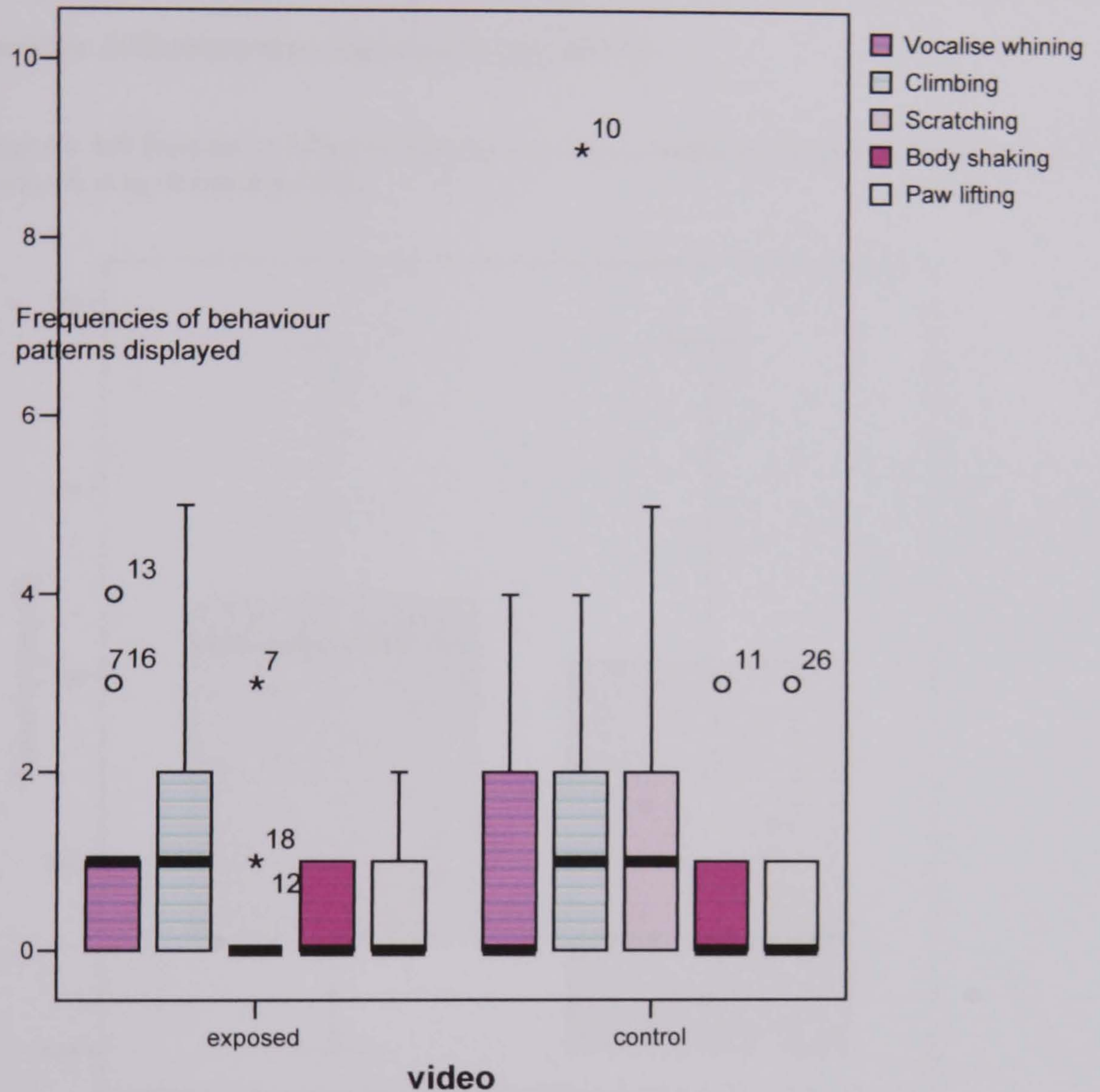
6.3.1.6 Other behaviour patterns

Several of the other patterns were recorded in only one puppy or in none, and so were not analysed further (jumping, digging, yawning, urinating, defecating). Whining, Climbing, Scratching, Body Shaking and Paw lifting were performed by more than 25% of the puppies, and were compared between the video-exposed and control groups by Mann-Whitney U-tests (Table 6.21). Scratching was performed more often by the control group (median=1, compared to median=0 for video-exposed), but this difference was not significant (P=0.07). The other measures, Whining, Climbing, Body Shaking and Paw lifting, appeared to be unaffected by treatment (Figure 6.8).

Table 6.21 Mann-Whitney U-values and Asymp. Sig (2-tailed) values for the behaviour patterns: Vocalise whining, Climbing, Scratching, Body shaking and Paw lifting, calculated for the effects of exposure to video.

Behaviour patterns	Mean exposed group	Mean control group	U	P
Vocalise whining	14.58	12.42	70.5	0.42
Climbing	13.69	13.31	82.0	0.89
Scratching	11.15	15.85	54.0	0.07
Body shaking	13.31	13.69	82.0	0.88
Paw lifting	13.15	13.85	80.0	0.84

Figure 6.8 Boxplot representing effect of exposure to video images on frequencies of single behaviour patterns displayed. Single points represent individual scores more than two interquartile ranges from the median.



6.3.2 Exploring objects

The visits made to the four objects were examined for the effects of prior exposure to the video, and gender.

6.3.2.1 Latency to contact the first object

The first object contacted was almost invariably the vacuum cleaner (12/13 in both video-exposed and control groups); the other two puppies contacted the fan first. This may have simply reflected the positions of the objects in relation to the start point and the observer (Fig. 6.1), although the vacuum cleaner did appear, for some unexplained reason, to be particularly attractive. Although the control group, and males, were quicker to contact their first object than the exposed group (Fig. 6.9, Table 6.22a and 6.22b) or females (Fig. 6.10, Table 6.22a and 6.22b), neither difference was statistically significant.

Figure 6.9 Boxplot of effect of exposure to video images on latency to approach first object in seconds (Log10 transformed).

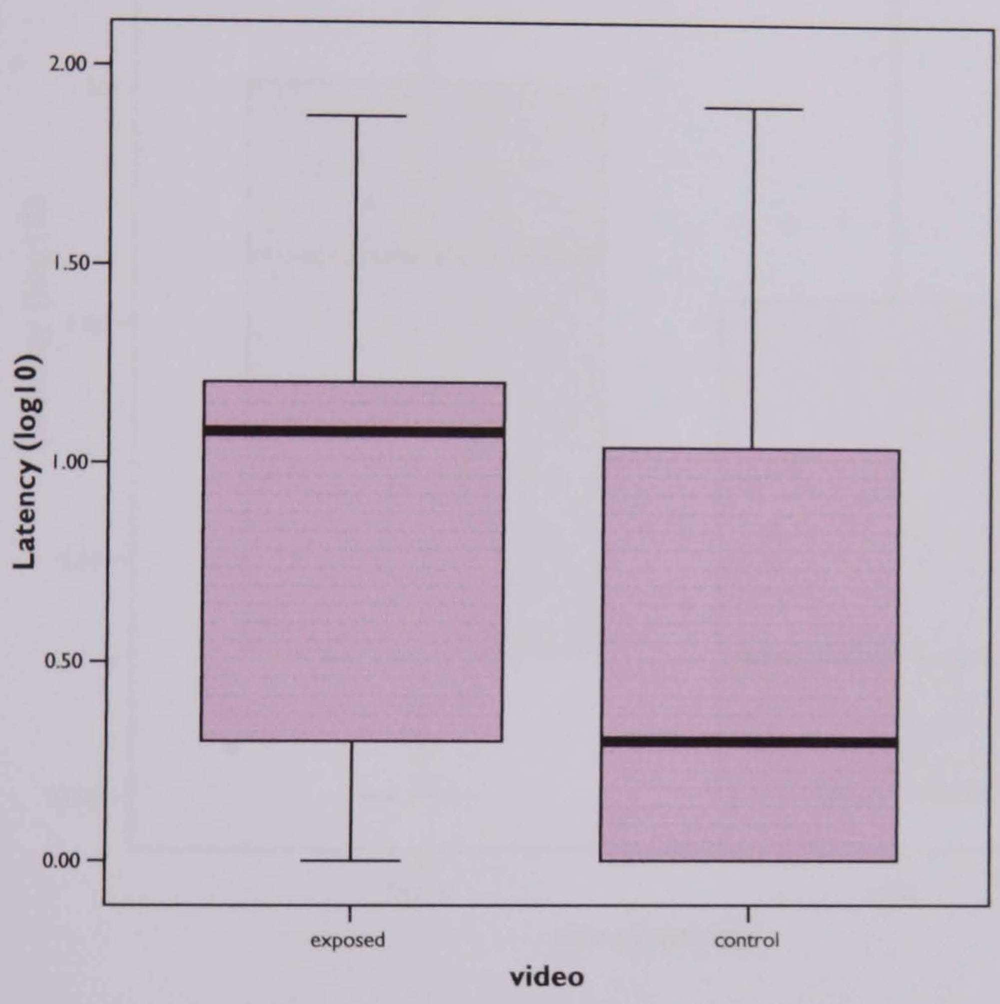


Table 6.22a ANOVA table for Latency to approach first object. The residual Error Mean Square was used to calculate F-ratios for the effects of Video, Gender and Litter; for Breed, Litter was used as the error term, since each litter could belong to only one breed (i.e. a nested term)

Factor	Mean Square	d.f.	F	P
Video	1.02	1	2.35	0.14
Gender	0.72	1	1.65	0.22
Error	0.43	18		
Breed	-	3	-	-
Litter(Breed)	0.06	2	0.14	0.87

Figure 6.10 Boxplot of effect of exposure to video images on latency to approach object in seconds split by males and females. (Log10 transformed)

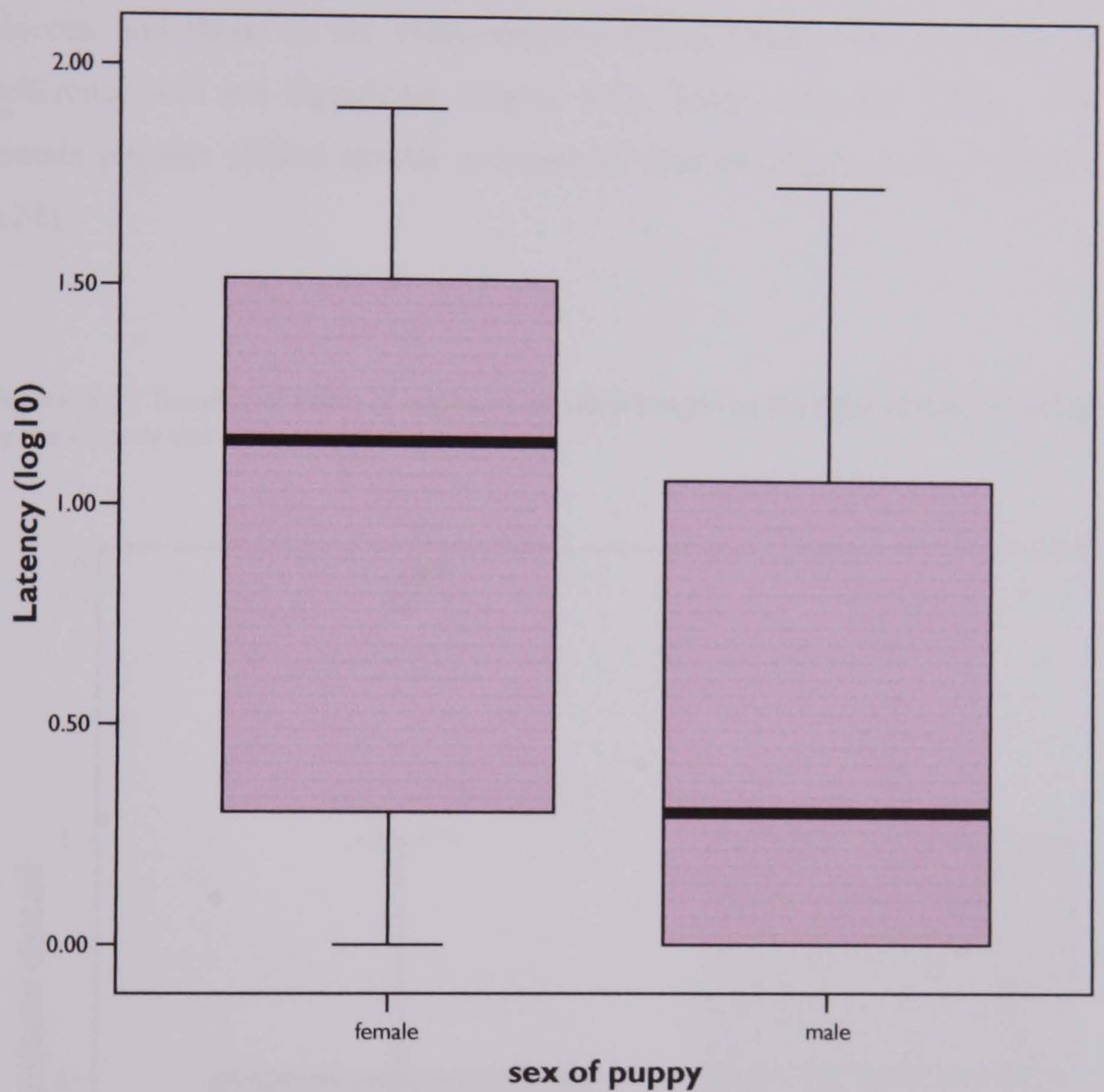


Table 6.22b Mean latencies and standard errors to contact the first object (seconds), back transformed from log 10-transformed data. Since back-transformation generates asymmetric standard errors, positive and negative values are shown separately.

Exposure to video		**Gender of puppy**	
Exposed (N=13) Mean Standard error (+) Standard error (-)	 7.76 4.51 2.83	Female (N=11) Mean Standard error (+) Standard error (-)	 7.89 6.07 3.44
Control (N=13) Mean Standard error (+) Standard error (-)	 3.09 1.71 1.1	Male (N=15) Mean Standard error (+) Standard error (-)	 3.05 1.55 1.03

6.3.2.2 Number of objects visited

On average, the puppies in the control group visited between two and three of the objects, and those in the video-exposed group visited less than two, but this difference was not significant (Figure 6.11, Table 6.23 and 6.24). Male and female puppies visited similar numbers of objects (Figure 6.12, Table 6.23 and 6.24).

Figure 6.11 Boxplot of effect of exposure to video images on the total number of objects visited by the control and video-exposed group.

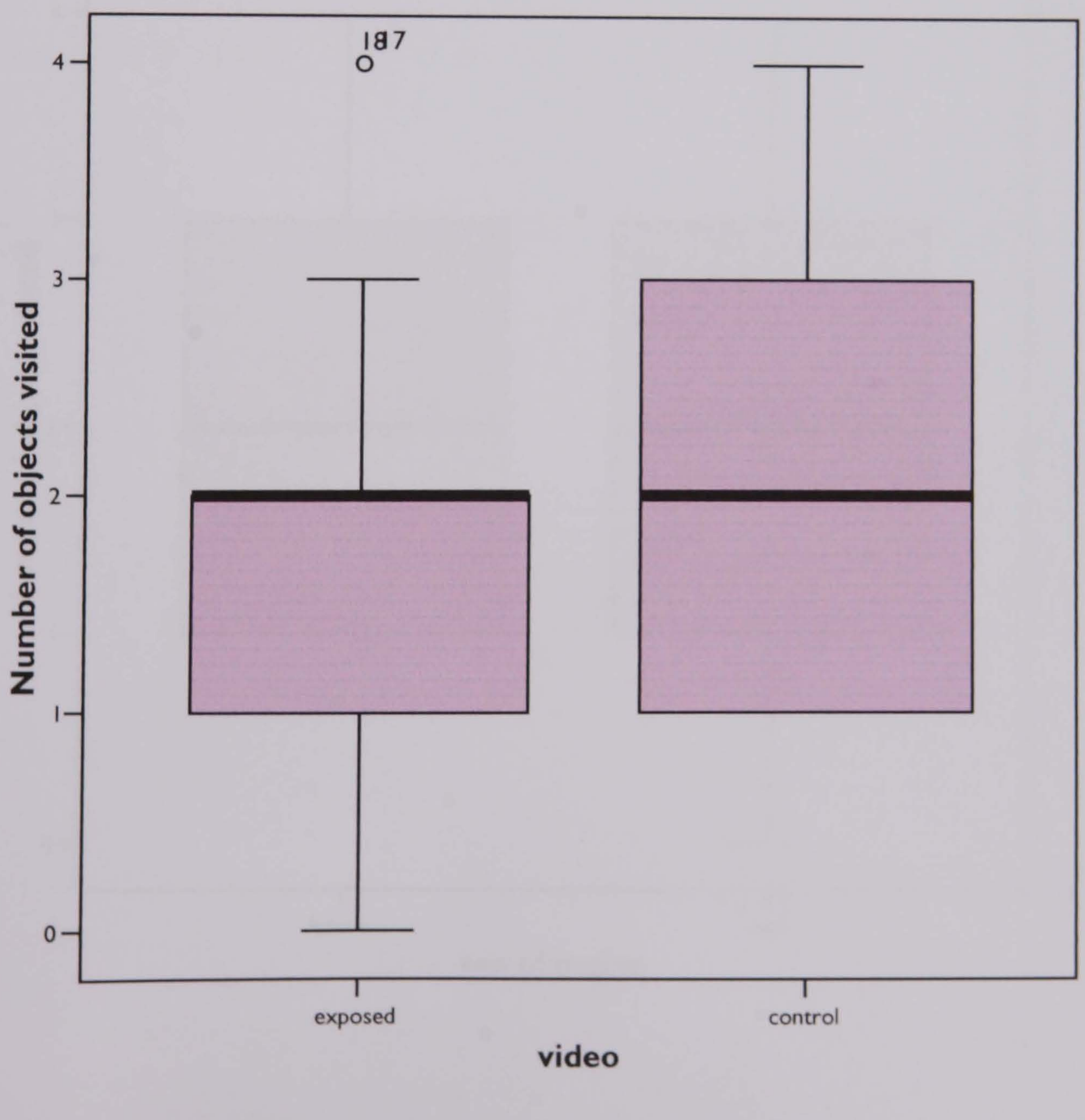


Table 6.23 ANOVA table for amount of objects visited. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	1.42	1	0.96	0.34
Gender	0.05	1	0.34	0.86
Error	1.47	18		
Breed	2.38	3	6.30	0.39
Litter(Breed)	1.10	2	0.37	0.69

Figure 6.12 Boxplot of the number of objects visited per group by males and females.

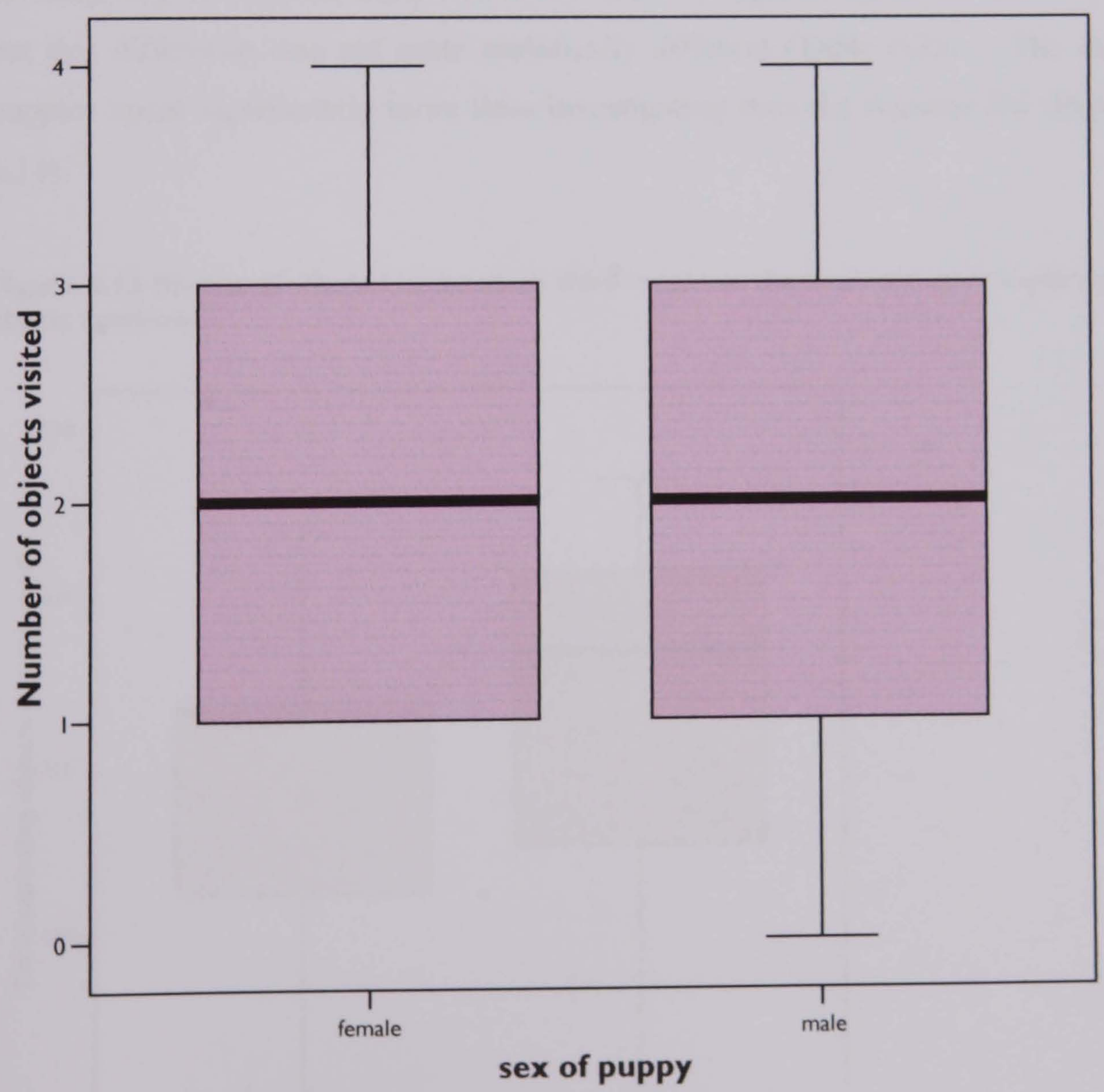


Table 6.24 Means and standard errors for number of objects visited and standard errors, calculated for the effects of exposure to video and gender.

Exposure to video		**Gender of puppy**	
Exposed (N=13) Mean Standard error	 1.94 +/-0.37	Female (N=11) Mean Standard error	 2.23 +/-0.46
Control (N=13) Mean Standard error	 2.42 +/-0.35	Male (N=15) Mean Standard error	 2.13 +/-0.33

6.3.2.3 Time spent investigating objects

The puppies exposed to the video spent about half the amount of time investigating the objects, compared to the control puppies (Fig. 6.13, Table 6.26), but this difference was not quite statistically different (Table 6.25). The male puppies spent significantly more time investigating than the females did (Figure 6.14).

Figure 6.13 Boxplot of effect of exposure to video images on the total time spent exploring all objects combined.

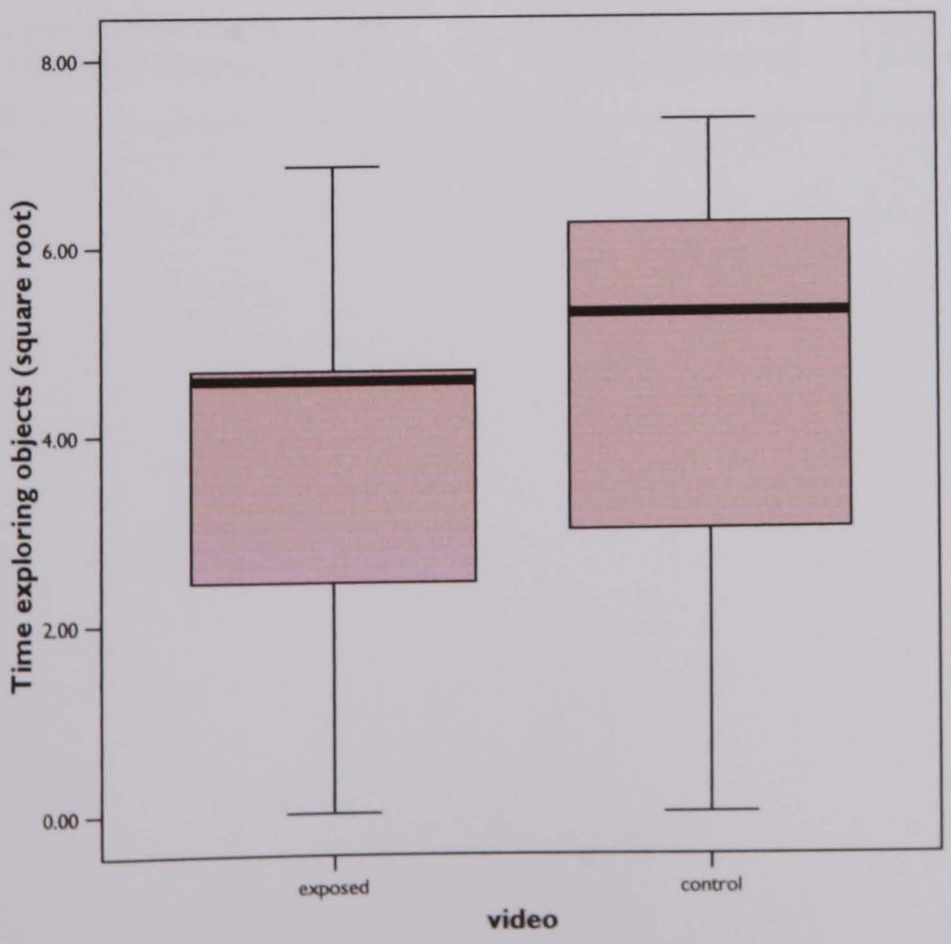


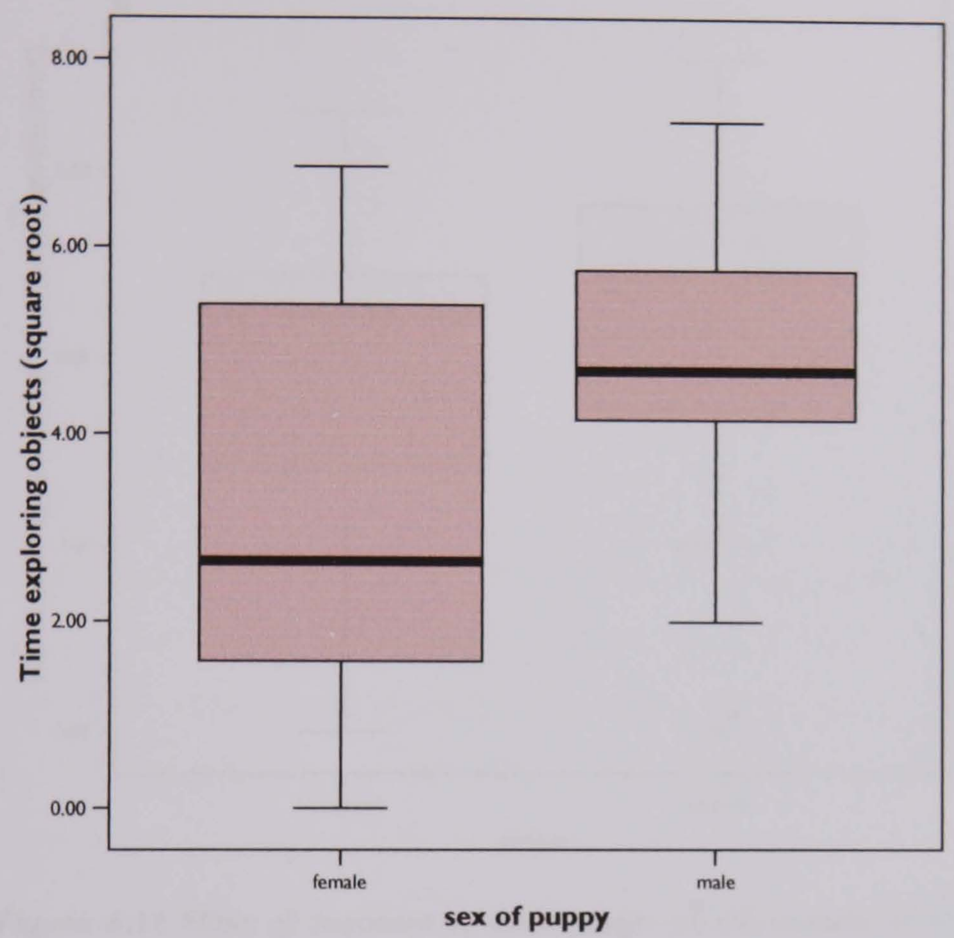
Table 6.25 ANOVA table for amount of time exploring all objects combined together. See caption to Table 6.5 for details of calculation of F-ratios.

Factor	Mean Square	d.f.	F	P
Video	9.58	1	2.61	0.12
Gender	17.95	1	4.88	0.04
Error	3.68	18		
Breed	0.77	3	0.11	0.95
Litter(Breed)	6.58	2	1.79	0.20

Table 6.26 Mean durations in contact with the objects (seconds) and standard errors, back-transformed from square-root transformed data. Since back-transformation generates asymmetric standard errors, positive and negative values are shown separately.

Exposure to video		Gender of puppy	
Exposed (N=13) Mean Standard error (+) Standard error (-)	 11.21 4.2 3.54	Female (N=11) Mean Standard error (+) Standard error (-)	 8.58 4.75 3.71
Control (N=13) Mean Standard error (+) Standard error (-)	 20.96 5.43 4.8	Male (N=15) Mean Standard error (+) Standard error (-)	 24.98 5.49 4.95

Figure 6.14 Boxplot of effect of exposure to video images on time exploring objects per group males and females.



6.3.2.4 Number of visits to objects

The total number of visits to all objects was marginally, but not significantly, higher in the control group of puppies (Figure. 6.15, Table 6.27). Males made slightly more visits to objects than females did (Figure 6.16), but by comparison with the durations, it appears that the main difference between males and females was that the males spent longer investigating once they had contacted an object.

Figure 6.15 Boxplot of total number of visits to all objects (Square-root transformed) by the control and exposed groups.

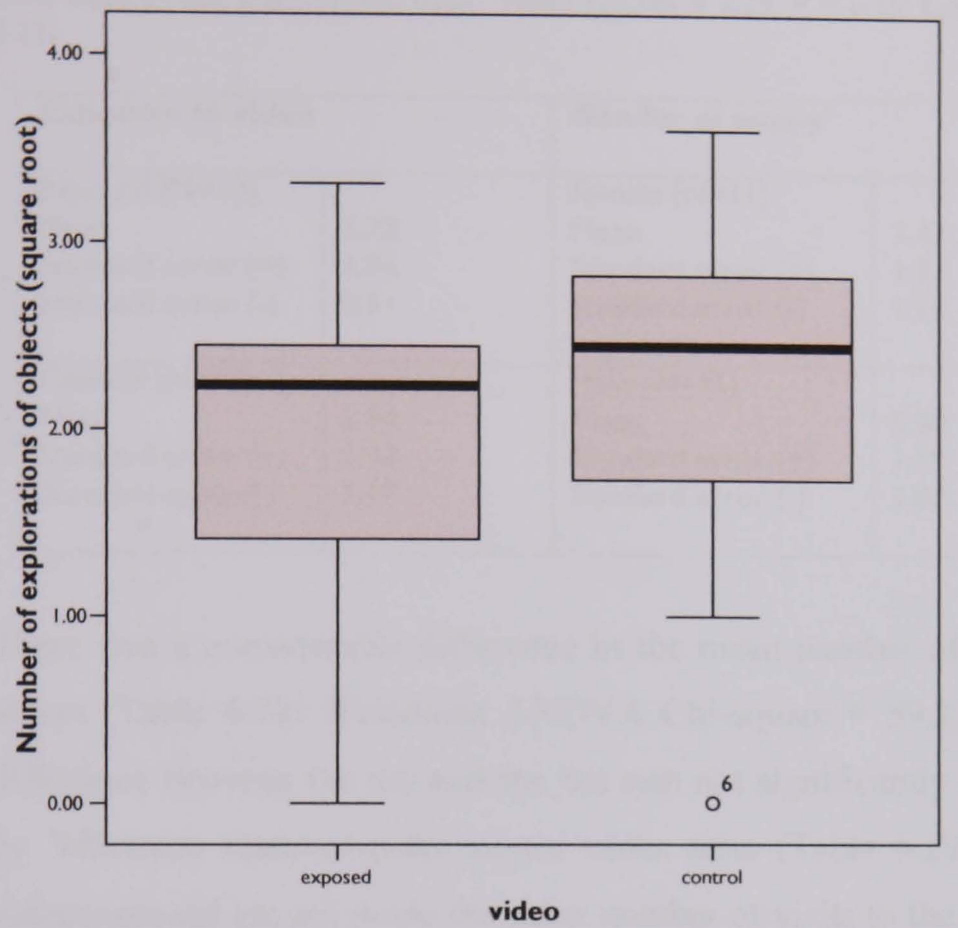


Figure 6.16 Effect of exposure to video images on the number of explorations of objects per group by females and males.

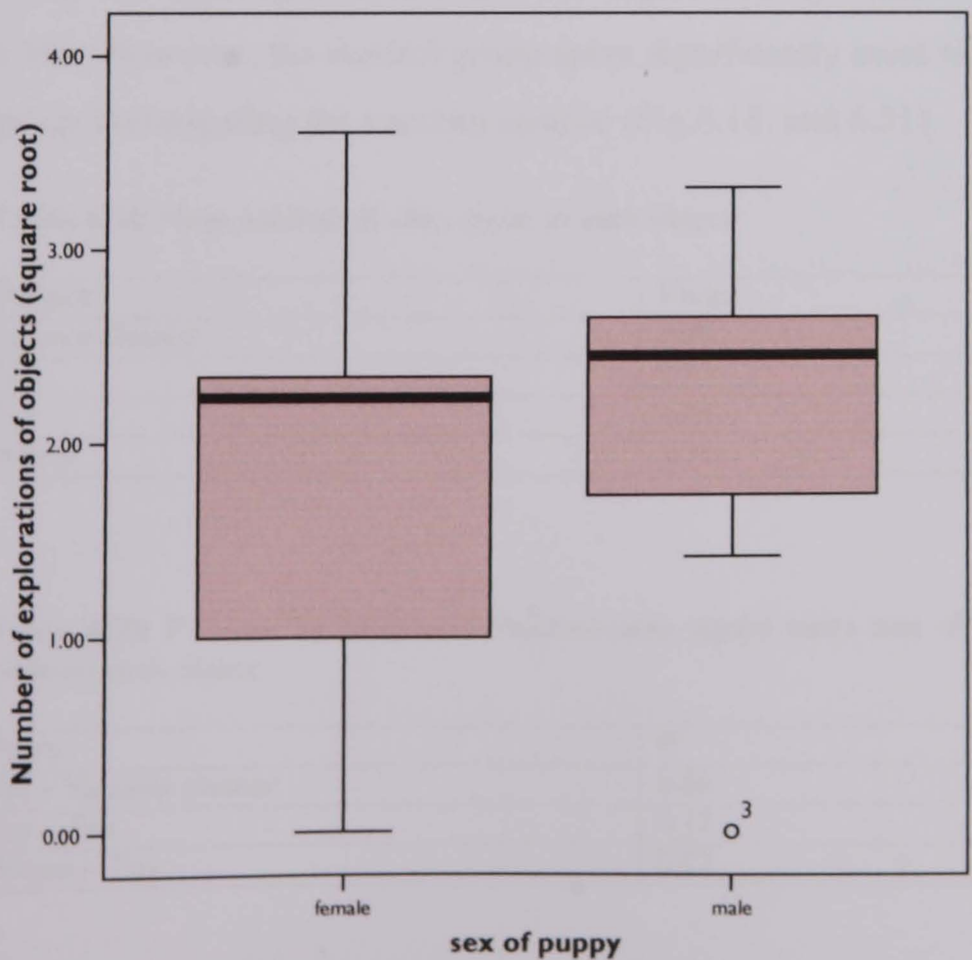


Table 6.27 Mean numbers of contacts with the objects and standard errors, back-transformed from square-root transformed data. Video F(1,18) = 2.29, P = 0.15: Gender F(1,18) = 1.00, P = 0.33)

Exposure to video		**Gender of puppy**	
Exposed (N=13) Mean Standard error (+) Standard error (-)	 3.28 1.06 0.91	Female (N=11) Mean Standard error (+) Standard error (-)	 3.47 1.37 1.15
Control (N=13) Mean Standard error (+) Standard error (-)	 5.54 1.30 1.17	Male (N=15) Mean Standard error (+) Standard error (-)	 5.30 1.19 1.07

There was a considerable difference in the mean number of visits made to each object (Table 6.28*)* (Friedman ANOVA Chi-square = 59.2, P<0.001); only the difference between the toy and the fan was not significantly different at P = 0.05 by Wilcoxon matched-pairs signed ranks tests (Table 6.29). The control and video-exposed groups made the same number of visits to the wheel. The puppies from the control group made slightly more visits to the other three objects (Figure 6.17), but these differences were not significant by Mann-Whitney tests (Table 6.30). However, the control group spent significantly more time than the exposed group investigating the vacuum cleaner *(*Fig.6.18, and 6.31).

Table 6.28 Mean number of visits made to each object

Object	**Mean**
Vacuum cleaner	3.90
Fan	2.33
Toy	2.06
Wheel	1.71

Table 6.29 P values by Wilcoxon Matched-pairs signed ranks test of mean number of visits made to each object

Pairs	**P**
Fan – Vacuum cleaner	0.00
Toy – Fan	0.12
Wheel – Toy	0.02

Figure 6.17 Total numbers of visits means and standard deviations to different type of objects, by the exposed and control group. Vacuum cleaner: N=26, mean=3.46, Standard deviation= +/-1.84. Fan: N=26, mean= 0.92, Standard deviation=+/-1,32. Toy: N=26, mean= 0.54, Standard deviation=+/- 0.91. Wheel: N=26, mean= 0.23, Standard deviation=+/-0.43.

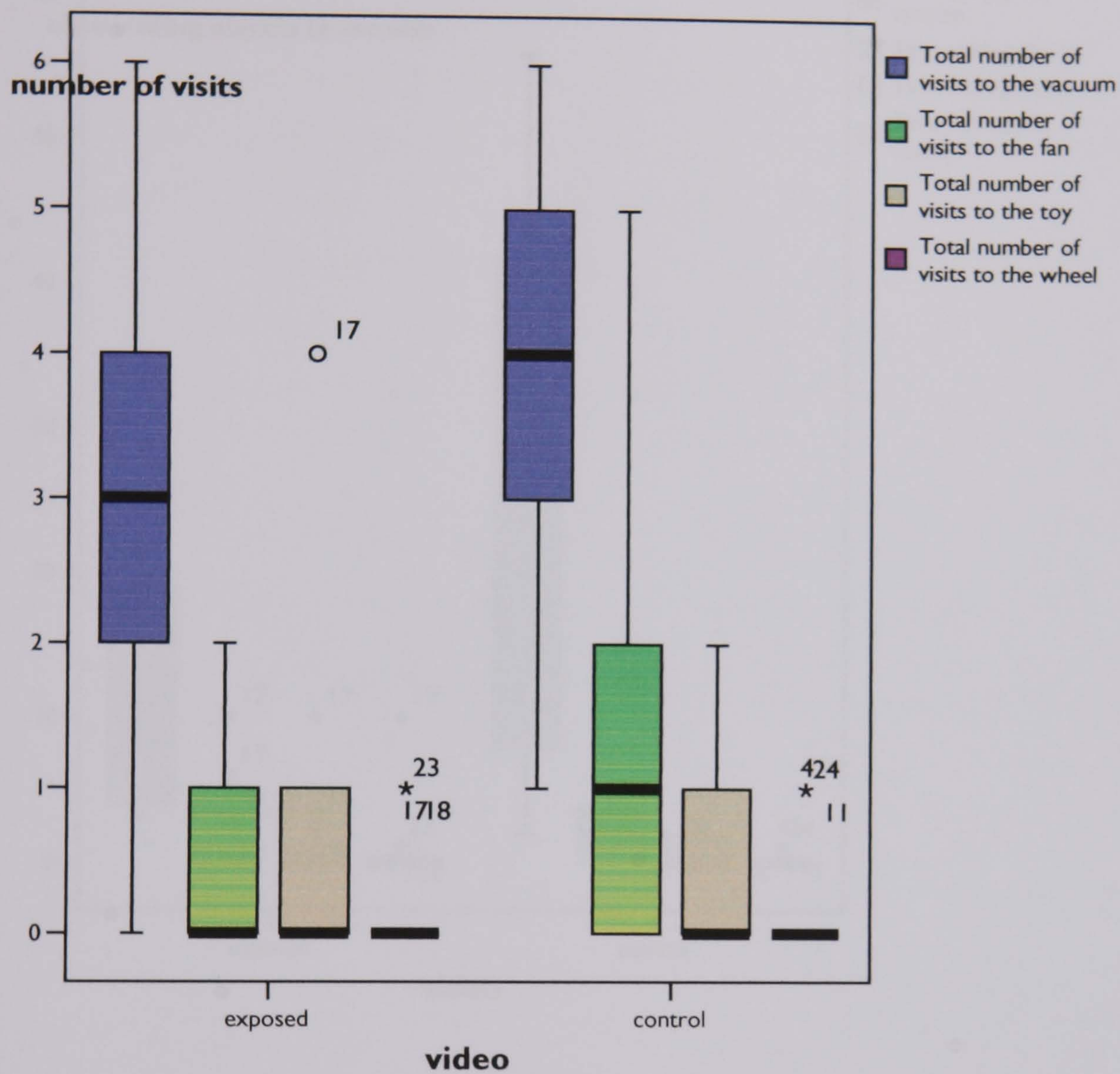


Table 6.30 Mean ranks for the objects visited split per object, calculated for the effects of exposure to video.

Object	Mean exposed group	Mean control group	Mann Whitney U	P
Vacuum cleaner	11.62	15.38	60	0.20
Fan	11.92	15.08	64	0.25
Toy	12.62	14.38	73	0.49
Wheel	13.50	13.50	84.6	1.00

Figure 6.18 Boxplot of time visiting each of the objects, split by the control and exposed group.

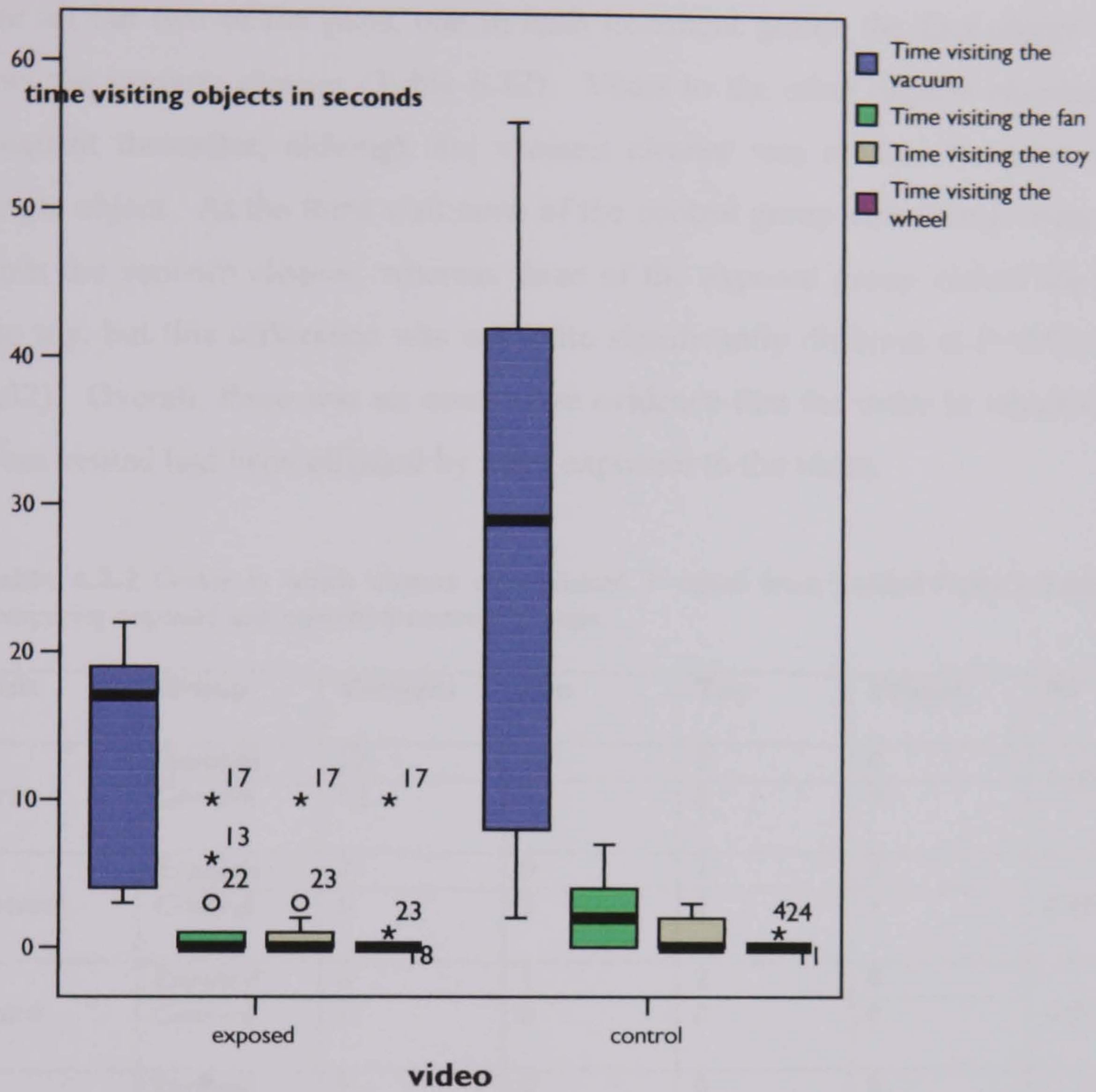


Table 6.31 Mean ranks for the time visiting the objects split per object, calculated for the effects of exposure to video.

Object	Mean exposed group	Mean control group	Mann Whitney U	P
Vacuum cleaner	10.42	16.58	44.5	0.04
Fan	12.15	14.85	67.00	0.33
Toy	12.73	14.27	74.50	0.56
Wheel	13.62	13.38	83.00	0.92

6.3.2.5 Order in which objects were visited

For all but two of the pups, one in each treatment group, the first object visited was the vacuum cleaner (Table 6.32). Visits to the other objects became more frequent thereafter, although the vacuum cleaner was always the most visited single object. At the third visit none of the control group visited any object apart from the vacuum cleaner, whereas three of the exposed group visited the fan or the toy, but this difference was not quite significantly different at P=0.05 (Table 6.32). Overall, there was no conclusive evidence that the order in which objects were visited had been affected by prior exposure to the video.

Table 6.3.2 Order in which objects were visited. P-values from 2-sided Fisher's Exact Tests comparing exposed and control treatment groups.

Visit	Group	Vacuum	Fan	Toy	Wheel	P=
First	Exposed	12	1	0	0	1.000
	Control	12	1	0	0	
Second	Exposed	7	0	1	2	0.437
	Control	5	3	2	1	
Third	Exposed	6	1	2	0	0.074
	Control	11	0	0	0	
Fourth	Exposed	5	2	0	0	0.603
	Control	4	2	2	1	
Fifth	Exposed	5	1	1	0	0.648
	Control	5	3	0	1	

6.3.4 Gender differences

To summarize the gender differences, between males and females, there was no statistically significant difference in the amount of objects visited ($F(1,18)= 034$; $P\ 0.86$) (Figure 6.16, Table 6.33), or the latency to approach the first object (Table 6.33, Figure 6.10,); although the males approached the first object sooner compared to the females, this was not statistically different ($F(1,18)=1.65$, $P=0.22$) However, they spent statistically significantly more time exploring the objects ($F(1,18)\ 4.88$; $P\ 0.04$) (Table 6.33, Figure 6.14).

Table 6.33 Means for Number of objects visited, Number of explorations of objects, Latency to approach first object and total time exploring objects and standard deviations for males and females.

Measure	Females (N=11)	Males N=15)
Mean number of objects visited Standard deviation	2.23 +/-0.46	2.13 +/-0.33
Mean number of explorations of objects Standard deviation	3.47	5.30
Mean latency to approach first object (s) Standard deviation	7.89	3.05
Meantime exploring objects (s) Standard deviation	8.58	24.98

6.4 Discussion

6.4.1 Behaviour patterns

These results suggest that a dog's capacity to remain in emotional homeostasis at 7 to 9 weeks of age can be increased by exposure to video images during the period of parasympathetic dominance between 3 and 5 weeks of age. The control puppies scored higher than the exposed puppies for several postures (ear position, body position crouched) and locomotion (tail movement fast, run) associated with stress (a *et al* 1997, 1997b, 1998). The results might also suggest that exposure to video images results in the formation of maintenance stimuli that are associated with a positive emotional state, and in the formation of a broader maintenance set, reducing the chance that stimuli that are encountered will be unfamiliar, thereby increasing the dog's capacity to maintain emotional homeostasis when in an unfamiliar environment or when confronted with an unfamiliar stimulus, and influencing the need to display exploration behaviour. The control group visited more objects and more different type of objects, although as expected from the results of experiment one, this was not significantly different. In addition, they inspected the vacuum cleaner for significantly longer and showed a shorter latency to approach the first object. The combination of these results seems to suggest that the exposed puppies had a lesser need to assess the environment for risks because of their higher level of familiarity with the stimuli in the environment, resulting from the exposure to the video images. Unfortunately of the four objects, the exposed group also visited the vacuum cleaner most often, which suggests that this object was intrinsically more attractive. This difference might have obscured any difference between exposed and control group in their responses to the objects included or not included on the videotape.

Crouching appears in anxiety or fear provoking situations (Overall 1997, Beerda *et al* 1997a, King *et al* 2003) and has been suggested to reflect a tendency to escape (Schilder & Van de Borg 2004). The level of crouching increases with the level of anxiousness. Very low postures may indicate high levels of acute stress, and a moderate lowering of the postures moderate levels of experienced stress (Beerda *et al* 1997a). Pulling back of the ears is linked with fear (Beerda *et al* 1997a). A repetitive wagging of the tail is associated with higher levels of excitement (Beerda *et al* 1997a) and agonistic behaviour (Scott & Fuller 1966). The body positions of the control group were more often associated with fear.

No significant differences between the control and exposed group in the amount of displacement activities (see Table 6.2 for list of displacement activities and stress parameters measured) or stress parameters elicited by the test situation were found. A possible explanation for this could be that the time period during which the behaviour of the puppies was measured was too short to present significant differences. As the puppies are placed in an unfamiliar environment without their littermates for the first time, it can be assumed that this was mildly stressful for all puppies. In an experiment conducted by Levine (1960), he measured the adrenocortical response of handled and non handled rats to an electric shock. The results showed that, although both groups had similar blood levels of adrenal steroids before the shock, the handled rats showed a much higher increase in adrenal steroid level during the first 15 minutes after the shock compared to the non-handled group. However, when the non-handled rats had achieved the same level of adrenal steroids they displayed this high level for a much longer period than the handled rats. Levine (1960) suggests that the fast HPA system reaction in the handled rats is more adaptive because it is more consistent with an appropriate functioning stress system, decreasing the risk of developing psychosomatic effects (e.g. suppression of the immune system) caused by exposure to prolonged periods of stress. If the period of observation was prolonged and in addition physiological stress measures were taken (e.g. saliva cortisol levels) perhaps differences in the amount of displacement activities or stress parameters shown by the exposed and control group would have reflected a differences in the emotional state or gradation of level stress of the puppies.

6.4.2 Exploration

Without making any assumptions about the underlying emotions driving the exploratory behaviour so far, because exploration can be instigated by motivational systems that are independent of fear or anxiety (e.g. curiosity) (Roy & Chapillon 2004), the results of this experiment show that the control puppies display a higher level of exploratory behaviour, possibly caused by the higher level of novelty of the situation. This strengthens the conclusion of Chapter 5, that in dogs, exposure to video images between 3 and 5 weeks of age results in the formation of cognitive representations that are generalised to the real world, and can thus be used to increase a puppy's knowledge of the world, decreasing the chance that unfamiliar stimuli, which might cause a loss of emotional homeostasis, are encountered. However, although less likely, given the direction of the results of the exploration measures, it cannot be totally excluded that all the puppies found the situation equally novel, but that the exposed puppies responded more confidently to that novelty. Distinguishing between these two explanations is not possible because of the strong bias in the exploratory behaviour towards the vacuum cleaner.

Some authors would suggest that an increased level of exploration results from the fact that a novel situation has rewarding properties, leading to the puppies demonstrating a higher level of curiosity-driven exploratory behaviour (Barnett & Cowan 1976) or that the level of deprivation experienced by the control group compared to the exposed group, has resulted in an extension of the approach period, and therefore that the puppies were actively seeking stimulation (Fox 1971, Bateson 1981). In contrast to the puppies tested in the experiment described in Chapter 5, that were 36 days old, the puppies in this experiment were tested between 51 and 61 days. At this age exposure to novelty should cause a negative affective state and an approach-avoidance conflict, resulting from the development of the fear response (Freedman *et al* 1961). The results of the experiment described in this chapter suggest that the increased motivation to explore is associated with fear.

It is suggested by Koolhaas *et al* (1999) that the level of aversiveness of a novel stimulus, and whether fear is evoked, is determined by the cognitive appraisal of the stimulus rather than by its physical characteristics. The theory of the 'Two dimensional defense system', (McNaughton & Corr 2004), proposes that the hippocampus is directly involved in some emotions and places it at the centre of a system with interconnected structures that respond to signals of novelty, punishment and non-reward, and generates outputs including inhibition of prepotent behaviour, enhancing attention and arousal, and increasing risk aversion in conflict situations. A functional, behavioural and pharmacological distinction is made between fear and anxiety. Fear involves fight, flight, freezing and has the function of moving an individual away from danger, whereas in an approach-avoidance situation, anxiety has the function of moving the individual towards danger. Thus, anxiety occurs when entering a dangerous situation and is displayed as a risk assessment approach or withholding entrance (passive avoidance), whereas fear is a form of active avoidance which operates when leaving a dangerous situation (McNaughton & Corr 2004). The experiment described in this chapter could be classified as a forced open field test in which the individuals have no possibility to withdraw into a familiar environment. This might imply that the higher level of exploratory behaviour displayed by the control group is an expression of risk assessment of the stimuli resulting from a higher level of anxiousness or fear.

This is supported by the significant differences in type of locomotion. The control puppies ran significantly more whereas the sample puppies walked significantly more. Although movement is involved in the exploratory and the fear system, rapid movement is regarded to be more indicative of fear (Mackay & Wood-Gush 1980). From research conducted to explore the effect of early handling on emotional reactivity using a several session procedure, it was concluded that locomotion during session one of exposure to an open field represents a high emotional reactivity level and during the following tests sessions a low emotional reactivity level (Roy & Chappillon 2004). During the first session no differences were found between handled and control rats for parameters like locomotion and rearing, but they became significantly different during the third session. The locomotion of the handled rats increased only slightly. In the control rats motionless time increased inversely to locomotion (Roy & Chappillon 2004). In this experiment there was only one test session. The higher level of locomotion displayed by the control group can therefore be interpreted as a higher level of emotional reactivity.

Latencies to explore a novel object or area in elevated plus maze or open field tests are often used as a measure reflecting anxiety or fear, (Augustsson & Meyerson 2004), being interpreted as the more confident animals showing the shorter latencies to approach the new object or enter the unfamiliar environment. In dogs, anecdotal observations suggest that dogs that show body postures associated with fear show increased latency times (King *et al* 2003). The findings in this experiment are in contradiction with these observations. The control group displayed significant more body positions associated with fear and shorter latencies to approach than the exposed group and more time exploring objects, although the latter two were not statistically significant, with the exception of exploration of the vacuum cleaner which was explored for significantly longer by the control group. This suggests that the shorter latency to approach the first object might reflect an increased level of anxiety or fear than in the exposed group, instead of a higher level of confidence.

The significantly higher amount of exploration of the objects by the males is also consistent with the hypothesis that the level of exploration in the conducted experiment reflects a higher level of anxiety or fear, and could be interpreted as a risk assessment behaviour. Gray (1987) reports that in a large variety of species (rats, foxes, cats, cockerels) males are generally found to be more sensitive to stress and more fearful than females . When exposed to challenges, for example, male rodents show a higher behavioural reactivity than do females (Gray 1987). However, the interpretation of the emotional levels between individuals and between males and females is extremely complicated because of all the methodological differences between different studies.

In summary, it can be concluded that in puppies exposure to video images between the age of 3 and 5 weeks results in the puppies being more confident, when exposed to an unfamiliar environment at 7-8 weeks of age.

Part 2:

Chapter 7: Separation anxiety in dogs. The role of emotional homeostasis and the sensitive period of behavioural organization in its development

(This chapter is based on the paper: Appleby D. and Pluijmakers J. (2003) Separation anxiety in dogs. The role of emotional homeostasis and the sensitive period of behavioural organization in its development; published in The Veterinary Clinics of North America Small Animal Practice, 33, 321-344 and presented at the Companion Animal Behaviour Therapy Study Group – Study Day 2004, Birmingham, UK).

7.1 Introduction

Problems involving destruction, vocalisation and house soiling by dogs that occur during the owner's absence are common in the pet population (Borchelt & Voith 1982, Takeuchi *et al* 2000, Bradshaw *et al* 2002a) and constitute a significant proportion of the caseload of the behaviour specialist (McCrave 1991). Until relatively recently the term separation anxiety was used generically to describe all separation related problems (Heath 2002). However, there are causes that are unrelated to anxiety (Borchelt & Voith 1982, McCrave 1991, Heath 2002) and previous papers and publications have categorised them (Borchelt & Voith 1982, McCrave 1991, Blackwell *et al* in press). In particular McCrave (1991) produced an influential paper that identifies differentials for the motivations of the three most commonly reported separation-related behaviours (Overall *et al* 2001) (Table 7.1). As a consequence of this classification new generic terms were introduced (Blackwell *et al* in press) and it is now common practice to refer to a separation problem followed by a description of the diagnosed motivation (Blackwell *et al* in press), one of which is separation anxiety.

In this chapter a short overview of the literature regarding separation problems and separation anxiety will be given, after which a model for the diagnosis and treatment of separation anxiety in dogs will be introduced. Components of this model are tested in the analyses of clinical data described in the following chapters. The effectiveness of the proposed treatment regimes (section 7.11) has not yet been evaluated.

Table 7.1 Differential diagnosis for separation problems. From: McCrave (1991)

Differential diagnoses for separation problems based on the symptoms (in bold):		
House soiling	**Destruction**	**Vocalisation**
House breaking	Play behaviour	Reaction to external stimuli
Submissive/excitement	Puppy chewing	Socially facilitated
Urine marking	Reaction to arousing stimuli	Play/aggression
	Over activity	
Fear induced	Fear response	Fear induced
Separation anxiety	Separation anxiety	Separation anxiety

7.2 Diagnosing separation anxiety

It is generally recognised that successful treatment of separation anxiety requires careful consideration of the history of the dog and the presenting signs, followed by diagnosis based on empirical evidence (Borchelt & Voith 1982, McCrave 1991, Pageat 1998, Mills & Sheppard 1999, King *et al* 2000). However, the process is made difficult by a lack of consensus about how separation anxiety should be defined (Appleby & Pluijmakers 2003).

The symptoms or combination of symptoms commonly reported during owner absence are destruction, house soiling and vocal behaviour (Borchelt & Voith 1982, McGrave 1991, Overall 1997, Pageat 1998) indicative of distress (Overall *et al* 1999). Less reported but welfare-significant symptoms (Overall *et al* 2001) can also occur, possibilities include: withdrawal, in-appetence, hyperventilation, salivation, gastrointestinal disorders (vomiting/diarrhoea) (Takeuchi *et al* 2000), increased and repetitive motor activity, such as pacing and circling, and repetitive self-directed behaviours, such as over-grooming or self mutilation (Borchelt &

Voith 1982, Pageat 1998, King *et al* 2000). Dogs with the condition can become anxious and agitated or display depressed behaviours in response to stimuli associated with the owner's departure (McCrave 1991, Simpson 2000).

In one recent study the median age of onset of separation anxiety was over 1.5 years (Takeuchi *et al* 2000). The significance of breed differs between studies: although an increased prevalence of the problem has been reported in mixed breeds (McCrave 1991), a study of a general population found only weak evidence for such a bias (Bradshaw *et al* 2002a). The problem is reported more often in males than females (Beaver 1999, Podberscek *et al* 1999, King *et al* 2000, Takeuchi *et al* 2000, Gualtier 2001, Flannigan & Dodman 2001, Bradshaw *et al* 2002a). Prolonged periods without separation from the owner, a prolonged period without the person to whom the dog is attached, periods of kennelling (Voith & Borchelt 1985), a house move with the owners (Flannigan & Dodman 2001, Seksel & Coyle 2001) and time spent at a shelter (Voith & Borchelt 1985, McCrave 1991, Serpell & Jagoe 1995) have all been cited as causes of separation anxiety.

In the broadest definition of separation anxiety the condition is described as problematical behaviour motivated by anxiety that occurs exclusively in the owner's absence or virtual absence (Borchelt & Voith 1982, Overall 1997). A more specific definition of separation anxiety (Gaultier 2001) requires ongoing attachment to the maternal or primary caregiver or person to whom this attachment is transferred after homing (Voith & Borchelt 1985, McCrave 1991, Pageat 1998, King *et al* 2000, Gaultier 2001). This definition is borrowed from human psychology and attachment theory in human and non-human apes (Serpell & Jagoe 1995). It has been suggested that an emotional bond (Bowlby 1969) allows the infant a secure base from which it can explore its environment (Harlow & Zimmerman 1959, Schaffer & Emmerson 1964, Bowlby 1969, Pageat 1998) and develop all of its behaviour (Pageat 1998). The potential for an attachment bond to develop is thought to be increased if owners also form a strong attachment to their dogs, because they respond to, and therefore reinforce, dependent behaviour (Pageat 1998).

7.3 Hyperattachment

Some authors in the field of pet behaviour have suggested that hyperattachment is a necessary condition for separation anxiety (Voith & Borchelt 1985, McCrave 1991, Pageat 1998, King *et al* 2000, Gaultier 2001). This has been subdivided into primary and secondary hyperattachment (Pageat 1998, Gaultier 2001). Primary hyperattachment is the continuance of the primary attachment bond to an individual beyond puberty, which constitutes the specific definition of separation anxiety and correlates with a perpetuation of other characteristics of immaturity (Gaultier 2001). Secondary hyperattachment can develop at any age and is described as dependency on one or more persons in the dog's 'family' circle. A dog suffering from an emotional disorder, such as phobia or loss of primary attachment figure, may develop this type of attachment (Pageat 1998, Gaultier 2001).

Typical manifestations of hyperattachment are: the organisation of all activities around the attachment figure when they are present (Pageat 1998), following from room to room (Beaver 1999), owners not able to go to the bathroom without their dogs wanting to follow them (Pageat 1998, Heath 2001), wanting to sleep next to them (Pageat 1998), leaning on them (Voith & Borchelt 1985), constantly wanting to be held (Beaver 1999) and displays of distress if separated from the owner when they are at home, which may involve destruction at the point of access (Lindell 1997). They also stand out from the normal dog population in respect of the effusive greeting behaviour at the time of the owner's return (Borchelt & Voith 1982, McCrave 1991, Overall 1997, Simpson 2000).

There are arguments against hyperattachment being a necessary condition for separation anxiety. These include the observation that dogs that are 'spoilt' and encouraged to have a very close relationship with their owners do not necessarily develop separation problems (Borchelt & Voith 1982, McCrave 1991, Overall 1997, Flannigan & Dodman 2001). Several authors have commented that only some dogs that display separation anxiety in the broader sense display symptoms of hyperattachment when the owners are at home (Overall 1997, Simpson 2000).

Destruction and vocal behaviour motivated by separation anxiety is routine in the sense that it is likely to occur every time the dog is left alone and separated from the attachment figure(s), as it occurs more frequently than the intermittent behaviour that occurs with other motivations like boredom (McCrave 1991, Lindell 1997). Some authors have suggested that destruction orientated towards doors and windows that give access to the direction by which the owners left is indicative of separation anxiety (Borchelt & Voith 1982, McCrave 1991) and barrier frustration (Lindell 1979), which is consistent with hyperattachment. Destructive behaviour involving items impregnated with the owner's scent such as shoes, papers, bedding and television controls also occurs (Lindell 1979), and has been attributed to disorganisation of exploratory behaviour related to seeking the owner by olfaction (Pageat 1998). Vocalisation when separated from the owner is thought to develop from puppy vocalisation during distress and affiliative behaviour and is generally higher in pitch, uses repeated sub-units, has little variation in tone and occurs at a greater rate, when compared with 'normal' dogs (Overall *et al* 1999).

The timing of onset of symptoms when left is significant, typically within the first thirty minutes and often almost as soon as the dog is left (McCrave 1991). They rapidly mount in magnitude and reach a peak within 30 minutes (Hothersall & Tuber 1979, Borchelt & Voith 1982, McCrave 1991, Beaver 1999, Hetts 1999, Lindsay 2000a), followed by a gradual adaptation period and a steady decline in distress from the level of arousal caused by departure, or re-arousal due to external stimulation, in addition to an internally controlled 20-30 minutes cyclic component (Lund & Jorgensen 1999). Symptoms can persist until the owner returns but the dog may recover and relax sooner (Borchelt & Voith 1982, McCrave 1991).

The Opponent-process theory (Solomon & Corbit 1974) offers a useful construct for understanding the adverse separation reactivity in dogs (Lindsay 2000a). According to this theory a hypothetical neural system regulates emotional arousal and prevents affective extremes from occurring as the result of attractive or aversive stimulation. Feelings of well-being and comfort are shadowed by hedonically opposite effects such as feelings of contact need. In terms of the phenomena of dependence, repeated stimulation of these feelings results in the gradual attenuation of dependent behaviour.

When a separation-anxious dog is comforted by social contact and security, opposing affects are generated and become problematical when the dog is left alone, when it becomes overwhelmed by loss of security and control. Repeated stimulation of these processes results in a condition of perpetual social attention seeking and neediness and the repeated stimulation of positive social effects strengthens the underlying anxiety or fear. As a consequence, when the dog is left alone the aversive emotions reoccur. The process continues for as long as the dependence is not effectively treated by broadening the dog's maintenance set, and fear and anxiety are reduced through systematic desensitisation and counter conditioning (Lindsay 2000a).

7.4 Fear, Anxiety and Phobia

A possible explanation for any failure of co-occurrence of hyperattachment and anxiety when separated from the owners is that separation anxiety describes more than one category of motivation, in which case another generic term may be necessary, within which separation anxiety is a subgroup. Sub classification is made difficult by the fact that separation anxiety is correlated with fear, but the link is poorly understood (Overall 1997). Although anxiety, fear and phobia are said to be distinct in some way and may not be driven by identical mechanisms (Lindsay 2000a) they are probably related at the neurochemical level (O'Farrell 1992).

Fear together with anxiety, have often been considered as motivators (Boissy 1995). They are defined as emotional states that are caused by the perception of any factual danger or possible danger or non-reward (Gray 1987) that threatens the well-being of the individual, characterised as a feeling of insecurity (Gray 1987, Boissy 1995) and distress (Lindsay 2000a). Phobia occurs when fear does not extinguish but remains at the same high level, even though the conditioned stimulus is never paired again with the noxious unconditioned stimulus (O'Farrell 1992), because the sensation of fear becomes the unconditioned stimulus (Overall 1997).

To alleviate distress in aversive situations that are a threat to homeostasis, animals display an adaptive response to recent or anticipated danger which involves two interdependent facets: psychobehavioural changes that nullify the effects of the trigger, and neuroendocrine adjustments necessary to maintain internal homeostasis (Boissy 1995). Two main systems are involved, the autonomic nervous system and the hypothalamic-pituitary-adrenocortical system (McFarland 1999). Examples of situations that cause a feeling of insecurity and induce hormonal signs of stress include mother-infant separation and exposure to novelty (McFarland 1999). Anticipation of distress requires a predictable relationship between a cue and the stressor (Fox 1971, Gray 1987, O'Farrell 1992, Overall *et al* 2001, Manning & Dawkins 1992) and response can be dependent upon cues that lack distinctiveness or upon patterns or sequences of events that are difficult to identify (O'Farrell 1992, Manning and Dawkins 1992) which can include owner absence if fear-eliciting stimuli have previously occurred in that context (Voith & Borchelt 1985). Control over the effect of the stressor is associated with lesser signs of distress (McFarland 1999). Activation of the HPA axis does not seem to occur when the animal is in a familiar situation, in which it has a tried and tested coping strategy available for dealing with any anticipated challenge in that situation, and where the actions taken are expected to deliver the results (Toates 1999).

7.5 Maintenance stimuli

It seems unlikely that the different models for separation anxiety, as described above, could develop without being interpretations of the same process. Therefore, is it possible that there is a mechanism that sits comfortably with both schools of thought? Rather than being exclusively caused by being left unattended, as suggested by many authors, separation anxiety can be defined as '*apprehension due to removal of significant persons or familiar surroundings*' (Dorland's Medical Dictionary 1989).

In the dog, which is a social pack animal (Fox 1978), a greater predisposition for problems associated with owner absence may have been unwittingly developed as a consequence of selecting for neotenised, affectionate and socially dependent behaviour (Fox 1978, Serpell & Jagoe 1995). However, formation and continuation of dependence results from an ongoing conditioning process, during which response patterns become attached to the cues provided by the social and non-social objects in the animal's environment (Fox 1978, Cairns 1966, Scott 1992). Therefore, what is often interpreted as attachment or bonding is actually a high level of conditioned dependency required for emotional homeostasis, defined as stability in the normal neurophysiological states of the organism (Dorland 1989). The significant factors in the extent to which dependency upon any one stimulus develops are salience, duration of exposure (Bateson 1981, Pageat 1998) and stimulation (Cairns 1966, Gubernick 1981, Gross 1996). Removal of an object which the response system of the animal has been strongly conditioned to depend on for the maintenance of homeostasis, is associated with a significant disruption of its behaviour. The degree of disruption is correlated with the likelihood of behaviour to reintroduce the maintenance stimulus. This in turn will decrease disruption and increase the display of the behaviour aiming to achieve reintroduction of the stimulus on subsequent occasions (Cairns 1966).

What is described as maternal attachment is inevitable because of the availability and salience of the stimulus and the sensory and cognitive development at the time of exposure (Fox 1978, Scott 1992) and absence of opportunity to attach to other social or environmental stimuli due to limited mobility. The apparent attachment to or dependence on the maternal figure (Takeuchi *et al* 2000) is not primarily an affectional bond but a way for the individual to maintain behavioural organisation (Cairns 1966, Gubernick 1981), in effect what the puppy needs for the maintenance of a sense of well-being, or homeostasis of the autonomic nervous system (McFarland 1999, Cairns 1966, Bourdin 1999). This stability results in the confidence to explore and develop parasympathetic responses to other stimuli through learning (McCune et al. 1995). Exposure to experiences and learning to cope reduces emotionality when exposed to novel or challenging stimuli (Fox 1978, Serpell & Jagoe 1995). The process results in reduced dependence upon the initially very narrow and salient stimulus set necessary for the maintenance of homeostasis and behavioural organisation (Bateson 1981), associated with proximity to the dam, littermates and nest site (Cairns 1966, Gubernick 1981).

Dependence upon the dam is reduced as she becomes less responsive and less tolerant after weaning, and thereby less salient (Pageat 1998), which suggests that dependence upon specific stimuli for behavioural organisation is also unlearned (Whoolpy & Ginsberg 1967, Fox 1971, 1978, Bateson 1981). However, a puppy may remain dependent upon the maternal figure if the process is disrupted (Pageat 1998). This can occur if the individual is over-protected or socialised with its owners and not given the opportunity to develop independence (Fox 1978, Pageat 1998. Illness during puppyhood and/or nursing is similarly disruptive (Serpell & Jagoe 1995). Owners can unwittingly encourage dependence through reinforcement of care soliciting behaviours, and reinforcement of sympathetic automatic responses to challenging or fear eliciting stimuli, the probability of which is predisposed genetically (Murphree & Dykman 1965) and/or through stimulus deprivation in early life (Scott & Fuller 1965, Fox 1978, Serpell & Jagoe 1995, Appleby *et al* 2002). Conversely, a lack of nurturing stimuli or their premature withdrawal can result in an inability to learn normal social responses (Overall *et al* 2001).

7.6 Development of maintenance stimuli

During the second stage of the sensitive period of behavioural organisation (three to five weeks of age), described in Chapter 3, a puppy starts to broaden its maintenance set. The initially reflexive organisation of behaviour is replaced by behavioural organisation through associative learning, and the presence of maintenance stimuli and composition of the maintenance set becomes an important factor for maintaining emotional homeostasis.

After four weeks of age attachment to both animate and inanimate stimuli occurs (Elliot & Scott 1961, Gurski *et al* 1980, Scott 1992). The length of association with an animate or inanimate object and its relative cue weight determines the development of dependent behaviour (Cairns 1966). The process is quick, for example Scott (1962, 1992) found that site attachment was formed in 20 minutes (see also Elliot & Scott 1961, Cairns & Johnson 1965, Cairns & Werboff 1967, Gurski *et al* 1980). Available data support the concept that animals tend to remain in the presence of objects to which they have been exposed (Boissy 1995).

Several studies, some restricted to puppyhood but with implications for later life, have been concerned with the effects of environmental and social experience on behavioural organisation and the alleviation of distress. The effect of these and potential for inducing dependence can be ranked.

1. Isolation in an unfamiliar and uncomfortable location causes more distress vocalisation than isolation in an unfamiliar but comfortable location (Gurski *et al* 1980).

2. Isolation in an unfamiliar location results in higher levels of distress vocalisation than isolation in a familiar location in puppies (Elliot & Scott 1961) but the opposite is true in older dogs (Tuber *et al* 1982). This could be due to the fact that puppies are more dependent upon the stimuli associated with their limited experience and recent reinforcement of contact/care soliciting higher vocalisation. Vocalisation in a familiar environment in adult dogs could be the consequence of previous reinforcement, higher expectation and frustration in that location.

3. Food (Harlow & Zimmermann 1959, Pettijohn *et al* 1977) and toys (Pettijohn *et al* 1977) are less effective in the amelioration of distress than warmth and comfort (Harlow & Zimmermann 1959, Pettijohn *et al* 1977).

4. Isolation and segregation in a familiar location causes more distress vocalisation than retention in the same location with a familiar conspecific (Elliot & Scott 1961).

5. Food and toys are less effective in the amelioration of distress vocalisation than the presence of a familiar or unfamiliar conspecific (Pettijohn *et al* 1977)

6. Familiar or unfamiliar conspecifics reduce distress vocalisations less effectively than a human companion. The effect of the latter is proportionate to the level of interaction (Pettijohn *et al* 1977, Tuber *et al* 1982). This is consistent with research that shows that attachment in children is not dependent on care-giving, but on responsiveness to infant behaviour and the provision of stimulation (Schaffer & Emmerson 1964).

7. Another human example suggests that the presence of an unfamiliar person benefits confidence less than the presence of a familiar person (Ainsworth *et al* 1978).

We propose that the dog population can be divided into three groups according to the maintenance stimuli they depend on. These stimuli can change with time, although the probability of change is dependent upon several factors: (i) the degree of dependence on and salience of existing stimuli, (ii) the availability of existing stimuli, and (iii) how these factors compare with the properties of new stimuli. Therefore movement between groups can occur in response to events.

Group A: Those that do not develop autonomy, due to continuing primary hyperattachment.
Group B: Those that transfer their dependence to one or more stimuli, normally social, through need or an increase in the stimuli's salience and/or availability.
Group C: Those that learn to depend upon a range of stimuli without any narrow set of social or environmental stimuli becoming exceptionally salient.

If homeostasis is disrupted a dog may try to re-establish it by attempting to achieve proximity to one or more maintenance stimuli, which might be a salient human companion. The extent to which proximity to salient social maintenance stimuli is displayed is dependent upon a dog's expectation that it will be left. Animals that are seldom left or recognise a context in which they are unlikely to be left, such as after a certain time at night, seem to show less need to stay in proximity, generally or at specific times, than those that are left frequently (Appleby & Pluijmakers 2003). Conversely the need for proximity seems to increase if the owner's departures are unpredictable (Simpson 2000), as is sometimes the case with shift workers. Separation from emotionally rewarding stimuli is frustrating, has a punishing effect and anticipation of it can result in anxiety (Gray 1987). In turn this can lead to an increase in the vigour or depressed behaviour associated with maintaining proximity at its withdrawal or anticipation of withdrawal (Lindsay 2000a, Gray 1987). The extent to which these behaviours are displayed is affected by the extent to which homeostasis is disrupted.

Symptoms of distress when left unattended often start after the owners or one owner has been at home for a period of time, such as during a holiday (Borchelt & Voith 1982, Gray 1987). This can be explained as transference from group C to group B due to long exposure to the person(s) who in some instances also become more salient, e.g. if the owner lies with the dog for long periods of time if unwell, or more stimulating, e.g. through increased activity or stimulation together.

Maintenance set disruption, fear and anxiety that a fear-eliciting event may occur during owner absence, does not necessarily result in attempts to remain in contact with them (Lindsay 2000a, Overall *et al* 2001). Instead the dog may seek maintenance from inanimate stimuli, and as a consequence may attempt to escape (Beaver 1999), by any door or window, rather than specifically the one that would give access to the owners. They may try to increase homeostasis by digging into locations that offer opportunities for hiding in, or for gaining access to rooms shut off from them that they associate with maintenance stimuli, such as the owner's bedroom, hence occasionally the owner may find torn carpet, or scratched furniture (Lindell 1997, Appleby 1997, Hetts 1999). The extent to which fear is expressed when exposed to the same stimuli when the owner(s) are present may be reduced (Hetts 1999) because the set of maintenance stimuli is more complete and behaviour more ordered.

7.7 Disruption of Homeostasis

Disruption of homeostasis can result from internal or external stimulation, or both. The potential for disruption of homeostasis increases with the magnitude of challenge, which is influenced by:

1. The loss of several less salient or one or more major stimuli from the maintenance set, leading to behavioural disturbance, disruption of responses to situations and events, and a feeling of loss of control that can cause anxiety (Cairns 1966, Gubernick 1981, Boissy 1995). It is well recognised that the removal of salient social maintenance stimuli is a precursor for fear (O'Farrell 1992). The reintroduction of these stimuli, or the introduction of stimuli comparable to the original(s), or the learning of new maintenance stimuli, allows return to homeostasis and the reorganisation of behaviour.

2. The presence of novel stimuli leads to a negative emotional state, which requires the animal to compare the event with events experienced in the past (Bateson 1981, Boissy 1995). Behavioural arousal caused by the exposure to novelty is said to be similar to the arousal caused by an electric footshock

(Dantzer 1986). However, reaction to novelty normally decreases with repeated exposure to an earlier novel environment (McFarland 1999, Domjan 2000).

3. Animals may react fearfully towards a stimulus because of its physical characteristics (i.e. intensity, duration, suddenness) or because it is associated with a threatening event as a result of learning (Gray 1987).

The effect of these factors can be combined and can accumulate through a process of sensitisation (Domjan 2000). The extent to which disruption occurs is attenuated by the strength of the maintenance provided by the stimuli present in that context.

7.8 Behavioural responses to disruption of homeostasis

The type and magnitude of neuroendocrine arousal and the expression of behavioural signs associated with disruption of homeostasis are determined by: (i) psychological factors (Boissy 1995), such as the composition of the stimulus set the animal depends on, and the state of the neuroendocrine system when confronted with a challenging stimulus. The potential is influenced by:

1. Both phenotype and any underlying pathologies that could play a role (Pageat 1998, Overall *et al* 2001).

2. The amount of control the animal can exert on a challenging stimulus or threatening environment by the display of suitable behaviours (Henry 1980).

3. The physical properties of the triggering stimulus (Boissy 1995) (e.g. suddenness, intensity).

The animal's ability to predict and control a threatening event determines the neuroendocrine pattern and intensity of emotion experienced (Weiss 1972, Henry 1980, Boissy 1995). As long as the animal is 'only' challenged in its control, the medullosympathetic system is dominant (Henry 1980). Catecholamines are released in situations that call for attention and vigilance. The loss of control or the perspective of failure to meet expectations causes an activation of the HPA axis (Henry 1980).

The behavioural response to aversive events varies greatly and depends on whether threat is present or anticipated (Boissy 1995) and the intensity of emotion stimulated (Archer 1979). Low fear levels enhance activity e.g. moving around is generally an active behavioural strategy of coping which leads to a decrease of the HPA axis arousal (Dantzer 1986). Intermediate levels normally lead to conflict between the expression of fear and activity (e.g. exploratory behaviour is reduced). Intense fear disrupts behaviour or inhibits it totally (Gray 1987, Boissy 1995). In relation to separation anxiety, destruction and vocalisation are usually said to be attempts to regain contact with the owner by escaping from confinement and following or by distressed/relocation vocalisation (Lindell 1997, Overall *et al* 1999, Podberscek *et al* 1999). These behaviours could be interpreted as an attempt to cope by regaining control and indicative of a low level of arousal. In contrast, inappropriate defecation and urination may be symptomatic of a higher level of arousal, generalised anxiety (Podberscek *et al* 1999), or an intense reaction to a threatening stimulus (Beaver 1999) and could occur if the dog finds it has no control over the arousing stimulus because of the lack of a successful coping strategy.

7.9 Diagnosis

Different treatments may be appropriate, depending on whether the dog is classified as a member of group A, B or C, and can be more or less essential for establishing or re-establishing homeostasis and resolution of the animal's distress from which the problem arose (Table 7.2). Classification and the magnitude of symptoms also determine how and the extent to which the treatment programme should be phased and which, if any, drugs will be most suitable to support therapy.

It is hypothesised that after all the symptoms are listed and classified, the diagnosis can be further refined using the following criteria:

- The onset (O'Farrell 1992, Overall 1997, Landsberg *et al* 1997), duration and intensity of the symptoms displayed.
- Behaviour of the dog when the owner is present (McCrave 1991, Pageat 1998, King *et al* 2000, Simpson 2000, Flannigan & Dodman 2001).
- Departure and greeting behaviours (McCrave 1991, Pageat 1998, King *et al* 2000, Simpson 2000, Flannigan & Dodman 2001).
- Detailed analysis of the displayed symptoms (McCrave 1991, Overall *et al* 2001).

The listing of the symptoms provides pointers towards the possible causes of the problem behaviour (Overall *et al* 2001) and the accompanying level of anxiety. A broad range of symptoms might be indicative of multiple causes and/or a high level of arousal.

For members of group A that have not learnt to depend on a broad stimulus set, the presence of the owner, on to whom maternal dependence has been transferred, is necessary for emotional homeostasis. Virtual or actual separation from the owner or its anticipation causes a reduced sense of control, anxiety and disruption of behaviour. Destruction typically involves attempts to regain contact e.g. at doors and windows that would give access to the owner. Anxiety during the owner's absence increases the potential for fear in response to stimuli causing or associated with threat. Treatment for anxiety caused by the absence of the owner requires a reduction of dependence upon them and increasing dependence upon other stimuli for emotional homeostasis. It is often appropriate for treatment to be phased, each stage of which is introduced gradually. If a problem of response to fear stimuli coexists it should be treated separately, and consideration given to doing so prior to addressing anxiety caused by the owner's absence.

For dogs in group B disturbance of homeostasis and the experience of loss of control can result from (i) the removal of one salient stimulus, normally social (ii) removal of several less significant stimuli from the maintenance set, normally social (iii) a change in the need of the animal to rely on the maintenance set, for example as a result of feeling threatened by an aversive or novel stimulus, or as the result of the process of ageing. If disruption results in excessive dependence upon a person or persons, rather than environmental stimuli, which as argued above is likely, anxiety when the dog is left unattended increases the potential for fear.

For treatment to be successful, fear eliciting stimuli that cause or contribute to the disturbance of homeostasis have to be identified and removed or their effect reduced. The model predicts that balance in the maintenance set has to be restored by either (i) reintroduction of maintenance stimuli (ii) reducing the dependency on one specific or several stimuli (ii) increasing the dependence on alternative stimuli or (iii) a combination of these.

Removal of maintenance stimuli from dogs in group C should not cause disturbance of homeostasis, because the breadth of the overall set of social and environmental stimuli means the dog has sufficient stimuli available to maintain control of the parasympathetic system. However, members of this group could become fearful or phobic as a result of experience of a noxious event, which may or may not have been associated with and triggered by the absence of the owners. If the dog tries to cope, destruction of random objects might be caused as a result of trying to escape or hide. If the level of anxiety is high, symptoms such as defecation and urination are possible. Systematic desensitisation and counter conditioning responses to fear eliciting stimuli form an essential part of treatment (Overall 1997, Landsberg *et al* 1997). The level to which the dog's response to its stimulus set is disrupted by anticipation and/or generalisation to other stimuli has to be evaluated and treated if necessary (Toates 1999).

Table 7.2 Differential symptoms i.e. those that are not general to all three groups

Group	Onset	Behaviour when owner present	Departure- greeting behaviour	Symptoms when owner absent
A	From puppyhood on. The timing of onset of symptoms when left is significant, typically every time, within the first thirty minutes and often almost immediately after actual or virtual removal of the specific social stimulus the dog is dependent upon.	Organisation of all activities around a specific social stimulus. Following about the house. Physical contact need e.g. leaning on owner, sleeping next to owner, wanting to be held. Demanding for needy attention/affection seeking behaviour. Exploratory behaviour dependent on presence of specific social stimulus the dog is dependent upon.	Distress signs (e.g. trembling, shaking, howling, withdrawal) when departure is anticipated. Possible attempts to prevent departure. Depression or appeasement behaviour possible as result of anticipation of punishment when owner returns.	Destruction typically involves attempts to regain contact with the owner and is orientated towards doors and windows that give access to the direction by which the specific social stimulus left. Destructive behaviour involving items impregnated with the owner's scent such as shoes, papers, bedding and remote controls. Vocalisation consistent with separation distress/relocation.
B	'Sudden' onset after removal of one salient stimulus, several less significant stimuli or a change in the need of the animal to rely on the maintenance set. Caused by e.g. rehoming, moving house, left in other room than normally, when frustrated because of deviation of normal patterns, after holiday, illness, ageing). Only when the dog is left in circumstances where its maintenance set is inadequate.	Dependency behaviours normally directed towards one or several social stimuli. However, dogs can also be dependent on non-social stimuli e.g. certain location in the house. Dependency towards social stimuli may increase if unpredictability of separation and frustration increases. The onset of display of dependent behaviour may occur as a consequence of increased need or increased salience of the stimulus.	Departure distress and excessive greeting normally but not necessarily directed at one or more social stimuli. Possible attempts to prevent departure. Departure distress, agitation or depression. Depression or appeasement behaviour possible as result of anticipation of punishment when owner returns.	If over dependent on social stimuli destruction typically occurs as a result of an attempts to regain access to the individual(s). Alternatively the dog may seek maintenance from inanimate stimuli, or if fearful escape, by any door or window. For example, they may try to increase homeostasis by digging into locations to hide in or to gain access to rooms shut off from them that they associate with maintenance stimuli, such as the owner's bedroom. Vocalisation consistent with separation

				distress/relocation (may not occur if cause of distress is fear of external stimuli or a reliance on non-social stimuli). Defecation and urination alone or in combination with other symptoms suggests the possible involvement of a fear-eliciting stimulus (e.g. noise phobia).
C	Onset coincides with a fearful or phobic experience of a noxious event, which may or may not be associated with and triggered by the absence of the owners.	No inappropriate dependency behaviours Reaction to fearful stimulus also displayed when owner present. The extent to which fear is expressed when the owner(s) is present may be reduced because the set of maintenance stimuli is more complete and behaviour more ordered.	Distress signs can develop resulting from an increase in predictability and anxiety if owner absence is associated with noxious stimuli.	Defecation and urination alone or in combination with other symptoms suggests the possible involvement of a fear eliciting stimulus (e.g. noise phobia). Destruction of random objects may be caused as a result of trying to escape or hide (coping strategy).

7.10 Treatment

Every case requires a treatment programme devised for the animal's needs, the owner's circumstances and the environment the dog is to be left in. The rationales discussed here provide the essence of what may have to be considered for conditioning relaxation that is not disproportionately dependent on social or non-social maintenance stimuli. The treatment programme summarized in Table 7.3 is based on a summary of the general treatment rationales reported in the literature. These have been divided according to their hypothesised relevance for the groups A, B and C. In addition, it is usually recommended that treatment should be phased to avoid an unintentional increase in anxiety, which might otherwise be induced by radical alteration of the dog's circumstances and relationship with its owner (Landsberg *et al* 1997). Separation distress and its consequences often continue while treatment is taking effect (King *et al* 2000) and owners should therefore be advised accordingly. However, where possible, short-term management, such as the use of a dog sitter when the dog must be left, can reduce the potential for this to occur (Hetts 1999).

7.11 Reducing the salience of the person(s) on whom the dog is dependent and developing alternative maintenance stimuli: proposals for treatment regimes

The aim of the treatment rationales described in this section is to compose a balanced maintenance set. The salience of the stimuli the dog is over dependent upon are decreased to provide the individual with the capacity to stay in emotional homeostasis when these stimuli are absent. Depending on the analysis of the situation by the behaviour counsellor (e.g. who is the dog over dependent upon, social or non-social stimuli or combination) the optimal combination of treatment rationales is combined and applied for dogs in group A and B who are over dependent upon one social stimulus (Group A) or several social or non-social stimuli.

7.11.1 Ignoring attention seeking behaviour

Attention-seeking behaviour can be associated with distress during owner absence (Overall 1997, Lindsay 2000a). As it is indicative of over-dependence and sympathetic arousal, it is usually recommended that such behaviour should be ignored, to avoid unwitting reinforcement (Podberscek *et al* 1999, King *et al* 2000, Simpson 2000), but that vocal or physical rejection should not be used because reinforcement will result from the attention given. It is often proposed that interaction by touch, voice and eye contact should be initiated and concluded by the owner at times when the dog is relaxed, to reinforce relaxation and develop independent behaviour (O'Farrell 1992, Appleby 1997, Landsberg *et al* 1997, Podberscek *et al* 1999, Simpson 2000). In some cases there may be a potential for dependence to transfer to a new social stimulus. If this occurred it would be necessary for all family members to control interaction in the same way. The addition of scheduled, regular sessions of attention which the dog can predict may help it to relax (Overall 1997, Simpson 2000). They may also improve clients' compliance because it is in keeping with their perception of pet-ownership. Owners may need to be warned that attention-seeking behaviour will increase before it extinguishes because absence of an expected response will increase the vigour of the behaviour and an owner response will unwittingly reinforce it, making it likely to reoccur (Lieberman 1992). The model predicts that ignoring attention seeking behaviour is an essential part of the treatment of most dogs in Group A and B. Their over dependency makes them often very frantic about receiving attention from the owner(s). If the dog is over dependent upon a non-social stimulus to maintain emotional homeostasis (e.g. its crate) it will not display attention seeking behaviour that is symptomatic for over dependency, and this treatment rationale and the rationales described below which are aimed at reducing over dependency upon social stimuli could be omitted.

7.11.2 Reducing physical contact

If a dog tends to remain within a metre of an owner or in physical contact whenever they settle, this is considered indicative of over-dependence (Voith & Borchelt 1985, McCrave 1991, King *et al* 2000, Overall *et al* 2001). Preventing the dog from sitting on furniture next to the owner or on their lap may reduce both the reinforcement of dependent behaviour, and the contrast between owner presence and absence (Voith & Borchelt 1985, Podberscek *et al* 1999, Nack 1999, King *et al* 2000). Conversely, attention given when the dog chooses to lie at a distance from the owner in a relaxed manner is considered to develop independent behaviour (Askew 1996, Hetts 1999).

7.11.3 Dividing tasks

If the dog appears to be dependent upon a particular individual for activities that enhance attachment, such as playing, feeding, walking, training, some authors recommend that these should be shared by other members of the household where possible (O'Farrell 1992, Pageat 1998). The feeding of gratuitous titbits as opposed to rewards can be stopped so as to reduce the salience of the provider (Podberscek *et al* 1999).

7.11.4 Stimulating independent behaviour

Self-rewarding activities when the owner is present can help to develop independent behaviour. Examples include encouraging the dog to lie on its bed with a chew (Nack 1999) to play with toys that cause it to work for food to be released (O'Farrell 1992, Landsberg *et al* 1997, Beaver 1999, Podberscek *et al* 1999, Takeuchi *et al* 2000), and games that encourage it to search for food or toys during walks or in the owner's yard.

7.11.5 Sleeping location

Although sleeping with the owner is not thought to be causal (Overall 1997, Simpson 2000) in cases where the dog sleeps in the owner's bedroom because it is distressed when separated from him/her it is thought advisable that it is conditioned to be able to sleep in another location (Podberscek *et al* 1999). This can be achieved by moving it from the owner's bed, if it sleeps on it, and on to a bed of its own, which in turn is gradually moved out of the room. Subsequently a dog- or child-gate can be used across the open bedroom door, and when the dog is ready a series of relocations used to gradually move the dog towards where it will ultimately be expected to sleep (Podberscek *et al* 1999).

7.11.6 Canine companion

If separation anxiety is caused by over-dependence on a canine companion the treatment principles can be adapted to reduce the salience of this dog and the development of alternative maintenance stimuli. In most cases owners may not realise that this may have been an issue until after the demise or permanent departure of the dog on to which dependence was placed. In these circumstances over-dependence may have transferred to another social stimulus, such as an owner, prior to the problem being presented.

7.11.7 Providing maintenance stimuli during owner absence

The reduction of disproportionate dependence on social and non-social stimuli cannot be addressed without developing the dog's capacity to maintain emotional homeostasis through alternative stimuli, although stimuli associated with social contact can be utilized (Podberscek *et al* 1999). The model suggests that for some cases of Group B dogs, reintroducing the non-social maintenance stimulus/stimuli the dog is dependent upon to maintain emotional homeostasis when left alone can solve the separation problem. For example: reintroducing the crate, replacing the dog into the room he was in normally when left alone.

7.11.8 Relaxation cues associated with maintenance stimuli

Relaxed behaviour in the owner's presence (parasympathetic autonomic response) may become associated with a visual, auditory or olfactory stimulus, which can then be used to trigger relaxation during the owner's absence, by putting the relaxation cues in place before departure (Askew 1996, Landsberg *et al* 1997, Podberscek *et al* 1999, Hetts 1999, Simpson 2000). The model predicts that this would be most effective for Group A and B dogs.

Food items such as chews and palatable food pieces hidden in a toy can both generate relaxation and become relaxation cues during owner absence, if they are introduced gradually. As with other relaxation cues that are purposely developed rather than pre-existing, they are usually introduced when the dog is relaxed and the owner is present but not interacting with it. Subsequently they are often used in conjunction with systematic desensitisation sessions and then to stimulate relaxation when the dog is left unattended (Appleby 1997, Landsberg *et al* 1997, Beaver 1999, Podberscek *et al* 1999, Takeuchi *et al* 2000). The item can be removed when access to the owner is re-established during therapeutic sessions and when the owner returns home during actual use. Failure to show interest in food items during separation from the owner is indicative of sympathetic arousal (King *et al* 2000, Simpson 2000).

DAP ™ (Dog Appeasing Pheromone) is a synthetic version of a secretion from sebaceous glands between the mammary glands produced during lactation (Mills 2002) that is atomized by a plug-in device. It is claimed to have a beneficial effect in the treatment of separation problems (Mills 2002). The indications for its use (Mills 2002) suggest that it stimulates relaxation. Whether the response is innate or learned through association with warmth, comfort and suckling has not been established.

It is often recommended that any stimuli which are normally associated with relaxation in the owner's presence, such as the sound of the television or radio, should be left on when the dog is left unattended, to provide continuity (Landsberg *et al* 1997, Beaver 1999, Podberscek *et al* 1999). Recordings of voices and sounds that occur when members of the household are at home can also be used for this purpose (Beaver 1999, Podberscek *et al* 1999). It is considered important that these are also used at times when the owner is present, to prevent their becoming a cue for imminent departure.

It has been proposed that an owner the dog is dependent on should leave cloths/blankets impregnated with his or her scent in the place where the dog is known to lie when left alone (Beaver 1999). Putting items with unwashed laundry for a few hours before each use will refresh the scent (King *et al* 2000).

7.11.9 Changing the environment

Fear and anxiety can be associated with areas of the home in which the dog has experienced these emotions (Beaver 1999). Providing an alternative location for the dog to settle in during owner absence should make stronger maintenance stimuli available for dogs in group A and B, or reduce the salience of or remove fear-eliciting stimuli for Group C and B dogs (treatments described by Beaver 1999, Hetts 1999). The change of location can be indefinite, or until a positive association with the original location has been developed. The original location can be adjusted to suit the individual's needs, for example by creating free access to a sound-reducing den to hide in if the dog reacts to fear eliciting sound stimuli as many dogs in Group C and a proportion of Group B dogs are predicted to do. Once associated with relaxation when the owner is present and subsequently during systematic desensitisation sessions (e.g. for dogs in Group A and B where over dependency is a main factor in the problem), confinement in a crate can be used for some dogs (Voith & Borchelt 1985). However, abrupt confinement may increase anxiety (Beaver 1999, Voith & Borchelt 1985, Landsberg *et al* 1997).

7.12 Systematic desensitisation to departure cues and separation from the owner

Systematic desensitisation techniques should form an essential part of the treatment of all three groups. However, the stimuli the dog has be desensitised to vary between groups. For Group A and B dogs desensitisation to cues that are associated with removal of the social stimulus the dog is dependent upon is essential. For dogs in Group C and for some dogs in Group B, namely those who transferred from group C to B, desensitisation should preferably be applied in combination with counter conditioning techniques to the fear eliciting stimulus.

7.12.1 Desensitisation to departure cues

While the dog is in a relaxed state, stimuli associated with owner departure, such as the sound of car keys, putting a coat and shoes on etc. can be performed when the owner is not leaving, but is instead performing activities associated with remaining at home. It is considered important that the dog remains relaxed, to which end the level of stimulation should be increased gradually. Owners can subsequently combine an increasing a number of stimuli (O'Farrell 1992, Landsberg *et al* 1997, Overall 1997, Pageat 1998, Hetts 1999,Takeuchi *et al* 2000, Simpson 2000).

7.12.2 Desensitisation to owner absence

Some authors have advocated training the dog to sit or lie at a distance from the owner in a state of relaxation (Voith & Borchelt 1985, O'Farrell 1992, Overall

1997, Landsberg *et al* 1997, Takeuchi *et al* 2000) which can be associated with the relaxation cue discussed above. Initially the owner should only take a few steps away, before returning to reward the dog. Provided the dog remains relaxed the distance and duration of absence are gradually increased; however, they should be increased on a variable schedule to prevent dog from predicting the owner's return, which might otherwise result in its behaviour being disrupted if its expectations are not met.

An alternative approach to 'training' that does not risk increasing the salience of the owner through the interaction involved requires the use of a child or dog gate (Podberscek *et al* 1999). Initially this is used to prevent the dog from following the owner from room to room as they move about the house. Since the dog can see the owner through the gate it is less likely to be distressed by its use than by a closed door. On some of the occasions when the owner stays in a room for an extended period of time the gate can be used to keep the dog in an adjacent room. The technique should be used for variable periods in conjunction with relaxation. Once it is evident that the dog been conditioned to relax in these conditions the gate can be repositioned so that it retains them in an area further from the owner. Subsequently the dividing doors can be left less ajar, and finally closed.

To achieve optimal progress during the treatment period, it is often recommended that the dog should only be subjected to separations it can tolerate (Voith & Borchelt 1985, O'Farrell 1992, Landsberg *et al* 1997, Beaver 1999). If longer separations are inevitable, the 'relaxation cue' should only be used during therapeutic separations. The dog can be placed in a different part of the house, if feasible, during separations that have to occur in the course of everyday events (Beaver 1999).

7.13 Leaving and returning rituals

Owner interaction pre-departure is thought to reinforce anxiety (Voith & Borchelt 1985, Pageat 1998, Takeuchi *et al* 2000). To avoid this, it is often recommended that interaction is withdrawn approximately 30 minutes before the owner leaves. The dog should be placed with relaxation cues in a place where it has learnt to be relaxed when separated from the owner when they are at home. When it is evident the dog is relaxed the owner can leave but without speaking (O'Farrell 1992, Landsberg *et al* 1997, Podberscek *et al* 1999, Hetts 1999, Takeuchi *et al* 2000). Excessive greeting behaviour displayed by the dog when the owner returns should be ignored so as to avoid the unwitting reinforcement of the associated emotional disturbance. Conversely, the owner should respond to and therefore reinforce relaxed greeting behaviour (Podberscek *et al* 1999, Hetts 1999), such as sitting. It may also be important to note that what appears to be excessive greeting behaviour can be appeasement caused by anticipation of owner aggression carried out as misguided attempts to punish.

7.14 Avoiding punishment

Punishment for perceived wrongful behaviour during the owner's absence is not thought to be an effective technique for changing that behaviour and should be avoided for the dogs in all three groups (Voith & Borchelt 1985, Overall 1997, Lindsay 2000a). The emotional state caused by anticipation of the owner's apparently unprovoked aggression is one of the most commonly cited reasons why separation related behaviour worsens (Voith & Borchelt 1985, Simpson 2000). Owners often believe that their dog "looks guilty" but this is misinterpretation of a posture motivated by fear (Voith & Borchelt 1985). It is therefore considered important that owners ignore any damage or soiling found on their return (Voith & Borchelt 1985, Podberscek *et al* 1999, King *et al* 2000).

7.15 Systematic desensitisation and counter conditioning to fear eliciting stimuli

In cases where distressed behaviour occurs because of fear of specific stimuli, and stimuli that have become associated with them, such as rain on windows as a predictor of thunder, or the owner's absence if it is associated with noxious stimuli, the dog's response can be altered through systematic desensitisation and/or counter conditioning. These processes involve either predisposing relaxation and gradually increasing the level of exposure to the stimuli, or pairing the stimuli with another, such as food, that results in a response that is incompatible with fear (Voith & Borchelt 1985, Lieberman 1992, Hetts 1999). The stimuli can be real or recorded and, whichever method is in use, must always be presented at a level that is within the dog's capacity to remain relaxed, and increase at a rate that is compatible with its continuing to develop an association with remaining relaxed. For dogs represented in group B it may also be necessary to address over-dependence on social stimuli. For dogs in groups A and B the involvement of fear eliciting stimuli has to be assessed and treated as necessary.

7.16 Drug support for behaviour modification

Choice of drug therapy is dependent upon the nature of the disturbance to homeostasis and the nature of action required; therefore an accurate diagnosis of anxiety, fear response to threat or combination of both is essential (Pageat 1998, King *et al* 2000, Simpson 2000). Whether drug support is used is dependent upon clinical judgment and the severity of the disturbance. It is recommended that it should always be used as an adjunct to behaviour therapy, as a means of achieving homeostasis more quickly, thereby increasing the likelihood of behavioural therapy being successful, and to prevent further disruption of homeostasis and in some cases block memory of the disruption (Askew 1996). For those cases in which a sound phobia is involved, and a short time management is necessary to limit the negative consequences of a phobic event, benzodiazepines can be used because of their memory blocking properties. For the long term treatment, in which the goal is to improve the response to behavioural therapy and to prevent the phobia from getting worse a mono-amine oxidase B inhibitor can be applied if the animal shows inhibited behavioural responses (e.g. shaking, dribbling) or an SSRI if the dog shows panic behaviour (e.g. dive under the bed) (Heath 2005). Tricyclic antidepressants as Clomipramine, is labelled for use in pets for the treatment of generalized anxiety and separation anxiety (Landsberg *et al* 2003). When used in combination with a program of behavioural therapy, Clomipramine has been shown to be effective for reducing signs of separation related anxiety of dogs which showed signs of over attachment to their owner(s) (Simpson *et al* 1997).

Table 7.3 Elements of treatment program

	Group A	Group B	Group C
Reducing the salience of the person(s) on whom the dog is dependent and developing alternative maintenance stimuli	Possible stage of introduction		
Ignore attention-seeking behaviour. All interactions are initiated and concluded by the owner at times when the dog is relaxed	phase 1	phase 1	
Schedule frequent and regular attention sessions	phase 1	phase 1	
Reduce physical contact (e.g. lying on lap)	phase 1	phase 1	
Decrease dependency on a particular individual by dividing tasks	phase 1	phase 1	
Stop feeding gratuitous titbits	phase 1	phase 1	
Stimulate independent behaviour by providing self-rewarding activities	phase 1	phase 1	
Change sleeping location. If the dog sleeps in the bedroom gradually move it to another location.	phase 3	phase 3	
Providing maintenance stimuli during owner absence			
Develop a relaxation cue when the owner is present associated with maintenance stimuli	phase 1 or 2	phase 1 or 2	
Provide relaxation cues during systematic desensitisation sessions (e.g. chew toy, DAP, television, voice recordings, clothes)	phase 2	phase 2	

Provide relaxation cues during owner absence	phase 3	phase 3	
Change the environment. Provide an alternative location to settle in during owner absence with stronger maintenance stimuli, or adjust present location to the dog's need		phase 1	phase 1
Remove fear eliciting stimuli if possible			phase 1
Systematic desensitisation to departure cues and separation from the owner			
Desensitise to departure cues	phase 2	phase 2	
Systematically desensitise to owner absence	phase 2	phase 2	
Stop the dog from following throughout the house	phase 2	phase 2	
Leaving and returning rituals			
Withdraw all interaction 30 minutes before leaving.	phase 1	phase 1	
Place dog in location it has learned to relax in.	phase 3	phase 3	phase 1
Ignore excessive greeting behaviour and reinforce relax greeting behaviour	phase 1	phase 1	
Systematic desensitisation and counter conditioning to fear eliciting stimuli			
Identify fear eliciting stimuli, and start systematic desensitisation and counter conditioning program during owner presence. This may also necessitate systematic desensitisation and counter conditioning to departure cues and separation from the owner if these have become a conditioned stimulus for fear.		phase 1	phase 1
Punishment (Always inappropriate and should always be stopped)	phase 1	phase 1	phase 1
Drug support (Where appropriate)	phase 1	phase 1	phase 1

Chapter 8: Separation problems and the role of emotional homeostasis. Validation of the groups A, B and C

(This chapter is based on a paper presented at the Australian Veterinary Association Conference, 2005, Gold Coast, Australia).

8.1 Introduction

According to the model proposed in Chapter 7, the composition of the treatment plan should be different, depending upon whether a dog is classified as a member of Group A, B or C, because different treatment rationales are predicted for re-establishing emotional homeostasis. This suggests that an association between symptoms in relation to the onset, duration, intensity and type of symptoms displayed, should exist in a clinical population displaying separation problems. In this chapter possible patterns of associations between symptoms are explored.

8.2 Materials and methods

A clinical population was used to explore patterns of associations between symptoms and to validate the existence of the proposed Groups A, B and C in data that had been collected prior to this project. Data for the years 1999 to 2004 from case histories involving canine separation problems referred by veterinary surgeons to one behaviour counsellor, David Appleby (DA), and diagnosed by DA as involving fear or anxiety, was collected from the history forms used during interview and treatment reports. Until relatively recently the term separation anxiety was used generically to describe problem behaviour involving destruction, vocalisation and house soiling by dogs that occur during the owner's absence. However, there are causes that are unrelated to anxiety such as boredom, reacting to stimuli outside the house, play behaviour, house breaking problems (McCrave 1991). These cases were excluded by DA as they are not supposed to be influenced consistently by the maternal environment, age at homing and exposure to urban environments (Appleby *et al* 2002). Only cases in which the dog belonged to the first owner or breeder, and the owner could recollect the age of homing of the puppy in weeks, and had seen the maternal environment of the puppy, were included. A cut-off point of twenty-eight weeks at the time puppies were obtained was applied to avoid distortion of the analysis by a small sample of puppies obtained from the breeder much older than the majority of the sample. The age of twenty-eight weeks was based on the cut-off point that had been used by DA for a population in a prior research project (Appleby *et al* 2002). The same population was used in this project as the comparison group.

After application of the exclusion criteria, records were extracted for 124 cases. They were analysed for their demographic characteristics (e.g. age, breed, sex) and for patterns of associations between symptoms. A total overview of all symptoms recorded from the interview forms and treatment reports are represented in Table 8.3. Out of this list key symptoms were selected which where explored for the positive relationships between variables (Table 8.4) using cluster analysis and cross tabulations. For the classification of cases into the three groups A, B and C (Table 8.1), two variables, onset of symptoms and frequency of symptoms, were constructed, and these were subsequently tested for associations with dependency and destructive behaviour Behaviours scored as dependency were: signs of hyperattachment to one owner, signs of hyperattachment to several owners, organisation of activities around owner and organisation of activities around several social stimuli. Behaviours scored as destructive behaviour were: destruction not specified when left, destruction of things in the house that do not move when left, destruction of movable objects, destruction of objects with the owner's scent, destruction as if to follow the departing owner, and destruction when left in any direction, not following the owner. The variable, onset of symptoms, was divided into the following four categories: from puppyhood, following a change in routine, following a change in location, and after a noxious event. Frequency of symptoms was divided into two categories; every time the dog was left and not every time the dog was left. Positive values for both these variables were available for 44 cases.

Cluster analysis and cross tabulations were used to analyse the data for links between the onset of symptoms, frequency of symptoms, and behaviour. For each analysis, the maximum number of cases for which that data existed was used. For the cluster analysis, the Jaccard method was used, which weights co-occurrences and ignores co-absences, to avoid the latter dominating the analysis.

Table 8.1 Characteristics of cases typical of group A, B and C

Group	Onset	Behaviour when owner present	Departure- greeting behaviour	Symptoms when owner absent
A	From puppyhood on. The timing of onset of symptoms when left is significant, typically every time, within the first thirty minutes and often almost immediately after actual or virtual removal of the specific social stimulus the dog is dependent upon.	Organisation of all activities around a specific social stimulus. Following about the house. Physical contact need e.g. leaning on owner, sleeping next to owner, wanting to be held. Demanding for needy attention/affection seeking behaviour. Exploratory behaviour dependent on presence of specific social stimulus the dog is dependent upon.	Distress signs (e.g. trembling, shaking, howling, withdrawal) when departure is anticipated. Possible attempts to prevent departure. Depression or appeasement behaviour possible as result of anticipation of punishment when owner returns.	Destruction typically involves attempts to regain contact with the owner and is orientated towards doors and windows that give access to the direction by which the specific social stimulus left. Destructive behaviour involving items impregnated with the owner's scent such as shoes, papers, bedding and remote controls. Vocalisation consistent with separation distress/relocation.
B	'Sudden' onset after removal of one salient stimulus, several less significant stimuli or a change in the need of the animal to rely on the maintenance set. Caused by e.g. rehoming, moving house, left in other room than normally,	Dependency behaviours normally directed towards one or several social stimuli. However, dogs can also be dependent on non-social stimuli e.g. certain location in the house. Dependency towards social stimuli	Departure distress and excessive greeting normally but not necessarily directed at one or more social stimuli. Possible attempts to prevent departure. Departure distress, agitation or depression.	If over dependent on social stimuli destruction typically occurs as a result of an attempts to regain access to the individual(s). Alternatively the dog may seek maintenance from inanimate stimuli, or if fearful escape, by

	when frustrated because of deviation of normal patterns, after holiday, illness, ageing). Only when the dog is left in circumstances where its maintenance set is inadequate.	may increase if unpredictability of separation and frustration increases. The onset of display of dependent behaviour may occur as a consequence of increased need or increased salience of the stimulus.	Depression or appeasement behaviour possible as result of anticipation of punishment when owner returns.	any door or window. For example, they may try to increase homeostasis by digging into locations to hide in or to gain access to rooms shut off from them that they associate with maintenance stimuli, such as the owner's bedroom. Vocalisation consistent with separation distress/relocation (may not occur if cause of distress is fear of external stimuli or a reliance on non-social stimuli). Defecation and urination alone or in combination with other symptoms suggests the possible involvement of a fear-eliciting stimulus (e.g. noise phobia).
C	Onset coincides with a fearful or phobic experience of a noxious event, which may or may not be associated with and triggered by the absence of the owners.	No inappropriate dependency behaviours Reaction to fearful stimulus also displayed when owner present. The extent to which fear is expressed when the owner(s) is present may be reduced because the set of maintenance stimuli is more complete and behaviour more ordered.	Distress signs can develop resulting from an increase in predictability and anxiety if owner absence is associated with noxious stimuli.	Defecation and urination alone or in combination with other symptoms suggests the possible involvement of a fear eliciting stimulus (e.g. noise phobia). Destruction of random objects may be caused as a result of trying to escape or hide (coping strategy).

8.3 Results

8.3.1 Characteristics of the total population

From the 124 analysed cases 107 dogs (86%) were pure-bred (Table 8.2) and 17 (14%) cross breeds or mongrels. Seventy-five were males (60%) of which 37 (49%) were neutered compared to 49 females (40%) of which 19 (39%) were neutered (Figure 8.1). The age at consultation varied between 8 to 156 months of age (Figure 8.2), median age 3 years. Age at consultation was similar between the four gender groups (Kruskal-Wallis Anova test, Chi-square =6.59, P=0.09). The mean household size was 2.6 of which 33% included one or more children; 34% of the dogs lived together with at least one other dog.

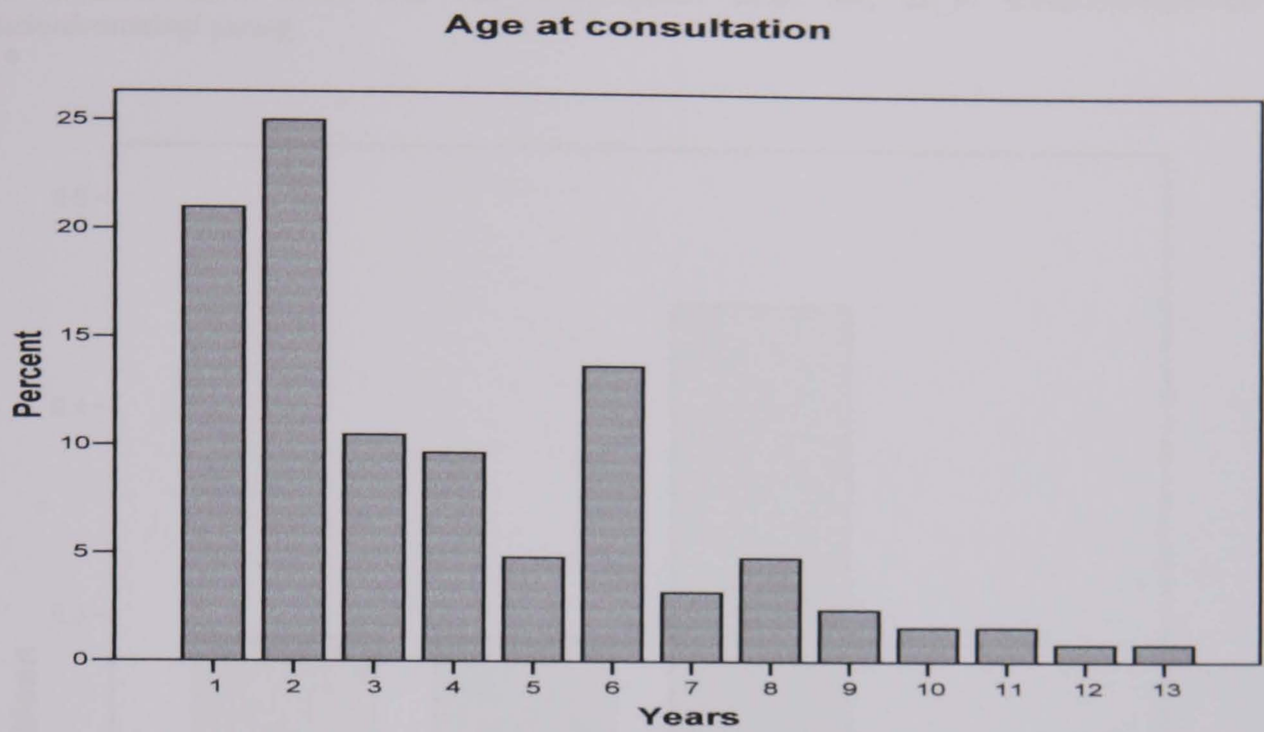
Figure 8.1 Gender of dogs displaying separation problems related to anxiety and fear.

Table 8.2 Frequencies of pure-breeds in the separation sample

Frequency	Breed
0.8%	Airedale Terrier
0.8%	Alaskan Malamute
0.8%	Bolognese
0.8%	Basset hound
2.4%	Border Collie
0.8%	Bordeaux Dog
0.8%	Bearded Collie
2.4%	Bichon Frisee

2.4%	Boxer
0.8%	Briard
1.6%	English Bull Terrier
0.8%	Staffordshire Bull Terrier
0.8%	Chesapeake Bay Retriever
13.7%	Cross breed/Mongrel
9.7%	Cocker Spaniel
0.8%	Cairn Terrier
0.8%	Dalmatian
1.6%	Doberman
0.8%	Standard Dachshund
2.4%	Fox Terrier
0.8%	Great Dane
1.6%	German Pinscher
4%	Golden Retriever
0.8%	Italian Greyhound
1.6%	German Shepherd Dog
0.8%	Irish Wolfhound
6.5%	Jack Russell Terrier
0.8%	Shiba Inu
1.6%	Labrador Retriever
0.8%	Lhaso Apso
3.2%	Lakeland Terrier
0.8%	Munsterlander
0.8	Old English Sheepdog
5.6%	Pointer
0.8%	Poodle
0.8%	Rottweiler
0.8%	Rhodesian Ridgeback
0.8%	Brittany Spaniel
0.8%	Standard Schnauzer
0.8%	Miniature Schnauzer
1.6%	Irish Setter
1.6%	Shar-pei
4%	Springer Spaniel
0.8%	Sussex Spaniel
0.8%	Tibetan Terrier
3.2%	Weimeraner
0.8%	Soft Coated Wheaten Terrier
0.8%	Whippet
3.2%	West Highland White Terrier
1.6%	Yorkshire Terrier

Figure 8.2 Age at consultation in years of dogs displaying separation problems related to anxiety and fear



The symptom most frequently reported was vocalisation when left (45%), followed by defecation and urination when left (29%) and destructive when left (29%) (Figure 8.3). Salivation/hyperventilation/vomiting/pacing was reported in 10% of the cases. The variables destruction not specified, destruction to movable objects, immovable objects and objects impregnated with the owners scent were combined into a new variable, destruction when left, before exploring the number of symptoms displayed per individual. Out of the four most frequently reported symptoms, 46% of the dogs displayed one symptom, 29% showed two symptoms and three percent, three symptoms (mean= 1.14) (Fig. 8.4). Twenty-two percent of the dogs showed a symptom that does not belong to the four most frequently reported symptoms. Forty seven percent of the dogs had been reported to show apprehension of at least one (25%), two (11.3%), three (6.5%) or four (4%) of the following stimuli: sudden loud sounds (e.g. firework and thunderstorms, other dogs and strangers or environmental stimuli, being all other animate and inanimate stimuli present in the living environment of the dog (e.g. traffic, children on roller skates, lawnmowers).

Figure 8.3 Proportion of cases (N=124) showing four types of symptoms when left. Des_all_l= Destructive when left, which is the total of destruction not specified, destruction to movable objects, immovable objects and objects impregnated with the owners scent.; Def_o_l= Defecation/urination when left; Voc_l=Vocalisation when left; Sal_l= salivation/hyperventilation/vomiting/ pacing

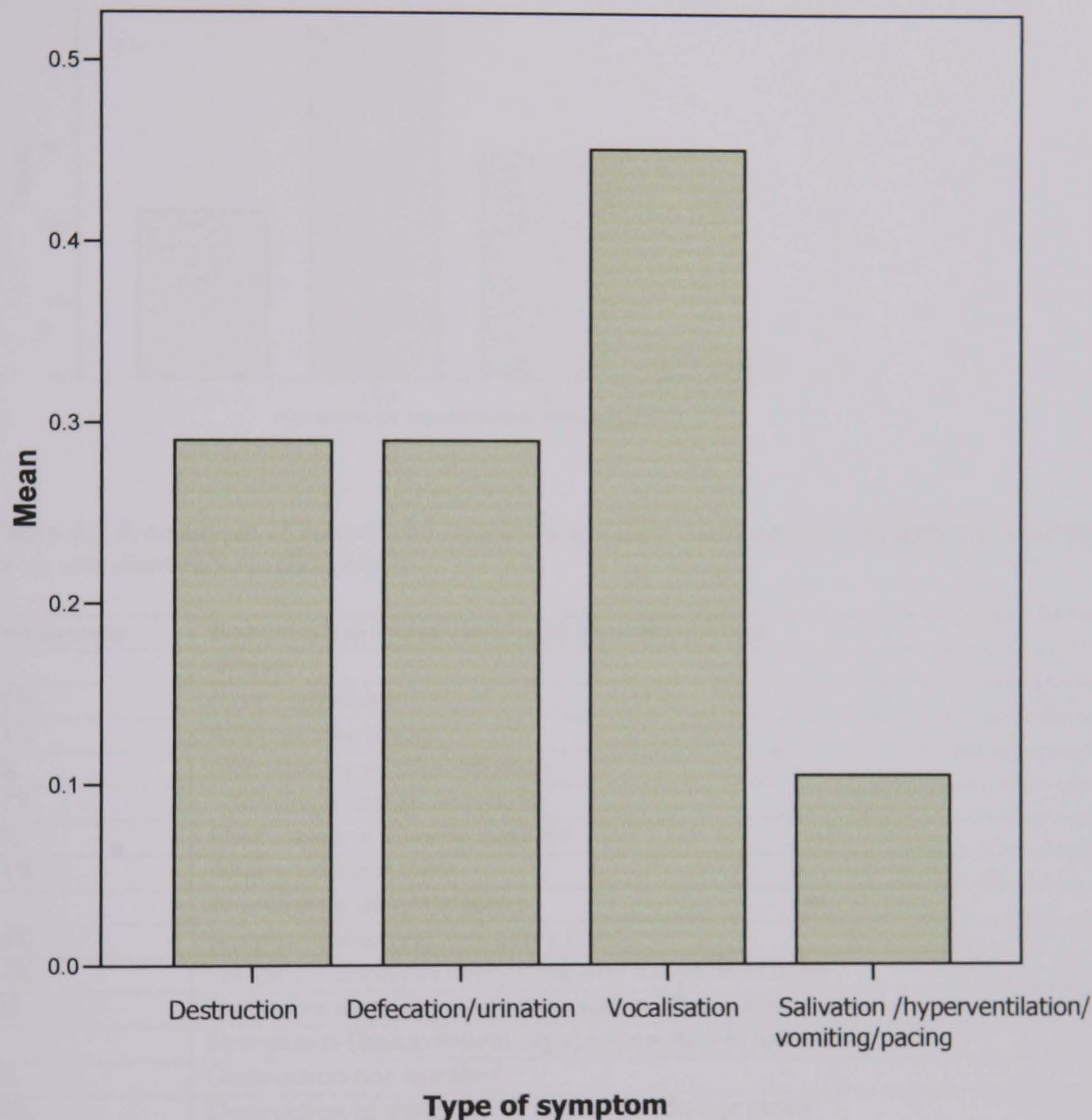


Figure 8.4 Percentage of dogs (N=124) showing, one, two or three combinations of the symptoms destructive behaviour, vocalisation, urination or defecation and salivation/hyperventilation/vomiting/pacing when left.

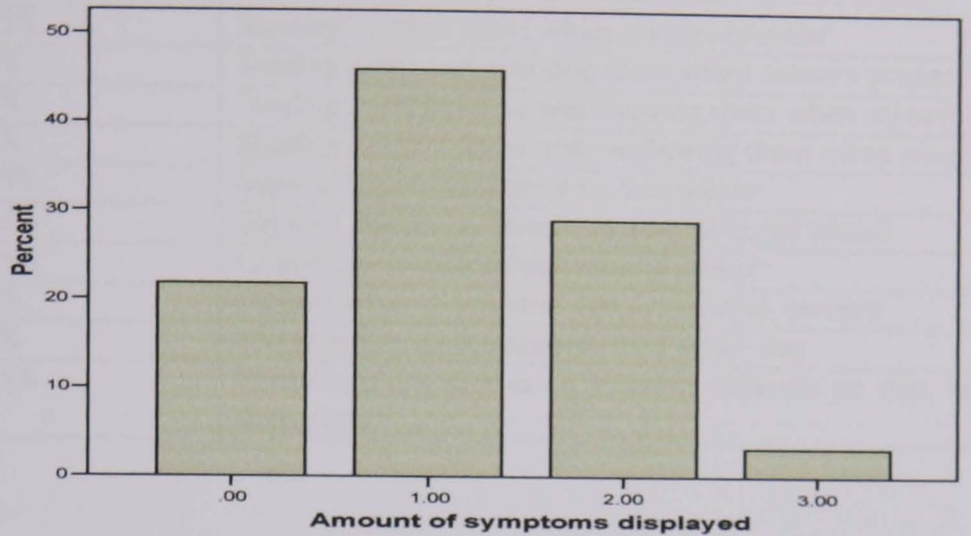


Table 8.3 Frequencies of behavioural symptoms and their characteristics recorded on interview forms and client reports (N=124).

Frequency	Behavioural symptoms and characteristics
	Onset
25%	From puppyhood
11%	After moving house
5%	After leaving another location
17%	Following a change in routine
4%	After illness of a family member
11%	After a noxious event
	Frequency of symptoms
26%	Symptoms displayed not every time
31%	Symptoms displayed every time after unspecified delay
8%	Symptoms displayed every time within 30 minutes
	Symptom (behavioural sign) when dog is left alone
5%	Destruction not specified
19%	Destruction of things in the house that do not move
14%	Destruction of things in the house that do move
13%	Destruction when left in any direction
4%	Destruction of objects impregnated with scent of owner
4%	Taking items that move, without destruction
4%	Taking items, without destruction, impregnated with scent of owner
11%	Salivation/hyperventilation/vomiting/pacing
29%	Defecation/urination only when left
45%	Vocalisation when left
29%	Destruction to door/window as if to follow departing owner
13%	Destruction to other door/window
2%	Digging in garden
13%	Digging into other locations
6%	Digging into objects in the house
7%	Depressed when left (in owner's opinion)
	Symptom (behavioural sign) when owner is present

2%	Tries to escape as owner leaves
1%	Tries to hide as owner leaves
7%	Excessive greeting on owner's return
18%	When present but separated from owner, destruction to internal doors
13%	Aggression to family members as they try to leave
36%	Nuisance behaviours that occur when owners present
15%	Stealing inedible items when owners present
3%	Stealing items and guarding them when owners present
7%	Stealing inedible items and chewing them when owners present
1%	Stealing inedible items and swallowing them when owners present
39%	Signs of hyperattachment to one owner
23%	Signs of hyperattachment to several social stimuli
15%	Organisation of activities around owner
7%	Organisation of activities around several owners
2%	Organisation of activities around other dog
15%	Preference to stay in a location different to that left in by the owner before departure

Co-occurrences of symptoms and signs were investigated by cluster analysis: because of the relatively small sample size the key symptoms were selected, and some symptoms or signs (Table 8.3) were combined into new variables (Table 8.4).

Table 8.4 Variables used for cluster analysis.

Variable name	Symptoms/signs combined in variable	Frequency in %
Onset from puppyhood	*(unchanged)*	25%
Onset after change in routine	Onset following change in routine Onset after illness of a family member	17% 4%
Onset after change in location	Onset after moving house Onset after leaving another location	11% 5%
Onset after a noxious event	*(unchanged)*	*11%*
Symptoms displayed not every time	*(unchanged)*	26%
Symptoms displayed every time	Symptoms displayed every time after unspecified delay Symptoms displayed every time within 30 minutes	31% 8%
Destruction not specified when left	*(unchanged)*	5%

Destruction of things in the house that do not move (when left)	*(unchanged)*	*19%*
Destruction of moveable objects	Destruction of things in the house that do move (when left) Taking items that move without destruction (when left)	14% 4%
Destruction of objects with owner's scent	Destruction of objects impregnated with scent of owner Taking items, without destruction, impregnated with scent of owner	4% 4%
Destruction as if to follow departing owner	*(unchanged)*	*29%*
Destruction when left in any direction not to follow the owner	Destruction when left in any direction Destruction to other door/window	*13%* *13%*
Digging	Digging into objects in the house Digging into other locations	6% 13%
Owner leaving	Tries to escape when owner leaves Aggression to family members as they try to leave	2% 13%
Defecation/urination when left	*(unchanged)*	29%
Vocalisation when left	(unchanged)	45%
Salivation/hyperventilation/vomiting/pacing when left	(unchanged)	11%
Preference to stay in a location different to that left in by the owner before departure	*(unchanged)*	*15%*
Depressed when left (in owner's opinion)	(unchanged)	7%

8.3.2 Patterns of associations between symptoms

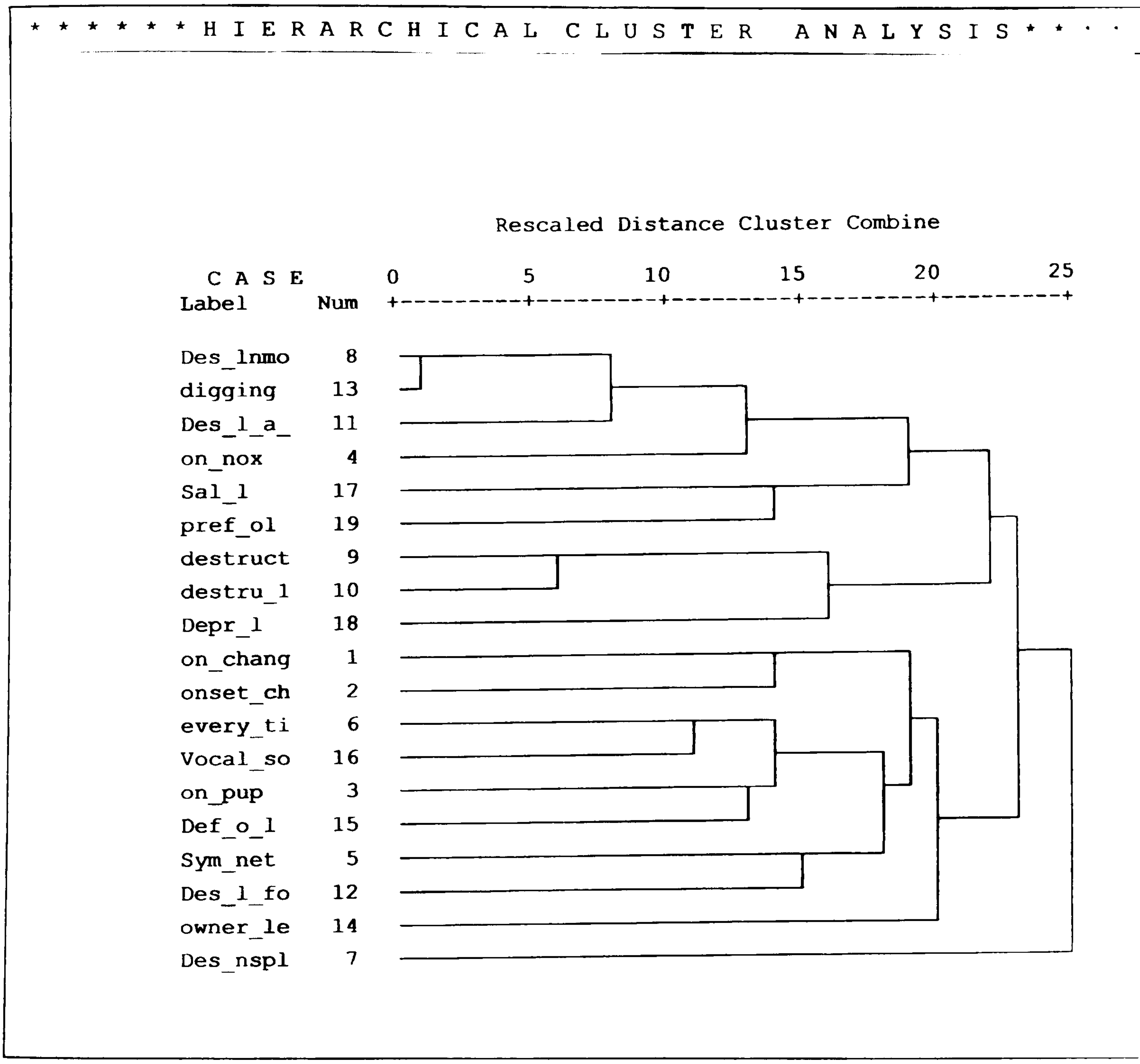
Cluster analyses were used to explore primary patterns of associations between key symptoms (Figure 8.5) in the total sample (N=124). Each cluster was then validated in turn for positive associations by Chi-square. The following symptoms were found to be significantly associated: (1) destruction caused to immovable objects with digging behaviour (Number of co-occurrences N1,1=14, Chi-square= 39.2, P<0.001). (2) destruction caused to immovable objects and digging behaviour (= cluster 1) with destruction caused in any direction (N1,1=10, Chi-square= 29.2, P<0.001). (3) Destruction to movable objects and destruction to objects impregnated with the owner's scent (N1,1=8, Chi-square= 32.8, P<0.001). (4) Salivation and preference to stay in another location (N1,1=6, Chi-square= 10.6, P= 0.001). (5) Onset after change in routine and onset after a change in location (N1,1= 8, Chi-square= 8.49, P=0.004).

In contradiction to what Figure 8.5 might suggest, displaying symptoms every time when left and vocalisation were not statistically significantly associated (N1,1=21, Chi-square= 1.73, P=0.19), nor was onset from puppyhood on with defecation/urination when left (N1,1=13, Chi-square= 3.34, P= 0.07).(Symptoms displayed every time and onset from puppyhood on (N1,1=14, Chi-square= 3.6, P=0.06).

It is inevitable for cluster analysis to simplify associations between symptoms, since the most closely associated symptoms are merged at each step, therefore positive associations between other symptoms were explored using cross tabulations. In addition, the behaviour when the owner is present, being excessive greeting, organisation or activities around one owner, organisation of activities around several social stimuli, hyperattached to one owner and hyperattached to several owners, were added to the analysis. The behaviours when the owner is present were not added as variables to the cluster analysis (Figure 8.5) because of the discussion in the literature about the extent to which they are characteristic for dogs displaying separation problems related to anxiety and fear.

Statistically significant associations were found between: (1) Destruction to immovable objects and objects impregnated with the owner's scent, with excessive greeting (N1,1= 4, Chi-square= 6.1,P= 0.01), (2) destruction to immovable objects and objects impregnated with the owner's scent with onset after a change in routine (N1,1= 8, Chi-square= 5.0, P= 0.03). (3) Vocalisation and onset after a change in routine (N1,1=17, Chi-square= 7.9, P= 0.01). (4) Destruction to immovable objects and digging behaviour and onset after a noxious event (N1,1= 9, Chi-square= 10.8, P= 0.00). (5) Destruction to immovable objects and digging behaviour and symptoms displayed every time (N1,1= 6, Chi-square= 4.1, P= 0.04), (6) Organisation of activities around significant social stimuli and vocalisation (N1,1=13, Chi-square=6.2, P= 0.01). (7) Hyperattached to one owner with vocalisation (N1,1=28, Chi-square= 5.4, P= 0.02). (8) Hyperattached to one owner with organisation of activities around one owner (N1,1= 11, Chi-square: 4.45, P= 0.04). (9) Hyperattached to several owners and onset from puppyhood on (N1,1=11, Pearson Chi-square= 3.94, P= 0.05). (10) Organisation of activities around several social stimuli with excessive greeting (N1,1=2, Pearson Chi-square=4, P= 0.05).

Figure 8.5 Dendrogram representing hierarchical cluster analysis (Jaccard method) of symptoms when the owner is not present.



Key to abbreviations:
Deslnmo: Destruction of things in the house that do not move, Des_l_a= Destruction when left in any direction, on_nox: onset after a noxious event, sal_l= Salivation when left; pref_ol= Preference to stay in a location different to that left in by the owner before departure; destruct= Destruction of objects with owner's scent ; destru_l= Destruction of moveable objects; Depr_l= Depressed when left; on_chang= Onset after change in location; onset_ch= Onset after change in routine; every_ti= Symptoms displayed every time; vocal_so= Vocalisation when left; on_pup= Onset from puppyhood; Def_o_l= Defecation/urination when left; Sym-net= Symptoms displayed not every time; Des_l_fo= Destruction as if to follow departing owner; owner_le= Owner leaving; Des_nspl= Destruction not specified.

8.3.3 Validation of groups

Out of a total of 44 dogs for which the variables onset and frequency of symptoms were available, the 11 dogs that showed onset after a noxious event, eight (73%) did not show symptoms every time they were left (Chi-square = 3.03, P=0.08), which tends to validate group C as being distinct from the other types of cases. In contrast with group A and B dogs, removal of maintenance stimuli from these dogs does not cause a disruption of homeostasis because of the breadth of their maintenance set. Members of this group can, however, become fearful or phobic as a result of experiencing a noxious event e.g. a firework. They only display the symptoms when exposed to the noxious stimulus. The link between the presence of separation problems from puppyhood (N=19) and symptoms occurring every time the dog was left, was weaker (Chi-square=2.32, P=0.13), but does tend to validate group A. A weak association was also found between dogs not displaying the symptoms every time and onset after a change of routine (Chi-square=1.83, P=0.18), one of the criteria for group B. However, there was no relationship between starting to display separation problems after a change in location and displaying those symptoms every time (Chi-square=0.11, P=0.7), so the coherence of group B is uncertain from this data and requires further analysis. The lack of the expected relationship between displaying symptoms every time and change in location suggest that other factors than a sudden loss of maintenance stimuli, or a combination of factors or additional aspects may cause a loss of emotional homeostasis e.g. external stimuli such as noises. Nevertheless, based on the available criteria, 38 of the 44 cases were classified as group A, B or C. Twelve dogs were classified as group A, seventeen as group B, and nine as group C. Six dogs could not be classified with certainty based on the available combination of variables.

8.3.4 Behavioural signs within groups

Digging behaviour is a typical symptom associated with group C as it is interpreted as an attempt of the dog to hide or escape from the noxious stimulus. Of the six dogs that were reported to display digging behaviour when left, five

(83%) were from Group C (Fisher's exact test, P=0.001). Group C was also associated with destruction caused in any direction (Fisher's exact test P=0.001). Only one-third of Group C dogs showed strong attachments to people in their households, compared to approximately three-quarters of dogs in the other groups (Fisher exact test P=0.04). Group C dogs attempt to re-establish emotional homeostasis not by seeking contact with the owner as the maintenance stimulus, or stimuli that are associated with the owner (lying on clothes, digging into the door in the direction the owner has left), but through seeking a place to hide, probably to decrease the intensity of the stimulus or to escape from it. The destruction caused in different directions results from different attempts to find a hiding place or escape route. These links further validate the coherence of Group C. However, no positive links could be found between destructive or attachment behaviour and allocation of dogs to Group A or Group B, which therefore may need further refinement. However, having excluded group C cases (new N=110), some predicted links between destructiveness and attachment could be detected within pooled Group A and B dogs. Specifically, destruction in the direction of the owner's departure was associated with hyperattachment ($N_{1,1}$= 25, Chi-square = 3.9, P<0.05), and destruction in any other direction was associated with attachment to more than one person in the household ($N_{1,1}$= 5, Chi-square = 4.7, P=0.03.)

8.4 Discussion

In this sample, males were outnumbered by females: this was also found in two other populations of dogs displaying separation problems in the UK; in 192 clinical cases (McPherson 1998) and 344 dogs whose owners reported separation-related behaviour when interviewed (Bradshaw *et al* 2002b). The latter study that used a non-clinical population does not have the design constraints that are bound to occur when using a clinical population. In agreement with the clinical population analyzed by McPherson (1998) most dogs in the present study were pure-bred, but this may simply reflect a higher probability for owners of pedigree dogs to seek clinical help. The incidence of displayed symptoms in this study was lower than in McPherson's study, but the frequency of readily identifiable fears was higher. McPherson found an average (median) of two symptoms and one fear in the majority of dogs compared to one symptom and two identifiable fears in the sample used for this study. These differences might be the result of the selection criteria applied to recruit the sample, as McPherson excluded cases in which symptoms are not expressed until more than 30 minutes after the departure of the owner: she also drew her cases from several clinics (including DA) so differences in methods of recording symptoms might be responsible. In contrast to both McPherson and Bradshaw et al, the most frequently recorded symptom in the present study was vocalisation, instead of destruction.

8.4.1 Characteristics and patterns of associations between symptoms of the total population

As predicted in the model proposed for diagnosing separation problems related to anxiety or fear in Appleby and Pluijmakers (2003), these results make it possible to conclude that combinations of symptoms can give a first indication of the cause of the problem behaviour, and can potentially be used to start refining the diagnosis and treatment plan. Statistically significant associations were found between (1) different characteristics of symptoms (2) causes of problem behaviour and behaviour when the owner(s) is present and (3) onset of symptoms and type of symptom.

8.4.1.1 Associations between different characteristics of symptoms

The association of destruction caused to immovable objects with digging, and the subsequent association with causing destructions in any direction; compared to the association between destruction to movable objects and destruction to objects impregnated with the owner's scent, illustrate differences in the strategies dogs use in their attempts to re-establish emotional homeostasis. The latter are trying to regain emotional homeostasis by getting access to objects associated with the maintenance stimulus, being the owner(s), and the other two are trying to escape from the environment or hide in any possible way, instead of trying to regain emotional homeostasis through getting access to maintenance stimuli. Dogs that are hyperattached to one owner appear to use vocalisations as a strategy to call the owner back (Borchelt & Voith 1982, Voith & Borchelt 1985, McCrave 1991, Serpell & Jagoe 1995) to regain emotional homeostasis. This is supported by the association between being hyperattached to one owner and displaying vocalisations as a symptom. The significant association between displaying symptoms every time when left and from puppyhood on illustrates that the development of a primary hyperattachment, during which the attachment to the bitch is transferred to the owner and the dog does not develop independency beyond puberty, may result in the development of separation problems related to anxiety or fear.

The links predicted between destructiveness and dependency on one or several owners was detected in the sample. Specifically, destruction in the direction of the owner's departure was associated with over dependency and destruction in any other direction was associated with dependency on more than one person in the household. In the literature it is suggested that destruction orientated towards doors and windows in the direction the owner has left, is indicative of over dependency and interpreted as an attempt to regain access to the owner (Borchelt & Voith 1982, McCrave 1991). A significant link between over dependency on several owners, resulting in destruction in several directions to regain access to maintenance stimuli, does not appear to have been reported before. It supports the differentiation described in the model (see page 125 -126) between dogs that display separation problem because they have formed a primary hyperattachment or secondary hyperattachment. Primary hyperattachment is described as the continuance of the infant-mother bond that is transferred on to the owner after homing. Secondary hyperattachment, on the contrary, can develop at any age and is not necessarily directed at any one social or non-social stimulus. The higher incidence of fearfulness in the separation sample used in this research, compared to the clinical population analyzed by McPherson, who only included cases which displayed symptoms every time within 30 minutes and are indicative of a hyperattachment (Overall 1997, Landsberg *et al* 2003), might have made this association apparent, although McPherson also found that many dogs are both dependent and fearful. The association between fear and dependency seems to be in line with the higher incidence of dogs vocalising when left alone, as this is usually interpreted as attempts to regain access to the owner (Overall *et al* 1999, Podberscek *et al* 1999).

8.4.1.2 Causes of problem behaviour and behaviour when owner is present

Analysis of the display of dependency behaviour of the dog when the owner is present have been shown to be possibly associated with the cause of the behaviour. Hyperattachment to one owner was statistically significant associated with organisation of activities around one owner; and organisation around several social stimuli with excessive greeting. Excessive greeting and organisation of activities around one owner might thus be an indication that problem behaviour is caused by the loss of one or more social maintenance stimuli, namely the owner(s).

The links predicted between destructiveness and dependency on one or several owners was detected in the sample of pooled group A and B dogs. Specifically, destruction in the direction of the owner's departure was associated with over dependency, and destruction in any other direction was associated with dependency on more than one person in the household. In the literature it is suggested that destruction orientated towards doors and windows in the direction the owner has left, is indicative of over dependency and interpreted as an attempt to regain access to the owner (Borchelt & Voith 1982, McCrave 1991). A significant link between over dependency on several owners, resulting in destruction in several directions to regain access to maintenance stimuli, does not appear to have been reported before. It supports the differentiation described in the model (see page 125 -126) between dogs that display separation problems because they have formed a primary hyperattachment or secondary hyperattachment. Primary hyperattachment is described as the continuance of the infant-mother bond that is transferred on to the owner after homing. Secondary hyperattachment, on the contrary, can develop at any age and is not necessarily directed at any one social or non-social stimulus. The higher incidence of fearfulness in the separation sample used, compared to the clinical population analyzed by McPherson, might have made this association apparent, although McPherson also found that many dogs are both dependent and fearful. The association between fear and dependency seems to be in line with the higher incidence of dogs vocalising when left alone, as this is usually interpreted as attempts to regain access to the owner (Overall *et al* 1999, Podberscek *et al* 1999).

8.4.1.3 Onset of symptoms and type of symptom

The combination of time of onset of symptoms and type of symptoms can give a first indication of the cause of the problem behaviour. Onset after a change in location is statistically significant associated with a change in routine suggesting that both coincide often, for example as a result of a house move with the owner.

The high percentage of dogs that start to display problem behaviour after a change in routine is noteworthy. Examples include, following a period of dog or owner illness, after summer holidays, change in daily schedule. Symptoms are often indicative of attempts to re-establish emotional homeostasis that involve stimuli associated with the owner(s), such as, taking/destruction of objects impregnated with the owner's scent and vocalisation, which, in the literature, is often interpreted as an attempt to restore or maintain contact with the owner (Borchelt & Voith 1982, Voith & Borchelt 1985, McCrave 1991, Serpell & Jagoe 1995, Bradshaw *et al* 2001). This is in line with the prediction made in the model of the role of emotional homeostasis in the development of separation problems related to anxiety and fear, described in Chapter 7, that the composition of the maintenance set remains flexible during the life of the individual (Cairns 1966, Appleby & Pluijmakers 2003), and that the value of a stimulus in the maintenance set is determined by factors such as duration of exposure and amount and quality of interaction (Cairns 1966, Appleby & Pluijmakers 2003). Increased exposure to maintenance stimuli because of a change in routine (e.g. holiday period) may result in an increase in the salience of the stimulus to such an extent that removal of the stimulus causes a loss of emotional homeostasis. In addition, a loss of maintenance stimuli because of being placed in another environment may result in an increased importance of and reliance on social maintenance stimuli e.g. the owner. The fact that the symptom vocalisation is associated with organisation of activities around a significant social stimulus and vocalisation is statistically significant associated onset after a change of routine seems to strengthen the assumption that the maintenance set stays flexible during an individual's life, and changes in the composition of the maintenance set may increase the need for other maintenance stimuli.

The association between onset after a noxious event and digging behaviour and destruction of immovable objects in any direction supports the hypothesis that in these cases the behavioural strategy for re-establishing emotional homeostasis of Group C dogs is one of escape from the environment or to try to hide to decrease the intensity of an occurring or predicted stimulus in the context of the owner's absence.

8.4.2 Characteristics and patterns of associations between symptoms of the groups

8.4.2.1 Group C

The data have demonstrated that dogs categorised as being in group C stand out from the total sample because they show the most significant relationship between the variables onset of behaviour and frequency, type and direction of destruction, and (lack of) over-attachment to people. As was hypothesized for these dogs, loss of emotional homeostasis is related to the occurrence of actual noxious stimuli e.g. fireworks, thunderstorms. They have a maintenance set that is adequate for coping with separation from their owner(s) and will only display the symptoms when its effect is challenged by exposure to a fear-eliciting event. Significantly, they may display a reaction to the noxious stimulus when the owner is present or even use the same or another coping strategy in the presence of the owner. However, this might be displayed to a lesser extent, because the presence of the owner(s) improves the quality of the maintenance set and increases the capacity to maintain emotional homeostasis. However, these dogs are not over-dependent upon their owner(s) and therefore no link with dependency was expected.

When left alone, the coping strategy of dogs in this group seems to be directed towards decreasing the intensity of the frightening stimulus; for example, by seeking a hiding place by digging into immovable objects, or escaping in any direction from the environment in which the noxious stimulus is perceived to occur, instead of trying to gain access to maintenance stimuli. Co-occurrence of destruction to immovable objects, destruction in any direction and digging shows that these behaviours can be alternatives but may also co-occur in the same dog. Other authors (Overall *et al* 2001) also indicate that dogs displaying symptoms of separation anxiety frequently show signs of noise phobia or thunderstorm phobia. Overall (1998) found that 40% of the dogs with a noise phobia and 8% with a thunderstorm phobia also had separation anxiety. Systematic desensitisation and counter-conditioning responses to fear-eliciting stimuli using CDs with high quality recordings form an essential part of treatment. In addition, the extent to which the dog's response to its maintenance set is disrupted by anticipation and or generalisation to other stimuli has to be evaluated and treated if necessary. These dogs may also try to seek more effective maintenance stimuli.

8.4.2.2 Group B

It was hypothesised that, in contrast to dogs in group A, which have not learned independence, dogs in group B have learned independence but have lost it at some point due to a change in the effectiveness of the maintenance set. For these dogs loss of emotional homeostasis can be caused by apprehension due to the loss of one or several significant social stimuli or familiar surroundings. In the absence of sufficient maintenance stimuli to maintain emotional homeostasis, the threshold for fear and separation distress may be considerably lowered, which might result in a highly aversive and generalized fear towards the environment (Harlow & Mears 1979).

The need for maintenance stimuli for dogs in group B may also be related to presence or anticipation of noxious stimuli. A dog that has previously had an appropriate maintenance set to cope with separation may learn to fear being left alone and show signs of anticipatory anxiety at times when it expects to be left. As a result, the animal might develop an increased dependency upon the owner(s) and transfer from group C to B and start to display separation anxiety every time it is alone (Lindsay 2000a). In addition, for dogs in group B, separation anxiety may result from a change in the value of individual stimuli in the maintenance set. The onset of symptoms of separation anxiety is common after periods of prolonged contact with family members e.g. after the children go back to school, or the owner goes back to work after a period of illness (Lindsay 2000a).

No association was found between timing of the onset of symptoms and frequency for the total group of dogs that had been hypothesised as belonging to group B. A possible explanation for this could be that owners have difficulty consistently recalling the exact onset of the symptoms. This could occur because owners have variable tolerance towards the problem behaviour and variable attitude towards the severity of the problem for the dog (Bradshaw *et al* 2002a).

To formulate a successful treatment program for group B-dogs, balance in the maintenance set has to be recovered by (1) reducing over dependence on the one or several stimuli, (2) reintroducing maintenance stimuli or (3) the introduction of new maintenance stimuli or (4) a combination of these. If applicable, the effect of a possible fear-eliciting stimulus has to be removed or reduced using systematic desensitisation and counter conditioning techniques. Dogs motivated by frustration are often successful social manipulators because they have learned that persistence in the face of non-reward and punishment is effective. Modifying their manipulative separation behaviour and substituting it with more obedient and cooperative behaviour is often an essential aspect of the treatment (Lindsay 2000b).

8.4.2.3 *Group A*

Dogs displaying the symptoms every time have previously been categorised as group A, i.e. where primary hyperattachment, defined as the continuance of a bond to an individual beyond puberty (Gaultier 2001), results in separation anxiety. Virtual or actual separation from the owner elicits anxiety, which increases the potential for fear to stimuli causing or associated with threat. In this study there was a trend for correlation between the presence of separation problems from puppyhood and symptoms occurring every time these dogs were left, although this was not as close as expected. Dogs belonging to group A were thought to cause destruction in the direction that would allow them to follow the owner because they are over dependent on them as a maintenance stimulus. It is often suggested in the literature that vocalization is an attempt to call the owner back (Overall *et al* 1999). This might, for dogs in group A, be a more common symptom.

Treatment primarily requires a reduction of the dependency on the owner and increasing dependence on other social and non-social stimuli. It is often appropriate for treatment to be phased. If fear of a stimulus coexists, this should be treated separately and consideration should be given to doing so before treating the anxiety resulting from the owners' absence.

8.5 Conclusion

The results support the model developed by Appleby and Pluijmakers (2003) which suggests that the population of dogs displaying separation problems related to anxiety and fear consist of subpopulations which differ in the cause of the loss of emotional homeostasis, symptoms displayed and behaviour when the owners are present.

The significant associations, found between (1) different characteristics of symptoms, (2) behaviour displayed when the owners are present and cause of the problem behaviour and (3) the onset of symptoms and type of symptoms, illustrate that they can be used to start diagnosis and refining the treatment plan. As predicted by the model (Table 8.1) significant associations have been found between symptoms or behavioural signs within group A, B and C. However the composition of group B needs more refinement. The methodology chosen might have limited the associations found. The aim of a treatment report and interview form is primarily to develop a diagnosis and treatment plan for the owner, and hence not all symptoms or details of symptoms might be written down. In addition the owners might not have reported all symptoms because they assume they are irrelevant to the problem or are not aware of them: this will be particularly true of separation disorders, in which by definition the key behaviours take place in the owner's absence and hence are usually unobserved.

Chapter 9: Early experiences and the development of separation problems related to anxiety and fear

9.1 Introduction

Surprisingly little is known about the impact of early experiences on the development of separation related behaviour problems (Serpell & Jagoe 1995). Research into the relationship between the developmental effects of a dog's early experiences and the appearance of behaviour problems later in life has been relatively limited (Appleby 2000, Serpell & Jagoe 1995), although it is generally accepted that there exists a sensitive period in early development during which experiences have a greater effect on establishing canine temperament and behaviour than those that occur in later life (Serpell & Jagoe 1995, Overall 1997).

The most important factors that have an effect on the level of experience a puppy receives are thought to be (i) the maternal environment, (ii) the extent of exposure to novel stimuli whilst in and after leaving the maternal environment and (iii) the age at which the puppy is moved from the maternal environment. The age at homing may affect development in its own right (i.e. the disruption itself may be more or less traumatic depending upon the age at which it happens), and it will also determine the quantity of exposure to stimuli received in both locations (summarized in Appleby 1999).

Jagoe (1993) conducted a retrospective survey of the owners of 737 adult dogs to investigate the possible long-term effects of early experience on the development of dog behaviour problems. He found that in separation-related behaviour problems, there was no significant relationship between the prevalence of any separation related behaviour problem and the source the puppy was obtained from, the age at which the puppy was obtained and the age at which the puppy was first taken out on a regular basis to busy urban environments. There was a significantly higher prevalence of separation-related barking in puppies that had been ill than in puppies that had remained healthy. Also, puppies first vaccinated between five to eight weeks had a significantly lower prevalence of separation-related destructiveness, while pups first vaccinated between nine and twelve weeks had a significant higher prevalence.

Several factors may play a role in the development of separation problems related to anxiety and fear. Firstly, it may depend upon the extent to which emotional reliance upon a stimulus or stimuli is formed. Whether a stimulus becomes part of a maintenance set and the degree to which dependency upon it develops is influenced by cue salience, duration of exposure, context (Cairns 1966), the stimulation the object provides (Cairns 1966, Gross 1996, Gubernick 1981) and the level to which the maintenance set has developed and already enabled behavioural organization (Scott 1962). Experiments at Bar Harbor showed that from around three weeks of age puppies become severely distressed when separated from their mother, littermates and nest site (Elliot & Scott, 1961, Scott 1962), e.g. when placed alone in a strange situation, and that the level of distress rises to a peak at around 7 weeks, after which it gradually declines. Further data support the concept that animals tend to remain in the presence of stimuli to which they have been exposed (Boissy 1995).

Secondly, exposure to experiences and learning to cope during early development may reduce emotionality when exposed to novel or challenging stimuli, by reducing dependency on the initially narrow and salient stimulus set necessary for the maintenance of homeostasis and behavioural organization, consisting mainly of the bitch, littermates, the nestsite and the breeder's family when the puppy grows up in a domestic maternal environment. Consequently, it is suggested that the canine population can be divided into three groups, termed A, B and C, according to the maintenance stimuli they depend on, a process which is influenced by (1) the degree of dependence on and salience of existing stimuli, (2) the availability of existing stimuli and (3) how these factors compare with the properties of new stimuli (Chapter 7, Appleby & Pluijmakers 2003).

Appleby *et al* (2002) conducted a study into the early experiences of dogs displaying symptoms of avoidance behaviour or aggression, and compared them with a clinical population of dogs that did not show such behaviour. The behavioural symptoms were tested for their association with the dog's maternal environment, the environment it experienced between three and six months of age and the age at which it was obtained. The main findings were that both a non-domestic maternal environment and no exposure to busy urban environments after vaccination significantly increased the likelihood of the development of problems relating to avoidance behaviour and fear related aggressive behaviour towards unfamiliar dogs and people. The relative effect of maternal environment, environment after vaccination and the age at which the puppy was obtained interacted; in general, the longer a puppy was in an impoverished environment, the more likely it was to show avoidance or fearful behaviour later in life. Obtaining puppies from a domestic environment prior to eight weeks of age and exposing them to busy environments before the end of the socialisation period was recommended by the authors as the best procedure for avoiding problem behaviours related to fearfulness and aggression towards people.

The purpose of the study described in this chapter was to subject the early life experiences of dogs displaying separation problems related to anxiety and fear to quantitative analysis, based on an analysis of clinical records similar to that conducted by Appleby *et al* (2002). Based on the work done by Appleby *et al* (2002) one could hypothesise that puppies which grow up in a deprived maternal kennel environment and do not receive regular exposure to busy urban environments before the end of the socialisation period, might also have a higher risk for developing separation problems related to anxiety and fear, even though the link between separation anxiety and fear is inadequately understood (Overall 1997). These factors could be influenced both by the age of homing of the puppy and the complexity of the environment after homing, as these will determine the amount of social maintenance stimuli that are available to the dog.

It was hypothesised that (1) puppies that are raised in a non-domestic maternal environment should show a higher incidence of separation problems related to anxiety and fear compared to puppies raised in a domestic maternal environment, due to their limited exposure to a variety of stimuli in a non-domestic maternal environment. Exposure to unfamiliar stimuli after homing might therefore be more aversive for puppies raised in a non-domestic maternal environment compared to puppies growing up in a domestic maternal environment. In addition, the dependency on the bitch and littermates might be higher compared to puppies exposed to a broader range of maintenance stimuli in a domestic environment, making the separation more aversive. The possible higher level of negative emotional arousal experienced during and after homing might increase the need for maintenance stimuli to provide a sense of security, and consequently strengthen the dependency on them. The repeated experiences of a negative emotional state when left alone could subsequently sensitize the puppy, resulting in an increased need to have access to the maintenance stimulus, and increased level of distress experienced when separated. (2) Puppies that have not been exposed to busy urban environments between three and six months of age at a regular basis should show a higher incidence of separation problems related to anxiety and fear, as exposure to a wide variety of stimuli increases the possibility for the dog to get familiar with the stimuli exposed to which can be protective against the development of fear- and anxiety based separation problems. (3) Dogs both raised in a non-domestic maternal environment and not exposed to busy urban environments between three and six months of age on a regular basis, should show the highest incidence of separation problems related to anxiety and fear. (4) The longer a puppy stays in a deprived non-domestic maternal environment the more likely it will be to develop separation problems related to anxiety and fear because of the higher chance of exposure to unfamiliar stimuli which might cause a fear reaction and the increased need for support from maintenance stimuli, the most likely being the owner(s).

If not being exposed to a variety of stimuli in the maternal and post vaccination environments is an important predisposing factor for the development of separation problems related to anxiety or fear, it is further predicted that (5) puppies in Group A should be over-represented in those dogs in the separation sample which have experienced non-domestic maternal environments and non-urban environments, and have displayed symptoms of separation problems from puppyhood on. (6) Dogs in group C, which only display symptoms when they are exposed to the actual noxious stimulus that they are fearful of, are predicted to be more likely than dogs in Group A to have received a domestic maternal environment and exposure to urban environments. The early environment typical of Group B is more difficult to predict, as some of this group consists of dogs that transferred from group C to B as a result of learning. Other Group B dogs might also actually be Group A dogs, which are mis-classified as B because their owners did not initially identify the separation problem, or because the negative influences of the deprived maternal environment did not become apparent until later. Therefore, no specific hypothesis for early experiences of Group B was formulated other than that (7) no specific association was expected. The relationship between symptoms and maternal environment, age obtained and exposure to busy urban environments post-vaccination are explored. No predictions were made as it was expected for these to interact and to be influenced by many other factors which can not be examined in detail using the available data (e.g. amount of time spent alone during the day, living environment when problem behaviour started.)

9.2 Materials and methods

Three groups of dogs were used, the clinical population described in Chapter 8, a control group and a comparison group. The control group consisted of dogs for which information was collected using questionnaires distributed through veterinary practices. The comparison group was formed from clinical cases displaying behaviour problems unrelated to anxiety or fear.

9.2.1. Clinical cases

A clinical population consisting of the 124 clinical cases seen by David Appleby (DA) between 1999 and 2004 for separation problems related to fear and anxiety, subjected to certain exclusion criteria was used. Separation problems might result from many factors. However, there are causes that are unrelated to anxiety such as boredom, reacting to stimuli outside the house, play behaviour, house breaking problems (McCrave 1991). These cases were excluded by DA as they are not supposed to be influenced consistently by the maternal environment, age at homing and exposure to urban environments (Appleby *et al* 2002). Only cases in which the dog belonged to the first owner or breeder, and the owner could recollect the age of homing of the puppy, and had seen the maternal environment of the puppy, were included. A cut-of point of twenty-eight weeks at the time puppies were obtained was applied to avoid distortion of the analysis by a small sample of puppies obtained from the breeder much older than the majority of the sample. The age of twenty-eight weeks was based on the cut of point that had been used by DA for a population in a prior research project. The same population was used in this project as the comparison group.

During the consultation the client's recollection of the age at which the puppy was obtained was recorded in weeks. The environment from which the dog was obtained was recorded as either domestic, meaning living in a residential part of the breeder's home, or non domestic, i.e. living in a kennel, garage, barn or shed. The clients' answers to questions about whether their puppy had been exposed to busy urban environments on a regular basis between three and six months were inspected. Dogs that had been exposed either to busy urban environments, or all their constituent elements at different times, such as walks along a busy road and car boot sales, were classed as exposed; others that had experienced none or only a few of the elements of urban environments, for example traffic but not large groups of people, were classed as unexposed. There was inevitably an element of subjectivity in these classifications, but these had been made by DA prior to the conception of this study, so they should have been unbiased. The number of adults, number of children and other dogs in the household, the age at which the dog was seen, and the symptoms displayed, were also recorded (see Chapter 8).

9.2.2 Control group

To recruit a control group a convenience sampling strategy was used. Questionnaires (see Appendix 5) were distributed to 40 veterinary practices in the UK with a covering letter (see Appendix 4) asking for the questionnaires to be given to owners attending the practice with dogs between 6 months and 8 years of age. From the questionnaire it was possible to determine:

- The age the puppies were obtained, the environment obtained from, their breed and gender; the age of the dog at the time the questionnaire was completed, and the way the dog was housed.
- The composition of the household of the owners: how many adults, children and other dogs and their living situation.
- If the dog, when left alone, would, on a regular basis, display any of the following behaviours: barking, howling, destruction, scratching that results in damage, or inappropriate defecation or urination in a dog that is otherwise housetrained. Dogs displaying any of these behaviours were excluded from the sample as the behaviours may be symptomatic of separation problems.

One hundred and sixty two forms were returned and 84 analysed. Forty six were discarded because the owners were not the first owner and another 32 were discarded because they were not completed correctly.

9.2.3 Comparison group

As a comparison group a subset of clinical cases seen by David Appleby between May 1996 and June 1999 that did not show problem behaviour related to fear and anxiety was used. This group (N=82) showed behaviour problems associated with a lack of control or attention seeking behaviour (e.g. pulling on the lead, recall problems, excitability, digging, chewing/scratching at objects when owners present). These problems are unlikely to be considerably influenced by the dog's maternal environment, age at homing or exposure to busy urban environment, as these problems are primarily due to reinforcement by the reaction of the owners when they are present (Landsberg *et al* 1997, Overall 1997) or are the result of inadequate or inappropriate training (Seksel *et al* 1999). The validity of the comparison group was established by comparing the environment they were obtained from and the age at homing with the control group.

9.2.4 Statistical analysis

For the comparison group a purposive sampling strategy was used, the criteria being a clinical population that did not show behaviour problems related to anxiety or fear. A sample recruited using this sampling strategy is not representative of the general population, since owners who seek help from a behaviour counsellor are a minority and therefore likely to be atypical. In addition, the convenience sampling strategy used to recruit the control group may not necessarily represent the general population, since not all dog owners attend veterinary surgeries, and possibly only the more responsible of these might complete a questionnaire. No "ideal" control group could be recruited, and as a result there was a risk of the analysis being confounded by the use of comparisons between populations with different characteristics. To investigate how likely this is, the control and comparison groups were compared. Chi-square tests were used to compare the control group with the comparison group for maternal environment and exposure to busy urban environments. Mann-Whitney U tests were used to compare the median ages of the dogs and the ages at which they had been obtained by their current owners. The clinical cases were compared with the control group for the maternal environment, age obtained and family composition after homing. For the effect of exposure to busy urban environments after vaccination and association between maternal environment and exposure to urban environment the clinical cases were compared with the comparison group. The relationship between symptoms and maternal environment, age at homing, and exposure to busy urban environments after vaccination was explored using the clinical cases, control and comparison groups using nominal regressions. If these at least approached significance (one or more terms $P<0.10$), Chi-square tests on contingency tables were used to test the effects of the two environments, and Kruskal-Wallis and Mann-Whitney tests to test the age at homing.

9.3 Results

9.3.1 The comparison and control group

There was no significant difference between the maternal environment the puppies in the comparison and control groups had been obtained from (P= 0.36) (Table 9.1). The control group was obtained at a slightly younger age (Fig. 9.1) but the median age for both groups was 8 weeks (Mann-Whitney U= 3031, P= 0.71).

9.3.2 Early experiences

9.3.2.1 Maternal environment

The separation group was tested against the control group and the comparison group for the environment they were obtained from. The separation group was slightly over represented in the domestic maternal environment, but no statistically significant relationship was found between the display of separation problems related to fear and anxiety and exposure to a domestic or non-domestic maternal environment. Fifty three percent (N= 66/124) of the dogs displaying separation problems were obtained from a domestic maternal environment, compared to 42% (N=35) from a domestic environment in the control group (P= 0.10). In the comparison group 48% of the dogs (N=40/82) were obtained from domestic maternal environment (P= 0.53) (Table 9.1). No evidence was found that non-domestic maternal environments predisposed these dogs towards such disorders.

Figure 9.1 Boxplot illustrating the age obtained of the separation, comparison and control group.

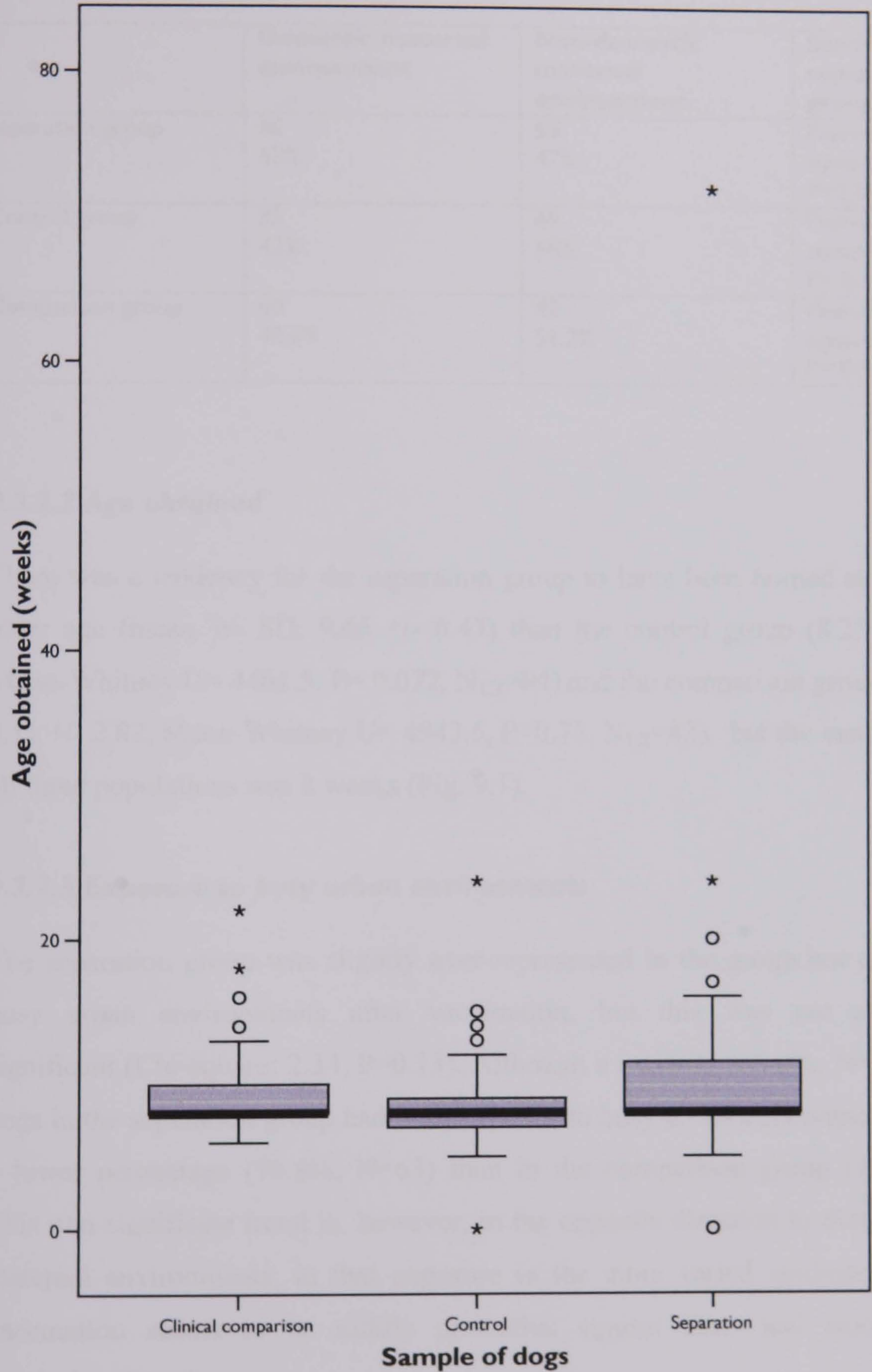


Table 9.1 Maternal environments of the separation, control and comparison group.

	Domestic maternal environment	Non-domestic maternal environment	Statistics cf. separation group
Separation group	66 53%	58 47%	Pearson Chi-square: 2.68 P=0.26
Control group	35 42%	49 58%	Pearson Chi-square: 2.68 P= 0.10
Comparison group	40 48.8%	42 51.2%	Pearson Chi-square: 0.39 P= 0.53

9.3.2.2 Age obtained

There was a tendency for the separation group to have been homed at a slightly older age (mean +/- SD: 9.65 +/- 6.43) than the control group (8.25 +/- 3.39, Mann-Whitney U= 4461.5, P= 0.072, $N_{1,2}$=84) and the comparison group (mean = 9.11 +/- 2.82, Mann-Whitney U= 4943.5, P=0.73, $N_{1,2}$=82), but the median age of all three populations was 8 weeks (Fig. 9.1).

9.3.2.3 Exposure to busy urban environments

The separation group was slightly over-represented in the group not exposed to busy urban environments after vaccination, but this was not statistically significant (Chi-square: 2.34, P=0.13). Although a majority (66.9%; N=83) of the dogs in the separation group had been exposed to busy urban environments, this is a lower percentage (76.8%, N=63) than in the comparison group (Table 9.2). This non-significant trend is, however, in the opposite direction to that found for maternal environments, in that exposure to the more varied environment post-vaccination seems to be mildly protective against fear- and anxiety-based separation disorders.

Table 9.2 Exposure to busy urban environments in the comparison group and separation group.

	Exposed to busy urban environments	Not exposed to busy urban environments
Separation group	83 66.9%	41 33.1%
Comparison group	63 76,8%	19 23.2%

9.3.2.4 Maternal environment and exposure to urban environments

The data for the separation group and the comparison group was analysed using cross tabulations to test for the relationship between the factors maternal environment and exposure to busy urban environments by the new owner after completion of vaccination. No significant association was found (Pearson Chi-square: 3.305, P= 0.35, d.f.=3) (Table 9.3) between the four possible combinations of environment, and the development of separation problems related to anxiety or fear. No particular combination of environments pre- and post-vaccination therefore appears to be especially protective or predisposing.

Table 9.3 Relationship between domestic maternal environment (Dom-env), non-domestic environment (Non-dom-env), exposure to urban environment (Exp. urban env.) and no exposure to urban environments or equivalent (no exp. urban env.) between the separation group and the comparison group.

Group	Dom-env + Exp. urban env	Non-dom-env + Exp. urban env	Dom-Env + No exp. urban env	Non-Dom-Env + No exp. urban env
Separation group (N=124)	46 37.1%	37 29.8%	20 16.1%	21 18.1%
Comparison group (N=82)	30 36.6%	33 40.2%	10 12.2%	9 11%

9.3.2.5 Age obtained, maternal environment and exposure to urban environments

The age of homing influences the duration of exposure to pre- and post-homing environments, therefore the separation group was compared with the control and comparison groups for age obtained and maternal environment, and for age obtained and exposure to busy urban environments using the comparison group. The age obtained was split into the categories obtained before 8 weeks, at 8 weeks and after 8 weeks. The majority of puppies from all three groups were obtained at 8 weeks (average 36%) or after 8 weeks (average 40%) whereas a minority had been obtained before 8 weeks of age. The differences between ages of homing on their own were not statistically significant between the three groups (Pearson Chi-square= 2.002, d.f.=4, P= 0.74) (Table 9.4).

When the effects of maternal environment were tested for puppies homed at different ages, puppies that grew up in a domestic maternal environment and were homed before eight weeks were statistically significantly more likely to develop separation problems related to anxiety and fear when tested against the control group (Chi-square= 3.89, P=0.04, d.f.= 1) but not against the comparison group (Chi-square= 0.43, P=0.51), and the equivalent test including all three groups was non-significant (Chi-square: 3.96, d.f.= 2, P=0.14). When they were homed at 8 weeks (Chi-square= 0.11, d.f.= 2, P=0.94) or after 8 weeks (Chi-square: 0.87, 2 d.f., P=0.65), all three groups were very similar in the proportion of separation cases (Table 9.5).

Table 9.4 Cross tabulations of frequencies of age obtained before 8 weeks, at 8 weeks and after 8 weeks. (Pearson Chi-square= 2.002, P= 0.74)

Group	**Homed before 8 weeks (N=68)**	**Homed at 8 weeks (N=105)**	**Homed after 8 weeks (N=117)**
Comparison group	18 22.0%	30 36.5%	34 41.5%
Control group	24 28.6%	30 35.7%	30 35.7%
Separation group	26 21.0%	45 36.3%	53 42.7%

Table 9.5 Relationship between age of homing and domestic maternal environment in the control, comparison and separation groups

Age at homing	Group	Domestic environment	Non-domestic environment
Before 8 weeks	Control group (N=24)	9 37,5%	15 62,5%
	Comparison group (N=18)	10 55.6%	8 44.4%
	Separation group (N= 26)	17 65.4%	9 34.6%
At 8 weeks	Control group (N= 30)	15 50%	15 50%
	Comparison group (N=30)	15 50%	15 50%
	Separation group (N=45)	24 53.3%	21 46.7%
After 8 weeks	Control group (N= 30)	11 36.7%	19 63.3%
	Comparison group (N=34)	15 44.1%	19 55.9%
	Separation group (N= 53)	25 47.2%	28 52.8%

The effect of exposure to busy urban environments appeared to be independent of age of homing: slightly more dogs in the separation group had not been exposed to such environments than in the comparison group, irrespective of whether the dogs had been homed before (Pearson Chi-square= 1.13, d.f.= 1, Fisher's Exact Test= 0.48), at 8 weeks (Pearson Chi-square=0.65, d.f.=1, Fisher's Exact Test= 0,57) or after 8 weeks of age (Pearson Chi-square= 0,74, d.f.= 1, Fisher's Exact Test= 0.5) (Table 9.6).

Table 9.6 Relationship between age of homing and exposure to busy urban environments for comparison and separation groups.

Age at homing	Group	Exposed	Not exposed
Before 8 weeks	Comparison group (N=18)	15 83.3%	3 16,7%
	Separation group (N= 26)	18 69.2%	8 30.8%
At 8 weeks	Comparison group (N=30)	25 83.35	5 16.7%
	Separation group (N=45)	34 75.6%	11 24.4%
After 8 weeks	Comparison group (N=34	23 67.6%	11 32.4%
	Separation group (N= 53)	31 58.5%	22 41.5%

9.3.3 Early environment of Groups A, B and C

Links between membership of groups A, B or C and early environment were explored using a nominal regression model, with group membership as the dependent variable, and exposure to a domestic or non-domestic maternal environment, and being or not being exposed to busy urban environment after vaccination as factors, and age of homing and age at consultation as covariates. The model approached significance (P=0.12) with age at consultation and post-vaccination environment the factors most likely to be linked to group membership (Table 9.7).

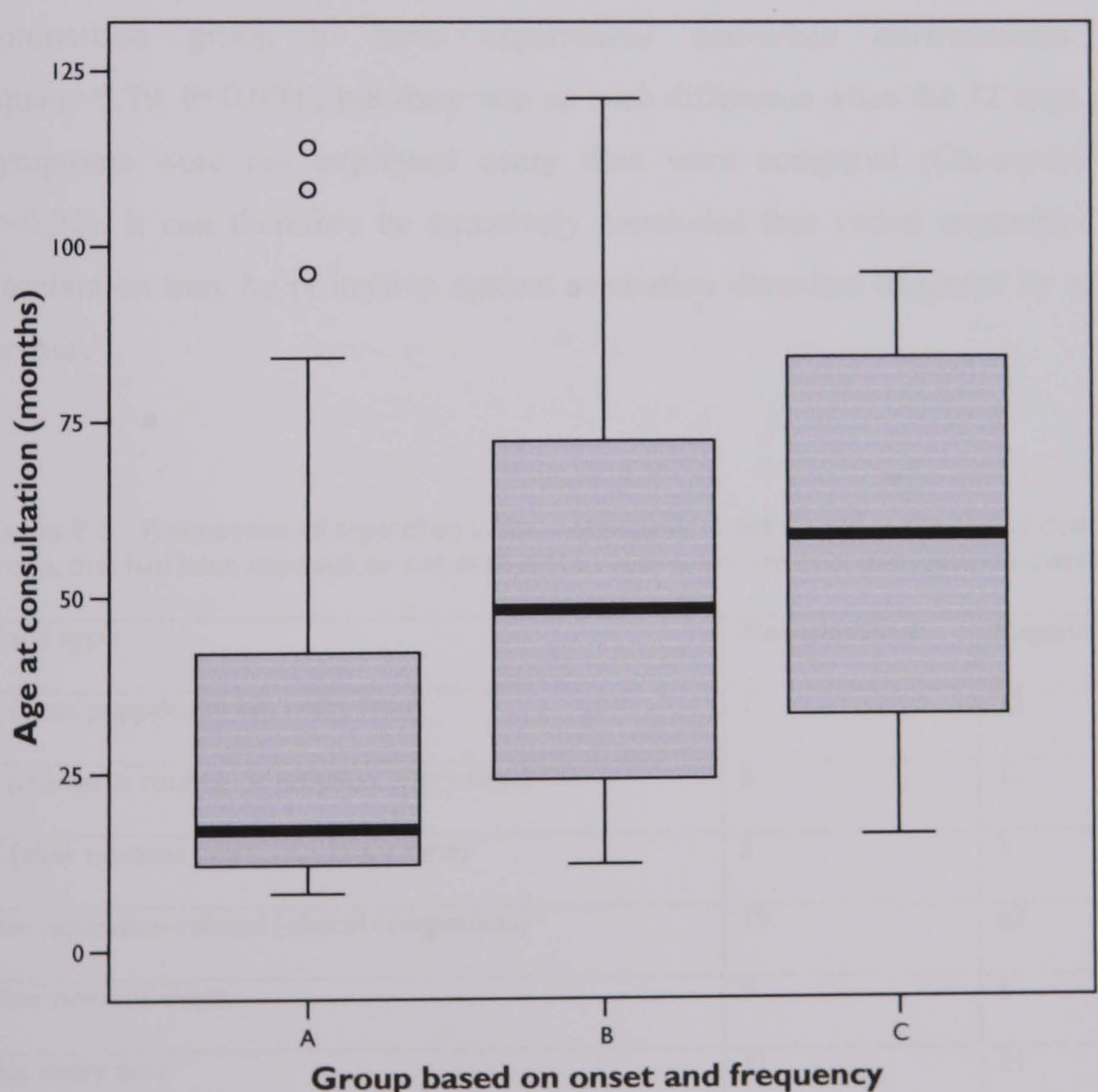
Table 9.7 Chi-square statistics from nominal regression of the effects of four factors on membership of type A, B or C separation disorders.

Effect	Chi-square	d.f.	P
Model	12.91	8	0.12
Age of homing	1.56	2	0.46
Age at consultation	5.51	2	0.06
Maternal environment	0.67	2	0.71
Post-vaccination environment	4.25	2	0.12

Removal of age of homing and maternal environment from the model improved the overall fit (Chi-square = 10.16, d.f.= 4, P=0.04) but not the fit with post-vaccination environment (Chi-square = 4.18, d.f.= 2, P=0.12).

The age at consultation was lower for group A (onset from puppyhood) (Fig. 9.2), as expected, since the delay between onset and consultation is likely to be reasonably constant.

Figure 9.2 Boxplot of age at consultation for the three groups of separation disorders A, B and C. Medians are significantly different by K-W test, Chi-square = 8.69, d.f.=2, P=0.01.



Removal of age of consultation from the model slightly strengthened the influence of the post-vaccination environment (Chi-square=4.71, d.f.=2, P=0.10) and so this relationship was explored further using crosstabulations. The proportion of dogs exposed to busy urban environments post-vaccination was similar in the A, B, and clinical comparison groups (Chi-square=0.30, d.f.=2, P=0.87), but differed significantly between the clinical comparison group and group C (Chi-square=5.77, d.f.=1, P=0.02) (Table 9.8, first three rows of data). In group C, almost two-thirds of the dogs had not experienced busy urban environments post-vaccination, compared to only about a quarter in the comparison group. Since the sample size of the C group is small (N=8), each of the two criteria for inclusion in group C were tested separately (Table 9.8, last two rows). Separation cases following noxious events (N=14) were significantly more likely than the comparison group to have experienced non-urban environments (Chi-square=9.79, P=0.002), but there was no such difference when the 32 dogs whose symptoms were not expressed every time were compared (Chi-square=1.49, P=0.22). It can therefore be tentatively concluded that varied experience post-vaccination may be protective against separation disorders triggered by noxious events .

Table 9.8 Frequencies of separation cases in groups A, B and C, and in the clinical comparison group, that had been exposed, or not exposed, to busy urban environments post-vaccination.

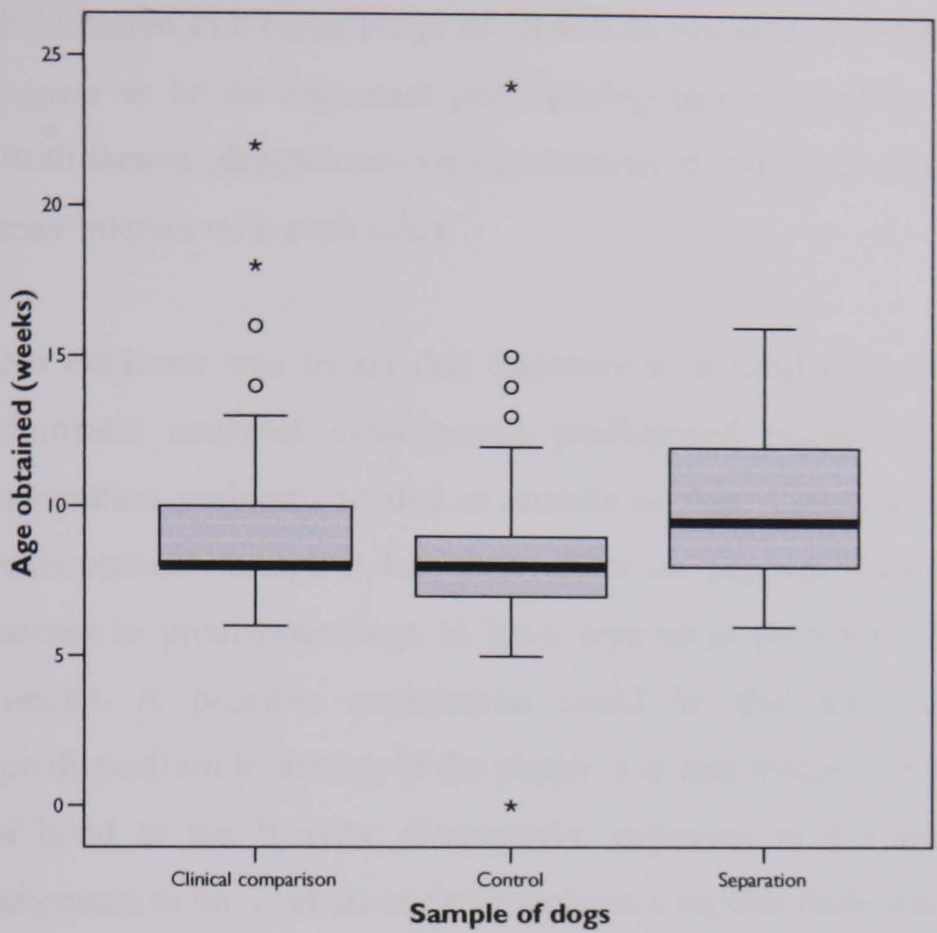
Case type	Not exposed	Exposed
A (from puppyhood on, every time)	7	22
B (change in routine or location, every time)	5	12
C (after noxious event, not every time)	5	3
Not separation-related (clinical comparison)	19	63
After noxious event	9	5
Not every time	11	21

9.3.4 Relationships between symptoms, maternal environment and age obtained

To explore relationships between symptoms of separation problems related to anxiety and fear, the maternal environment and age of homing, the separation group was compared with the control group and the comparison group using nominal regression and cross tabulations. The relationship between age obtained and symptoms was explored using the same nominal regressions, followed by Kruskal-Wallis and Mann-Whitney U tests. The symptoms tested were: destruction (in the direction of the owner's departure, in any direction, moveable objects, immoveable objects), urination/defaecation, salivation, aggression to family members when departing, vocalizations, and repetitive behaviour. Timing of onset of separation-related behaviour was also tested.

No significant links could be detected between maternal environment and the expression of individual symptoms. The age of homing (Figure 9.3) was significantly later in dogs where the onset of displaying symptoms had followed a change in routine (K-W Chi-square=7.61, d.f.=2, P=0.03). (Mean control group=8.25,—mean comparison group=9.11, mean separation group = 9.92), although the difference with the comparison group was marginally non-significant (Mann-Whitney U=771, P=0.08). Dogs displaying destructive behaviour towards immovable objects (e.g. sofa), had been homed at a slightly older age (mean 9.90 weeks) compared to the control group (Mann Whitney U: 584.00, P= 0.03) but not significantly later than the comparison group (Mann-Whitney U=662.5, P=0.17). There was a similar trend for dogs destroying moveable objects (mean age = 10.2 weeks: vs. comparison, U=515.5, P=0.09: vs. control, U=449, P=0.01).

Figure 9.3 Age of homing of dogs displaying symptoms after a change in routine.



9.3.5 Relationships between symptoms and exposure to busy urban environments post-vaccination

The comparisons of the separation group with the comparison group using binomial regression and cross tabulations, showed one significant association. The dogs in the separation group that had not been exposed to busy urban environments were more likely to cause destruction in the direction to follow the owner when left alone (Chi-square: 4.172, P= 0.04). No other symptom appeared to be associated with post-vaccination environment.

9.4 Discussion

These results illustrate that separation problems related to anxiety and fear have a multifaceted aetiology (Appleby & Pluijmakers 2003). A proportion of the dogs might display symptoms resulting from a negative emotional state caused by an excessive dependency on maintenance stimuli (e.g. the owner). However, learned fears of specific stimuli or contexts, resulting from inadequate socialization and habituation to a broad range of stimuli or negative experiences after homing, also appear to be an important predisposing factor (Appleby & Pluijmakers 2003). Both factors (dependency on maintenance stimuli and exposure to fearful stimuli) may interact with each other.

No evidence was found that exposure to a limited range of stimuli in a non-domestic maternal environment predisposed puppies to the development of separation problems related to anxiety or fear. Exposure to a domestic maternal environment though it has little effect on puppies homed at or after 8 weeks, seems to predispose dogs to have separation problems if they are homed at 7 weeks. A possible explanation could be that the early homing causes a predisposition to anxiety if the puppy is at that moment in the process of forming a bond to the breeder. Conversely, exposure to a variety of stimuli through exposure to busy urban environments on a regular basis post vaccination, seems to have a protective effect, independent of the age of homing. This effect was most apparent in dogs classified as Group C on the basis that their separation disorder was triggered by noxious events. No specific combination of maternal environments and environments post homing was found to be especially predisposing or protective against the development of separation problems related to anxiety or fear. Dogs in the separation sample that either started to display symptoms after a change in routine, or were destructive to immovable objects or movable objects, appeared to be homed at an older age.

9.4.1 The effect of the maternal environment

The weak association between maternal environment and the development of separation problems is in line with the results of the research conducted by Jagoe (1993) who also did not find an association between the type of maternal environment and display of separation problems.

The aversive experiences during the process of being placed in a rescue kennel has been proposed as an explanation for the higher incidence of separation problems in dogs obtained from shelters (McCrave 1991). This has been contradicted by studies illustrating that dogs obtained from rescue societies have only a slightly higher probability of displaying separation problems than dogs obtained from breeders (Bradshaw *et al* 2001). The extent of maintenance stimuli available for puppies raised in a non-domestic maternal environment is limited, and each stimulus is predicted to be more significant to maintain emotional homeostasis, because of the lack of exposure to a variation of stimuli and increased salience of the stimuli. However, no evidence was found that differences in the composition of the maintenance set of puppies obtained from a domestic or non-domestic environment, influences the composition and level of dependency on new maintenance stimuli after homing, or that the loss of more important maintenance stimuli causes a higher level of dependency on new maintenance stimuli.

Since many separation problems are suggested to result from strong attachment between the dog and the owner, McPherson (1998) explored how the dog-owner relationship between seven weeks and eighteen months of age influences the development of separation-induced behaviour, which was defined as any undesirable behaviour displayed immediately following separation from the owner that is not displayed in the owner's presence. Information was gathered from the breeders and subsequent owners of 23 Labrador Retrievers and 17 Border Collies. The amount of interaction between the owners and the dogs had no effect on the probability of developing separation induced behaviour at any age. However, exposure to a wide variety of stimuli through very extensive social referencing by the breeder at about seven weeks of age resulted in a tendency to display separation induced behaviour over the next six months, whereas extensive social experience after six months of age was associated with a reduction in the expression of separation induced behaviour (McPherson 1998). Among the possible explanations for the increased tendency to display separation induced behaviour, could be that the extensive social referencing around 7 weeks resulted in the dogs expecting social interactions to be frequent and varied, or that the puppies were developing an attachment to the breeder, who is present in a lot of situations (McPherson 1998) or, the social referencing might have been counterproductive and experienced as aversive, as these results were only found for the puppies that had received the highest score for social referencing. In addition, the annual review of cases of the Association of Pet Behaviour Counsellors (APBC) of 1995 showed that separation problems belonged to the four most common behaviour problems in puppies from a domestic maternal environment when obtained from up to 6 weeks to 16 weeks. This in contrast to puppies obtained from a non-domestic maternal environment, where separation problems did not appear in the top four of most common behaviour problems referred to members of the APBC (Magnus & Appleby 1995). Taken with the results described above, this suggests that further research into the formation of social bonds between dogs at people at the age of 7 weeks would be beneficial.

9.4.2 The effect of the environment after vaccination

The capacity of the maintenance set to maintain emotional homeostasis and prevent the experience of a negative emotional state after homing when exposed to an event that could have an aversive effect on the puppy (e.g. exposure to a novel environment or noxious stimulus) seems to have a more direct effect on influencing the predisposition to develop separation problems related to anxiety and fear. The capacity to maintain emotional homeostasis is influenced by many factors, such as the composition of the maintenance set (e.g. amount, variety, availability and dependency on stimuli), characteristics of the fear evoking stimulus or event (e.g. salience, intensity, novelty, frequency) and previous learning experiences (e.g. available coping strategies, generalization of fear to other context, reactions of the owners) (Appleby & Pluijmakers 2003) making it likely that the probability to develop separation problems is attributable to a complex interaction of many elements post homing.

Exposure to busy urban environments after vaccination seems to be mildly protective to the development of separation problems related to anxiety and fear. The majority of dogs in the separation sample (66.9%) and comparison group (76.8%) received exposure to busy urban environment, but 33% of the dogs in the separation sample had not been exposed to urban environments compared to 23% in the comparison group.

Separation cases in group C dogs, who started to display symptoms after a noxious event and only display symptoms when they are exposed to the noxious stimulus, were significantly more likely than the comparison group to have experienced non-urban environments which was the opposite of what was expected. These observations strengthens the assumption that fear of environmental stimuli and phobias (e.g. thunderstorm phobia, firework phobia) can be a primary factor in some dogs displaying separation problems related to anxiety and fear. Overall *et al* (2001), who conducted a study to determine if separation anxiety, thunderstorm phobia and sound phobia are associated in dogs, found that the probability that a dog would have separation anxiety, given that it also had a thunderstorm or noise phobia, was high. In addition it is suggested that there is a strong genetic effect that might predispose dogs to the development of a sound or thunderstorm phobia (Overall *et al* 1999). Dogs in group C have developed sufficient independency and are normally capable of maintaining emotional homeostasis when alone, except when exposed to the fear-evoking stimuli. If the noxious event becomes associated with being left alone and or conditioned to the location the dog was left in, anticipation of the noxious event occurring might result in a dog then displaying symptoms every time when left alone, i.e. transfer to group B.

Exposure to busy urban environments might have a protective effect for the development of separation problems because it increases the dog's capacity to maintain emotional homeostasis. The dogs are less fearful, which seems to reduce their dependency on the presence of maintenance stimuli to maintain emotional homeostasis in general and when left alone, plus it decreases the possibility to develop a learned fear, which subsequently sensitizes the dog and makes him anxious for being left alone and increases the need to seek contact with maintenance stimuli such as the owner.

9.4.3 Interaction between the effect of maternal environment and environment post vaccination

The prediction that the level of predisposition to develop separation problems is independent of the maternal environment but influenced by aversive learning experiences after homing, is further supported by the finding that no combination of domestic or non-domestic maternal environment and exposure to urban or non-urban environment was found to have a significant effect and to be unaffected by the age of homing.

The research conducted by Appleby (2000) into the relationship between canine aggression and avoidance behaviour and early experiences showed that both a non-domestic maternal environment and a lack of exposure to busy urban environments on a regular basis were significant predisposing factors for the development of inappropriate avoidance behaviour and some forms of aggression. This suggested the hypothesis that the highest incidence of separation problems related to anxiety and fear should be in the group of dogs that are raised in a non-domestic maternal and non-urban environment. Such a straightforward relationship was not found. Not in the whole separation group, nor in Group A, B or C.

9.4.4 Age at homing

The age at homing was found to interact with the maternal environment but not with exposure to urban environments after homing. Puppies raised in a domestic maternal environment and homed before eight weeks were significantly overrepresented in the separation group when compared with the control group but not with the comparison group. The proportion of separation cases was very similar for dogs homed at or after 8 weeks in the separation group, control and comparison group.

A possible explanation for the higher incidence of separation problems related to anxiety or fear in puppies obtained from domestic environments before eight weeks is that the separation from the breeder's home was experienced as more aversive than in puppies transferred as little as one week later. Ontogenetic changes may play a role. Elliot and Scott (1961) found that the display of separation distress starts at 3 weeks of age, peaks around 7 weeks and than quickly declines during the following weeks. In addition, puppies from a domestic environment are used to a higher level of stimulation from the environment than puppies from non-domestic environments, potentially making it more difficult for them to adapt when, for example, they are suddenly confined in a stimulus-poor environment. The way in which a puppy is exposed to separation and how separation distress is handled may affect how well a dog will learn to cope when left alone considerably (Lindsay 2000b). It is usually recommended that puppies should be exposed to separation experiences gradually, since they have a strong need for social contact with the owner. Adverse rearing practices e.g. locking the puppy in the garage or laundry room and letting it cry until exhaustion, excessive confinement and punishing the distressed puppy, might sensitize the dog to react negatively to subsequent separation experiences (Lindsay 2000b).

9.4.5 Symptoms

The age of homing was significantly later for dogs destructive to immovable objects and movable objects and for dogs that started to display symptoms after a change of routine. This probably is the result of an interaction with other unidentified factors, as there seems to be no logical explanation for this association.

Puppies not exposed to busy urban environments after vaccination were found to be more likely to cause destruction in the direction the owner has left. This might be the result of the dynamic interaction between fear and dependency. Maintenance stimuli provide the puppy with a base of security. When frightened they seek contact with the maintenance stimuli, e.g. the owner, or a familiar place. This not only reduces fear, but also strengthens the dependency on the maintenance stimulus. Consequently, fearful dogs are more prone to develop stronger dependencies on maintenance stimuli compared to non-fearful dogs, predisposing them to develop separation problems. For many fearful dogs, the owner is an important maintenance stimulus, so destruction the direction the owners have left, could be interpreted as being the result of their attempts to regain contact with them.

9.5 Conclusion

In natural conditions wolves go through a gradual process of social and territorial integration, during which the dependency on the bitch, littermates and nest-site is transferred to other animate and inanimate stimuli, making a gradual and perfect adaptation to the social group and environment possible (Lindsay 2000b). Most pet dogs go through an abrupt process in which they are removed from a familiar and secure environment and placed into an unfamiliar environment, without much effort being taken to make the transition from the breeder to the home as gradual as possible to minimize the effect of aversive experiences (Lindsay 2000b). The subsequent learning experiences in the post-homing environment appear to have a stronger predisposing effect for the development of separation problems related to fear and anxiety than does the maternal environment. The dogs displaying separation anxiety related to fear and anxiety seem to display behavioural signs of a negative emotional state resulting from a combination of over dependency on maintenance stimuli and fear of specific events that might generalize to other context or a combination of these. As puppies are normally exposed to periods of separation from their maintenance stimuli after homing, any predisposing or protective effect of the maternal environment may be largely overruled by the learning experiences after homing. The homing process may itself be predisposing if the process of establishing an attachment to the breeder is disrupted when the puppy is young (~7 weeks).

Chapter 10: General discussion

10.1 Introduction

This project focused on two types of behavioural problems in pet dogs (i) inappropriate avoidance behaviour, fear and fear related aggression and (ii) separation problems related to anxiety and fear. Both types form a considerable proportion of the caseload of the companion animal behaviour counsellor nowadays.

The established association between a lack of early life experiences and the development of inappropriate avoidance behaviour, fear and fear related aggression researched by Appleby and Bradshaw (Appleby 2000, Appleby *et al* 2002) formed the basis for the objective of the first part of this project, namely to find a practical method to decrease the likelihood of developing inappropriate avoidance behaviour, fear and fear related aggression, through increasing the variety of stimuli to which puppies that grow up in a restricted maternal kennel environment are usually exposed.

The aim of the second part was directed at the prevention of separation problems related to anxiety and fear, by exploring a possible association between restricted early life experiences and the development of the problem behaviour, which could result in the formulation of future guidelines for prevention. To improve the welfare of dogs that have developed separation problems related to anxiety or fear, a new model for the diagnosis and treatment of such problems was introduced and tested.

In this final chapter the main results and the extent to which the proposed concepts, models and hypotheses are supported will be examined and wider applications of this project are considered.

10.2 The concept of the sensitive period of behavioural organization and the role of emotional homeostasis

In Chapter 2 the current concept of the socialisation period in dogs was described, and then appraised for (i) the research methods used to examine the behavioural development of the dog during the socialisation period, (ii) the theoretical framework of imprinting used, and (iii) the practical appliance of some practices of puppy raising based on this research, which is suggested by some authors to be limited or detrimental to the welfare of puppies. The question was raised if this appraisal suggested the need for a new, more appropriate, theoretical framework?

Studies done by Freedman, King & Elliot (1961) on differences in approach behaviour in puppies at varying ages, Fox's (1971, 1978) work describing the neurological and heart rate development of puppies and Cairns (1966), exemplifying how stimuli an individual is exposed to can gain control over a behavioural response system, were reinterpreted. In combination with a consideration of how behavioural organisation may be influenced by the emotional development of the dog, a new concept of the sensitive period of behavioural organisation and the role of emotional homeostasis was formulated.

In this, it was suggested that for dogs to learn to maintain emotional homeostasis in a changing environment the three to five week period of development forms the foundation for the whole of the sensitive period. This concept is based on the parallels between changes in approach-avoidance behaviour towards novel objects and changes in heart rate and neurophysiological development, during which higher levels of neural organisation build upon more primitive mechanisms.

Based on the concept of the sensitive period of behavioural organisation, the hypothesis that exposure to video images during the period of parasympathetic dominance between 3 and 5 weeks would result in the formation of cognitive representations and increase the capacity to maintain emotional homeostasis when confronted with unfamiliar stimuli in familiar and/or unfamiliar environments was proposed, and was tested in the experiments described in Chapters 4, 5 and 6.

These experiments yielded three main results. First, there was an effect of pre-exposure to video images, because the exposed pups were generally less interested in new objects in general. They inspected fewer objects and inspected each object less often. These findings illustrate that stimulation by social and non-social stimuli in early life results in a lower motivation to explore novel stimuli or environments.

Secondly, exposure to video images appears to result in the formation of cognitive representations of the specific stimuli the puppy is exposed to. In the unfamiliar environment most of the exploratory behaviour of the exposed group was directed to the novel stimuli. This suggests that systematic exposure to video images has a specific effect and can be used to increase a puppy's knowledge of the world.

Thirdly, the results can be interpreted in terms of exposure to video images resulting in the formation of maintenance stimuli that are associated with a positive emotional state, and influencing the need to display exploration behaviour. The control puppies explored more objects, and their body postures and locomotion were those usually associated with fear, contrasting with those of the exposed group, which were not.

Summarised, these results suggest that a dog's capacity to remain in emotional homeostasis can be increased by exposure to video images during the period of parasympathetic dominance between 3 and 5 weeks of age. This exposure results in the formation of a broader maintenance set, reducing the chance that stimuli that are encountered will be unfamiliar, and increasing the dog's capacity to maintain emotional homeostasis when in an unfamiliar environment or when confronted with an unfamiliar stimulus.

10.3 Separation problems related to anxiety and fear

The call for formulating a new model for the diagnosis and treatment of separation problems, described in Chapter 7, was based on a lack of concurrence between the cases of dogs displaying separation problems seen in the behavioural practices of David Appleby and myself, and the descriptions found for the aetiology and diagnosis of separation problems in the literature. The new model is a synergy between (i) the "French" model (Pageat 1998), for which primary or secondary hyperattachment to social stimuli is an obligatory condition, (ii) the American model (Overall 1997), for which hyperattachment is not a necessary condition per se and introduces the role of fears and phobia's into the concept, and (iii) the model of emotional homeostasis and role of maintenance stimuli, presented in this thesis, in which separation anxiety is defined as: apprehension due to removal of significant persons or familiar surroundings.

The testing of the model for diagnosing separation problems related to anxiety and fear described in Chapter 8, illustrated significant associations between symptoms or behavioural signs, and also that the combination of onset of symptoms and type of symptom can potentially be used to start refining the diagnosis and treatment plan. The significant link between over-dependency on several owners, resulting in destruction in several directions to regain access to maintenance stimuli does not appear to have been reported before in the literature.

The data have demonstrated that the dogs displaying separation problems related to anxiety or fear, based on the described characteristics and patters on association between symptoms, can be categorized into the proposed groups A, B and C. Group C dogs stand out from the total sample most because they show the most significant relationship between the variables onset of behaviour and frequency, type and direction of destruction, and (lack of) over-attachment to people. The classification of group A and B is justified by the result, but Group B might need some more refinement. The development of separation problems because of the removal of significant environmental stimuli has been shown to be a relevant factor for some dogs in Group B.

In contradiction to the French model, it seemed that a proportion of the dogs might display symptoms resulting from a negative emotional state caused by an excessive dependency or hyperattachment on maintenance stimuli (i.e. the owner). However, learned fears of specific stimuli or contexts also appear to be an important predisposing factor, or possibly the most important factor, and both factors (dependency on maintenance stimuli and exposure to fearful stimuli) may interact with each other.

The analysis of the early life experiences of the dogs displaying separation problems related to anxiety and fear, further support the multifaceted aetiology of this type of behaviour problem. No evidence was found that exposure to a limited range of stimuli in a non-domestic maternal environment predisposed puppies to the development of separation problems related to anxiety or fear. Exposure to a domestic maternal environment, though it has little effect on puppies homed at or after 8 weeks, seems to predispose dogs to have separation problems if they are homed at 7 weeks. However, this interaction between the age at homing and the maternal environment but not found between age of homing and exposure to urban environments after homing.

The learning experiences in the post homing environment appear to have a stronger predisposing effect for the development of separation problems related to fear and anxiety than the maternal environment. The dogs displaying separation anxiety related to fear and anxiety seemed to display behavioural signs of a negative emotional state resulting from a combination of over dependency on maintenance stimuli and fear of specific events that might generalize to another context, or a combination of these. The homing process may itself be predisposing if the process of establishing an attachment to the breeder is disrupted when the puppy is young (~7 weeks).

In summary, the results of Chapters 8 and 9 confirm the multifaceted aetiology of separation problems related to anxiety and fear, and suggest that learned fears form a potential predisposing factor for the development of the problem behaviour. The model proposed helps to present an understanding of the stimuli a dog needs to maintain in emotional homeostasis, how fear and anxiety and interrelated with (over) dependency on maintenance stimuli, and how this can change as a result of events in the dog's situation. This should help in diagnosis and formulation of an appropriate treatment plan for the individual dog.

10.4 The conceptual framework of emotional homeostasis and the role of maintenance stimuli

Central to this thesis is the conceptual framework of emotional homeostasis and the role of maintenance stimuli in providing an individual with the capacity to cope and adapt in a changeable environment. It was not the aim of the project to test this total concept. Its relevance therefore in this context can only be described in relation to the prevention of inappropriate avoidance behaviour, fear and fear-related aggression, and the prevention, diagnosis and treatment of separation problems related to anxiety and fear.

It has been proposed that the dog's capacity to maintain emotional homeostasis develops throughout the sensitive period for behavioural organisation, as part of the processes whereby mental representations of stimuli are formed and linked to associations and responses. It was confirmed that during the three to five week period, exposure only to visual and auditory representations of stimuli is sufficient for those stimuli to become incorporated into the maintenance set, and potentially to become associated with parasympathetic activity, and that exploratory behaviour is subsequently influenced by the composition of this maintenance set when the puppy is exposed to a familiar or unfamiliar environment.

The suggestion that as behaviour becomes more organized, i.e. the maintenance set becomes more formed, attention to familiar stimuli decreases and increases towards unfamiliar stimuli, as described by e.g. Scott (1968) in dogs and by Kagan (1970) in children, is therefore supported.

From the results of the video experiments it can only be concluded that exposure to video images decreases the probability of unfamiliar stimuli causing a loss of emotional homeostasis. The extent to which the stimuli on the videotape helped the exposed puppies to maintain emotional homeostasis because they became dependent upon those stimuli is uncertain, but cannot be totally ruled out, as the model predicts that emotional dependence upon stimuli is formed and maintained by exposure.

However, the analysis of the sample of dogs displaying separation problems related to anxiety or fear illustrates that (i) the formation and continuation of dependence results from an ongoing conditioning process, during which response patterns become attached to the cues provided by the social and non-social objects in the animal's environment; and (ii) removal of an object which the response system of the animal has been strongly conditioned to depend on for the maintenance of homeostasis, is associated with a significant disruption of its behaviour. The classifications of the dogs into the three groups is consistent with the importance of the association between maintenance stimuli in helping the dog to remain in emotional homeostasis, and also the extent to which different type of stimuli (e.g. conditioned aversive stimuli, novel stimuli, innate fear eliciting stimuli) or situations (e.g. loss of emotional homeostasis) can cause a loss of emotional homeostasis and influence the level of dependency upon the maintenance set.

10.5 Neurological bases of behavioural development

A comparison of the early behavioural development of the dog with the development of behaviour in children during the first year of life, shows interesting parallels which might suggest directions for future research into the concept of behavioural organisation and the role of emotional homeostasis. In children during the first year of development some major changes take place: (i) the disappearance of neonatal reflexes e.g. the grasp reflex, (ii) the appearance of the fear of strangers and (iii) separation anxiety. This coincides in time with changes in the brain that permit brainstem reflexes to be increasingly inhibited by the cortex, processes in the hippocampus and prefrontal cortex facilitating the formation, storage and retrieval of memories, plus the strengthening of connections between the cortex and limbic system (Hershkowitz 2000).

Around three months of age children display a significant improvement in recognition memory, as indicated by a preference to look at a new object when confronted with both a familiar and an unfamiliar object, which indicates that it remembers the familiar object (Hershkowitz 2000). Recognition memory involves making a judgement whether or not the stimulus perceived is familiar. Recognition differs from recall in the sense that recall memory involves retrieving a representation of stimuli without the stimuli being present (Carver *et al* 2000). Experiments with infant monkeys have shown that for the type of recognition memory described, the hippocampus is essential. In humans the hippocampus shows maximal growth rate at around three moths, at the same time as the cortical visual system undergoes myelination to increase the capacity to process visual information efficiently. It approaches its adult volume at around seven to ten months, when major changes are taking place in the prefrontal cortex that increase synaptic flexibility. For example, there is a spurt in the differentiation of GABA-ergic inhibitory interneurons, synaptic density increases, and synaptic membrames show molecular changes in their composition (Hershkowitz 2000).

The universal appearance of signs of anxiety when approached by strangers or when separated from their caretaker suggests that there is a biological basis for its appearance (Hershkowitz 2000). When the working memory perceives a discrepancy between a situation in which the mother was present and one in which the mother has left, an emotional reaction might be elicited (Hershkowitz 2000). Hershkowithz (2000) describes the process as follows: "Stimulation of the amygdala activates the basal ganglia, hypothalamus and the hypothalamus-pituitary-adrenal (HPA) axis, leading to specific physiological responses, such as changes in heart rate, blood pressure, respiration, crying, facial expression, muscle activity and 'stress responses'. The strength of these reactions varies from individual to individual, depending on the sensitivity of the system to challenges and forms a neural basis of the infant's temperament. These responses are of clinical importance and are involved in psychosomatic interactions possibly even into adult life. A critical development at this stage is the integration of the limbic and endocrine system into the memory network. The capsula interna, which links the cerebral cortex reciprocally with the amygdala, develops mature myelin at 10 months, intensifying the connectivity between the two structures. Since the amygdale is also connected to the hypothalamus-pituitary-adrenal axis, the neural basis is established for the emergence of emotional responses to fear." (Herschkowitz 2000, pp 423).

Learning experiences during a sensitive period, i.e. a limited period in development when the brain is strongly affected by experiences, exert a long-lasting influence on the development of social and emotional behaviour (Knudsen 2004). For behaviour to develop normally, experiences must be of a particular kind and take place during a certain period (Knudsen 2004). Through processes as axon elaboration, synapse formation, and axon and synapse elimination, they customize a developing neural circuit to the needs of the individual, providing precise information about itself and the environment that cannot be entirely predicted and thus genetically encoded for (Knudsen 2004). Animals living in a complex environment may thereby develop a more complex CNS organisation and a permanently superior oxygen and nutrient supply to the brain (Bowen 2003).

Several parameters of behavioural development of the dog have been investigated and it is thought that the dog is neurologically mature at four weeks of age, with the exception of equilibration (Fox 1978). "The data on the developing canine brain serve to demonstrate the temporal coincidence of development and maturation of several interrelated structural and functional parameters. This coincidence, which occurs between the fourth and fifth postnatal week in the dog, may be termed a period of integration. It is at this time that several parts of the developing nervous system show both structural and functional integration, which marks the beginning of a relatively mature organizational level of activity. At this time, the organism begins to interact rather than react with conspecifics and through social experiences with both parent and peers develops emotional attachments to its own kind or to man. If denied human contact during this critical period from 4-12 weeks of age, it will subsequently avoid human contact (Scott & Fuller 1965). Such dogs are fearful of humans and are virtually untrainable. The fear period which develops after 8 weeks of age limits the capacity to develop new social attachments and essentially terminates this critical socialisation period. Thus even in a domesticated species, lack of exposure to man during this formative period (when brain centers are integrating and emotional reactions developing) will greatly limit the social potential of the species" (Fox 1978 p 156).

The parallels in the descriptions of the development of fear and separation anxiety in children and dogs, together with the research done by Fox (1971, 1978) into the timing of the neurological development of puppies, suggests that the practices of puppy raising are not optimally adjusted to the early onset of the sensitive period in development, for learning to occur that will maintain emotional homeostasis in a changing environment. Puppies are shown to start to display avoidance behaviour towards unfamiliar stimuli from five weeks on and can show fear from seven weeks on. They start to display distress when separated from familiar stimuli from four weeks onwards, quickly increasing to a peak around 7 weeks of age. This suggests that around five weeks their maintenance set is largely formed, and that the period of highest brain plasticity comes to an end, as the functional working memory, detecting discrepancies between expectations formed and the context exposed to, starts to result in a loss of emotional homeostasis, because preferential organisation has developed. To prevent the development of inappropriate avoidance behaviour, fear and fear-related aggression, and separation problems related to anxiety and fear, exposure to a wide variety of stimuli seems to be essential during the period of rapid neurological development up to five weeks. This should increase the puppy's knowledge of the world, and decrease the chance that encounters with novel stimuli will result in a loss of emotional homeostasis. In addition, learning to adapt to changes in the maintenance stimuli the puppy has already become dependent upon, is likely to be essential for optimal adaptation and the maintenance of emotional homeostasis.

10.6 Practical applications

This study has highlighted the importance of empirical research and integration of new insights, different school of thoughts and practical experiences into concepts. The reinterpretation of the old research done on the socialisation period in the nineteen-fifties and sixties, combined with the integration of more recent insights into the emotional development and use of video images to decrease fear in other species, has opened a new window of opportunities to decrease inappropriate avoidance behaviour and fear, and to increase the welfare of dogs. The findings are not only suitable to be applied by breeders of pet dogs, but also to dogs that have to learn to adapt to a possibly even higher level of variability in their environment, like working dogs such as Guide Dogs for the Blind, Rescue dogs, Military Working dogs etc.

The analysis of the relationship between early life experiences and the development of separation problems related to anxiety and fear, and the suggested importance of aversive learning experiences post homing in its development, should form the starting point for rethinking the current practices of homing puppies. In particular, separation from significant maintenance stimuli, and coping with variation of the composition of the maintenance set, can be introduced in a gradual way, and should possibly be started at the breeders, and gradually transferred to the environment of the new owner, thereby decreasing the possibility of forming fears for social or non-social stimuli.

Although the successfulness of the predictions for treatment from the proposed model for the diagnosis of separation problems related to anxiety and fear has not been evaluated, the merging of the French and American schools of thought, together with the introduction of new insights based on observations of companion animals' behaviour in clinical practice, into the new model is likely to become a practical tool to refine the diagnosis and treatment plan of dogs displaying separation problems related to anxiety and fear which can be applied in daily practice.

10.7 Future work

Although the results of the experiments during which puppies were exposed to video images have shown that systematic exposure between three and five weeks resulted in puppies being less fearful, a lot of additional research has to be done.

Firstly, the effect of exposure at other ages, such as after five weeks, or during the entire period from three weeks of age until the moment of homing has to be explored, to assess how the most optimal effect can be achieved. In addition, exposure during other moments of development would be a very interesting project to test the hypothesis that the most important phase to develop the capacity to maintain emotional homeostasis lies between three to five weeks of age.

Secondly, a structured assessment of the appliance of video images in combination with other techniques that are suggested to decrease emotionality, like early handling, environmental enrichment in the form of providing an enriched kennel environment with toys, and the effect of different ages of homing would be interesting to see which combinations of these would result in an even more beneficial effect.

Unfortunately, because of the lack of response to the questionnaires given to the puppy owners, aimed at assessing the long term effect of exposure to video images, a research project during which puppies are followed up after being homed is absolutely necessary to assess the long term effect of exposure to video images.

Finally, the amount of exposure of 30 minutes per day was chosen arbitrarily. A further refinement of the amount of exposure necessary, and exposure in groups or individually, could be beneficial in order to refine the practical application. Still to be assessed also is whether these techniques affect the emotional homeostasis of puppies living in domestic environments where there is naturally a greater range of stimuli.

To further evaluate the proposed model for the diagnosis and treatment of separation problems related to anxiety and fear, a retrospective or prospective study using a questionnaire during which a more detailed breakdown of symptoms and behaviour when the owners are present might result in more associations making a further refinement of the model possible. A logical further step of course is to assess the success of the treatment plan.

References

Ainsworth, M.D.S., Blehar, M.C., Waters, E. & Wall., S (1978) Patterns of attachment: A psychological study of the strange situation. Hillsdale: Erlbaum

Appleby, D.L. (1993) Socialisation and habituation. In: Fisher, J. (Ed.) The Behaviour of Dogs and Cats. Stanley Paul: London

Appleby, D.L. (1999) The relationship between canine aggression and avoidance behaviour, and early experience. MSc-thesis, Anthrozoology Institute, University of Southampton New College

Appleby, D.L., Bradshaw, J.W.S. & Casey, R.A. (2002) Relationship between aggressive and avoidance behaviour by dogs and their experience in the first six months of life. *Veterinary Record,* **150**, 434-438

Appleby, D.L & Magnus, E. (1995) Annual Review of Cases of the Association of Pet Behaviour Counsellors, UK

Appleby, D.L. & Pluijmakers, J.J.T.M. (2003) Separation anxiety in dogs: the function of homeostasis in its development and treatment. *The Veterinary Clinics of North America: Small Animal Practice*, 33, 321-344

Appleby, D.L. & Pluijmakers, J.J.T.M. (2004) The function of emotional homeostasis in its development and treatment of separation anxiety in dogs. Proceedings of the 2004 CABTSG Study Day, Attachment and its Effect on the Pet-Owner Relationship, Birmingham, 31st March 2004, pp 11-15

Archer, J. (1979) Behavioural aspects of fear. In: Sluckin, W. (Ed.) Fear in Animals and Man. Van Nostrand Reinhold: New York, pp 56-85

Archer, J. & Birke, L.I.A (1983) (Eds.) Exploration in Animal and Humans. Vans Nostrand Reinhold: Wokingham

Ashmead, D.H., Clifton, R.K. & Reese, E.P. (1986) Development of auditory localization in dogs: Single source and precedence effect sound. *Developmental Psychobiology*, **19**, 91-103

Askew, H.R. (1996) Treatment of Behavior Problems in Dogs and Cats. Blackwell Science: Oxford

Augustsson, H. & Meyerson, B.J. (2004) Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. *Physiology & Behavior*, **81**, 685-698

Barnard, C.J. & Hurst, J.L. (1996) Welfare by design: the natural selection of welfare criteria. *Animal Welfare*, **5**, 405-433

Barnett, S. A. & Cowan, P. E. (1976) Activity, exploration, curiosity and fear: an ethological study. *Interd Sci Rev*, **1**, 43-62

Bateson, P. (1979) How do sensitive periods arise and what are they for. *Animal Behaviour*, **27**, 470-486

Bateson, P. (1981) Ontogeny of behaviour. *British Medical Bulletin*, **37** (2), 159-164

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

Beerda, B., Schilder, M.B.H., van Hoof, J. A. R. A. M. & de Vries, H.W. (1997a) Manifestations of chronic and acute stress in dogs. *Applied Animal Behaviour Science*, **52**, 307-319

Beerda, B., Schilder M.B.H., van Hoof, J.A.R.A.M., de Vries, H.W. & Mol, J.A. (1997b) Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science*, **58**, 365-381

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., De Vries, H.W. & Mol, J.A. (1998) Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. *Physiology and Behaviour*, **66**, 233-242

Berlyne, D.E. (1960) Conflict, Arousal and Curiosity. McGraw-Hill: New York

Blackwell, E., Casey, R.A. & Bradshaw, J.W.S. (in press) A controlled trial of behavioural therapy for separation-related behavioural disorders in dogs. *Veterinary Record*

Bloomsmith, M.A., Keeling, M.E. & Lambeth, S.P. (1990) Videotapes: Environmental enrichment for singly housed chimpanzees. *Laboratory Animals*, **19**, 42-46

Boissy, A. (1995) Fear and fearfulness in animals, *Quarterly Review of Biology*, **70**, 165-191

Boissy, A. & Bouissou, M.F. (1995) Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. *Applied Animal Behaviour Science*, **46**, 17-31

Bolhuis, J. J. (1999) Early learning and the development of filial preferences in the chick. *Behavioural Brain Research*, **98**, 245-252

Bolhuis, J.J. & Honey, R.C. (1998) Imprinting, learning and development: from behaviour to brain and back. *Trends in Neuroscience*. **21**, 306-311

Borchelt, P. & Voith, V. (1982) Diagnosis and treatment of separation-related behaviour problems in dogs. *Veterinary Clinics of North America, Small Animal Practice*. **12**, 625-635

Bourdin, M. (1999) Development of the young dog and behavioral disorders. In: *Proceedings of the Second World Meeting on Ethology, Lyon*, pp 215-217

Bowen, J. (2000) The need for standards in socialisation. In: Casey, R. (Ed.) *Proceedings of the 2000 CABTSG Annual Study Day*: Preventing problems: a shared responsibility, Birmingham, April 5 2000, pp. 29-34

Bowen, J. (2003) The role of applied learning theory as a therapeutic approach in veterinary behavioural medicine. In: Heath, S. (Ed.) Proceedings of the 9th European Congres on Companion Animal Behavioural Medicine, Salzburg, pp 23-27

Bowlby, J. (1969) Attachment, Attachment and Loss. Volume 1, NewYork: Basic books

Bradshaw, J.W.S. (1992) Behavioural biology. In: C. Thorne (Ed.) The Waltham Book of Dog and Cat Behaviour. Pergamon: Oxford, pp 31-52

Bradshaw, J.W.S. (2002) unpublished manuscript. Anthrozoology Institute University of Southampton

Bradshaw, J.W.S, Blackwell, E. J., Rooney, N. J. & Casey, R. A. (2002b) Prevalence of separation related behaviour in dogs in southern England. In: Dehasse, J. & Biosca Marce, E. (Eds.) Proceedings of the 8th ESVCE Meeting on Veterinary Behavioural Medicine, Granada, Spain, pp 189-193

Bradshaw, J.W.S., McPherson, J.A., Casey, R.A. & Larter, I.S. (2002a) Aetiology of separation related behaviour in domestic dogs. *Veterinary Record*, **151**, 43-46

Cairns, R. B. (1966) Attachment behavior of mammals. *Psychological Reviews*. **73**, 409-426

Cairns, R.B. (1972) Attachment and dependency: a psychobiological and social learning synthesis. In: Gewirtz, J. L. (Ed) Attachment and Dependency. V. H. Winston: Washington DC pp. 29-80

Cairns, R.B. & Johnson, D.L. (1965) The development of interspecies social attachments. *Psychonomic Science*. **2**, 337-338

Cairns, R.B. & Werboff, J. (1967) Behavior development in the dog: an interspecific analysis. *Science*, **158**, 1070-1072

Campbell, W.E. (1975) Behavior Problems in Dogs. American Veterinary Publications Inc.: California

Carlson, N. R. (1998) Physiology of Behaviour. Allyn & Bacon: Massachusetts, USA, 6th edn.

Carver, J.L., Bauer, P.J., & Nelson, C.A. (2000) Associations between infant brain activity and recall memory. *Developmental Science*, **3** (2), 234-246

Chrousos, G. & Gold, P.W. (1992) The concept of stress and stress system disorders. *JAMA*, **267**, 1244-1252

Clarke, C.H. & Jones, R.B. (1999) Effects of prior video stimulation on open-field behaviour in domestic chicks. *Applied Animal Behaviour Science*, **66**, 107- 117

Clutton-Brock, J.(1984) Dog. In: Mason, I.L. (Ed) Evolution of Domesticated Animals, pp. 198-211. Longmann: London

Clutton-Brock, J. (1996) Origins of the dog: Domestication and early history. In: Serpel, J. (Ed.) The Domestic Dog: Its Evolution, Behaviour and Interaction with People, pp 6-20. Cambridge University Press: New York

Coppinger, R. & Coppinger, L. (2001) Dogs: A Startling New Understanding of Canine Origin, Behavior and Evolution. New York: Scribner Press

Corey, T.D. (1978) The determinants of exploration and neophobia. *Neuroscience & Biobehavioural Review*, **2**, 235-253

Crusio, W.E. & Van Abeelen, J.H. (1986) The architecture of behavioural responses to novelty in mice. *Heredity*, **56**, 55-63

Dantzer, R. (1986) Behavioural, psysiological and functional aspects of stereotyped behavior: a review and a reinterpretation. *Journal of Animal Science*, **62**, 1776-1786

Deag, J.M., Manning, A. & Lawrence, C.E. (2000) Factors influencing the mother-kitten relationship. In: Turner, D.C. & Bateson, P. (Eds.) The Domestic Cat: the Biology of its Behaviour, pp. 23-39. Cambridge University Press: Cambridge

DeFries, J.C., Weir, M.W. & Hegmann, J.P. (1967) Differential effects of prenatal maternal stress on offspring behavior in mice as a function of genotype and stress. *Journal of comparative and physiological Psychology*, **63**, 332-334

Denenberg, V.H. & Morton, J.R.C. (1962) Effects of environmental complexity and social groupings upon modification of emotional behavior. *Journal of Comparative and Physiological Psychology,* **55** (2), 242-246

Domjan, M. (2000) The Essentials of Conditioning and Learning. Belmont, USA: Austin, Wadsworth/Thompson, 2nd. ed.

Dorland's Pocket Medical Dictionary (1989) Philadelphia: W.B. Saunders Co

Einon, D. (1980) Environmental influences and exploration in the rat. *Applied Animal Ethology,* **6** (4), 384

Elliot, O. & Scott, J. (1961) The development of emotional distress reactions to separation and discomfort in the dog. *Journal of Genetic Psychology,* **99**, 3-22

Evans, C.S., Macedonia, J.M. & Marler, P. (1993) Effects of apparent size and speed on the response of chickens, *Gallus gallus*, to computer generated simulations of aerial predators. *Animal Behaviour,* **46**, 1–11

Evans, C.S. & Marler, P. (1991) On the use of video images as social stimuli in birds: audience effects on alarm calling. *Animal Behaviour,* **41**, 17–26

Fiala, B.A., Joyce, J.N. & Greenough, W.T. (1978) Environmental complexity modulates growth of granulecell dendrites in developing but not adult hippocampus of rats. *Experimental Neurology,* **59**, 372-83

Flannigan, G. & Dodman, N. (2001) Risk factors and behaviors associated with separation anxiety in dogs. *JAVMA,* **219** (4) 460-466

Fox, M. W. (1964) The ontogeny of behaviour and neurological responses in the dog. *Animal Behaviour, **12***, 301-310

Fox, M.W. (1969) Behavioural effects of rearing dogs with cats during the critical period for socialisation. *Behaviour,* **35**, 273-280

Fox, M. W. (1971) Integrative Development of Brain and Behaviour in the dog. University of Chicago Press: Chicago, USA

Fox, M.W. (1972) Understanding Your Dog. Coward, McCann and Geoghegan, inc.: New York

Fox, M. (1978) The Dog: Its Domestication and Behaviour. Garland STPM Press: New York, USA

Fox, M.W. & Bekoff, M. (1975) The Behaviour of Domestic Animals. In: Hafez, E.S.E. (Ed.) The behaviour of Domestic Animals, 3rd Ed. 370-409. Williams and Wilkins: Baltimore

Fox, M.W. & Stelzner, D. (1966) Behavioural effects of differential early experience in the dog. *Animal Behaviour,* **14**, 273-281

Fox, M.W. & Stelzner, D. (1967) Effects of early experiences in the dog. *Animal Behaviour,* **15**, 377 -386

Frank, H. & Frank, M. (1981) On the effects of domestication on canine social development and behaviour. *Applied Animal Ethology*, **8**, 507-525

Fraser, D. & Duncan, I. J. H. (1998) 'Pleasures', 'pains' and animal welfare: toward a natural history of affect. *Animal Welfare,* **7**, 383-396

Freedman, D.G., King, J.A. & Elliot, O. (1961) Critical Period in the Social Development of Dogs. *Science*, **133**, 1016-1017

Freeman, D. (1991) Barking up the Right Tree. Ringpress Books: Letchworth: Herts, UK

Frijda, N.H. (1988) De emoties: Een overzicht van onderzoek en theorie. Amsterdam, The Netherlands: Uitgeverij Bert Bakker

Fuller, J.L. (1967) Experimental deprivation on later behavior. *Science*, **158**, 1645-1652

Fuller, J.L. & Clark, L.D. (1966) Effects of rearing with specific stimulation upon the post isolation syndrome in dogs. *Journal of Comparative Physiological Psychology,* **61**, 258-263

Fuller, J.L. & Debuis, E.M. (1962) The Behaviour of Dogs. In: Hafez, E.S.E. (Ed.) The Behaviour of Domestic Animals. Baltimore: Williams and Wilkins. Cited in: Lindsay, S.R., Handbook of Applied Dog Behavior and Training: Adaptation and Learning. Vol. 1. Iowa: Iowa State University Press

Gaultier, E. (2001) Separation related behaviour problems: diagnostic criteria identification using a cluster analysis. In: Overall, K.L., Mills, D.S., Heath, S.E. & Horwitz, D. (Eds.) Proceedings of the Third International Congress on Veterinary Behavioural Medicine. Wheathampstead: Universities Federation for Animal Welfare

Goddard, M.E. & Beilharz, R.G. (1983) The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. *Applied Animal Behaviour Science*, **12**, 267-278

Goddard, M.E. & Beilharz, R.G. (1986) Early prediction of adult behaviour in potential guide dogs. *Applied Animal Behaviour Science*, **15**, 247-260

Goldstone, R.L. (1998) Perceptual Learning. *Annual Review Psychology*, **49**, 585-612

Gray, J. (1971) The Psychology of Fear and Stress. Cambridge University Press: Cambridge, UK

Gray, P. H. (1958) Theory and evidence of imprinting in human infants. *Journal of Psychology,* **46**, 155-166 cited in Scott, J.P., & Fuller, J.L., Genetics and the Social Behaviour of the Dog. University of Chicago press: Chicago

Greenough, W.T. (1975) Experiential modification of the developing brain. *American Science*, **63**, 37-46

Gross, R. (1996) Psychology: The Science of Mind and Behaviour. Hodder & Stoughton: London, UK

Gubernick, D. J. (1981) Parent and Infant Attachment in Mammals. In: Gubernick, D.J. & Klopfer, P. H. (Eds.) Parental Care in Mammals, pp 243-305. Plenum Press: New York, USA

Gurski, J.C., Davis, K. & Scott, J.P. (1980) Interaction of separation discomfort with contact comfort and discomfort in the dog. *Developmental Psychobiology*, **13,** 5, 463-467

Hadden, L.E. (2002) Modeling interactions between filial imprinting and a predisposition using genetic algorithms and neural networks. *Neurocomputing*, **42** (1-4), 215-237

Halliday, M.S. (1966) Exporation and fear in the rat. Symp. Zool. Soc. Lond. 18, 45-59

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

Harlow, H. F. & Mears, C. (1979) *The Human Model: Primate Perspectives*. Washington D. C.: V. H. Winston & Sons

Harlow, H. F. & Zimmerman, R. R. (1959) Affectional responses in the infant monkey. *Science,* **130**, 421-432

Hayes, C. (1951) The Ape in our House. New York: Harper, cited in: Scott, J.P. & Fuller, J.L., Genetics and the Social Behaviour of the Dog. University of Chicago press: Chicago

Heath, S. (2002) Dealing with separation problems in dogs. In: Scientific Proceedings of the 45th Annual Congress of the British Small Animal Association. Gloucester, UK: British Small Animal Veterinary Association, pp 536-538

Heath, S. (2005) Managing and treating canine noise phobias. In: Proceedings of the Australian Veterinary Behaviour Interest Group, Gold Coast, May 2005, pp. 56-60

Heffner, H.E. (1983) Hearing in large and small dogs: Absolute thresholds and size of the tympanic membrane. *Behavioural Neuroscience*, **97**, 310-318

Henry, J. (1980) Present concept of stress theory. In: Usdin, E., Kvetnansky, R. & Kopin, I. (Eds.) Catecholmines and stress: recent advances. Elsevier North Holland: New York, pp 557-571

Herschkowitz (2000) Neurological bases of behavioural development in infancy. *Brain & Development*, **23**, 411-416

Hetts, S. (1999) Pet Behavior Protocols: What to Say, What to Do, When to refer. AAHA Press: Colorado

Heymer, A. (1976) Vocabulaire éthologique. Paul Parey, Presses Universitaires de France: Paris, Berlin und Hamburg

Hothersall, D. & Tuber, D.S. (1979) Fears in companion dogs: Characteristics and treatment. In: Keehn, J.D. (Ed.) Psychopathology in Animals, New York, Academic Press, cited in: Borchelt, P.L. & Voith, VL: (1982) Diagnosis and Treatment of Separation-Related Behaviour Problems in Dogs. Veterinary Clinics of North America: Small Animal Practice, 12 (4) 625-635

Houpt, K.A. (1991) Domestic Animal Behavior. Ames: Iowa State University Press

Houpt, K.A. & Willis, M. B. (2001) Genetics of behaviour. In: Ruvinsky, A. & Sampson, J. (Ed..) The Genetics of the Dog, CABI Publishing: Wallingford, pp 371-400.

Hubrecht, R.C. (1995) Enrichment in puppyhood and its effects on later behaviour of dogs. *Laboratory Animal Science*, ***45***, 1, 70-75

Igel, G. J. & Calvin, A. D. (1960) The development of affectional responses in infant dogs. *Journal of Comparative and Physiological Psychology*, **53**, 302-305

Immelmann, K. & Suomi, S.J. (1981) Sensitive periods in development. In: Immelmann, K., Barlow, G.W., Petrovich, L. & Main, .M (Eds.) Behavioural Development. Cambridge University Press: Cambridge

Jacobs, G.H., Deegan, J.F., Crognale, M.A. & Fenwick, J.A. (1993) Photopigments of dogs and foxes and their implications for canid vision. *Visual Neuroscience*, **10**, 173-180

Jagoe, J.A. (1993) Behaviour problems in the domestic dog: a retrospective and prospective study to identify factors influencing their development. Department of Clinical Veterinary Medicine and St. Catharine's College University of Cambridge

Jones, R.B., Carmichael, N. & Williams, C. (1998) Social housing and domestic chicks' responses to symbolic video images. *Applied Animal Behaviour Science*, **56**, 231–243

Jones, R.B., Larkins, C. & Hughes, B.O. (1996) Approach/avoidance responses of domestic chicks to familiar and unfamiliar video images of biologically neutral stimuli. *Applied Animal Behaviour Science*, **48**, 81–98

Kabbaj, M. & Akil, H. (2001) Individual differences in novelty-seeking behavior in rats: a c-fos study. *Neuroscience*, **106**, 535-545

Kagan, J. (1970) Attention and psychological change in the young child. *Science*, **170**, 826-832

Kaiser, S. & Sacher, N. (2005) The effects of prenatal social stress on behaviour: mechanisms and function. *Neuroscience and Biobehavioural Reviews*, **29**, 283-294

Karn, H.W. & Munn, N.L. (1932) Visual pattern discrimination in the dog. *Journal of Genetic Psychology*, **40**, 363-374 cited in: Lindsay, S.R. Handbook of Applied Dog Behavior and Training: Adaptation and Learning. Vol. 1. Iowa State University Press: Iowa

Keeling, L.J. & Hurnik, J.F. (1993) Chickens show socially facilitated feeding behaviour in response to a video image of a conspecific. *Applied Animal Behaviour Science*, **36**, 223–231

Kellogg, W. N. & Kellogg, L. A. (1933) The Ape and the Child. New York: McGraw-Hill cited in Scott, J.P., & Fuller, J.L., (1965) Genetics and the Social Behaviour of the Dog. Chicago: University of Chicago press

Kempermann, G., Kuhn, H.G. & Gage, F.H. (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature*, **386**, 493-495

King, J., Simpson, B., Overall, K., *et al* (2000) Treatment of separation anxiety in dogs with clomipramine: results from a prospective, randomized, double-blind, placebo-controlled, parallel-group, multicentre clinical trail. *Applied Animal Behaviour Science*, **67**, 255-275

King, T., Hemsworth, P.H. & Coleman, G.J. (2003) Fear of novel and startling stimuli in domestic dogs. *Applied Animal Behaviour Science*, **82**, 45-64

Knudsen, E.I. (2004) Sensitive periods in the development of the brain and behaviour. *Journal of Cognitive Neuroscience*. **16**, 1412-1425

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt B.J., Van Reenen, G.G., Hoptster, H.,De Jong, I.C., Ruis, M.A.W. & Blokhuis, H.J. (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, **23**, 925-935

Landsberg, G., Hunthausen, W. & Ackerman, L. (1997) Behaviour Problems of the Dog and Cat. Butterworth Heinemann: Oxford, England

Landsberg, G., Hunthausen, W. & Ackerman, L. (2003) Handbook of Behavior Problems of the Dog and Cat. Saunders: Edinburg

Lehrman, D. S (1970) Semantic and conceptual issues in the nature-nurture problem. In: Aronson, L. R., Tobach, E., Lehrman, D.S., & Rosenblant, J. S. (Eds.) *Development and Evolution of Behaviour* pp 17-52. W.H. Freeman: San Francisco, USA

Leonard, J.A., Wayne, R.K., Wheeler, R.V., Guillen, S. & Vilá, C. (2002) Ancient DNA Evidence for Old World Origin of New World Dogs. *Science*, **298**, 1613-1616

Lester, D. (1967) Sex differences in exploration: toward a theory of exploration. *Psychol. Rec.*, **17**, 63-64

Levine, S. (1960) Stimulation in Infancy. *Scientific America*, **202**, 80-86

Lieberman, D.A. (1993) Learning: behavior and cognition. Brooks/Cole Publishing Company Pacific Grove: California, USA

Lindell, E. (1997) Diagnosis and treatment of destructive behaviour in dogs, *Veterinary Clinics of North America, Small Animal Practice*, **27**, 533-547

Lindsay, S.R. (2000a) Handbook of Applied Dog Behavior and Training: Adaptation and Learning. Vol. 1., Iowa State University Press: Iowa

Lindsay, S.R. (2000b) Handbook of Applied Dog Behaviour and Training, Volume 2: Etiology and Assessment of Behaviour Problems, Iowa State University Press: Iowa

Lorenz, K. (1935) Der Kumpan in der Umwelt des Vogels. *Journal fur Ornithologie*, **83**, 137-213, 289-413

Lund, J. D. & Jorgensen, M.C. (1999) Separation anxiety in pet dogs behaviour patterns and time course of activity. In: Proceedings of the First Conference of Veterinary Behavioural Medicine. Potters Bar, UK: University Federation for Animal Welfare, pp 133-142

Lundberg, A. & Keeling, L. (1997) High ranking hens are socially facilitated to dustbathe by a video image. In: Hemsworth, P.H., Spinka, M. & Kostal, L. (Eds.). Proceedings of the 31st. International Society for Applied Ethology, August 13th–16th, Prague, pp 32

Mackay, P.C. & Wood-Gush, D.G.M. (1980) The responsiveness of beef calves to novel stimulation: An interaction between exploration and fear. *Applied Animal Ethology*, **6** (4) 383-384

Maestripieri, D. (2000) Measuring temperament in rhesus macaques: consistency and change in emotionality over time. *Behavioural Processes*, **49**, 167-171

Maestripieri, D., Schino, P., Aureli, F. & Troisi, A. (1992) A modest proposal: displacement activities as an indicator of emotions in primates. *Animal Behaviour*, **44**, 967-979

Magnus, E. & Appleby, D.L. (1995) Annual report 1995. *Association of Pet Behaviour Counsellors*. APBC: London.

Manning, A. & Dawkins, M.S. (1992) An introduction to animal behaviour, 4 ed., Cambridge University Press: Cambridge

Markwell, P.J. & Thorne, C.J. (1987) Early behavioural development of dogs. *Journal of Small Animal Practice*, **28**, 984-991

McCrave, E. (1991) Diagnostic criteria for separation anxiety in the dog. *Veterinary Clinics of North America, Small Animal Practice*, **21**, 247-55

McCune, S., McPherson, J.A., & Bradshaw, J. S. W. (1995) Avoiding problems: the importance of socialisation. In: Robinson, I. (Ed) The Waltham Book of Human-Animal Interaction: Benefits and responsibilities of pet ownership pp 71-86. Pergamon: Oxford, UK

McFarland, D. (1999) Animal Behaviour: Psychobiology, Ethology and Evolution. Pearson Education Limited: Essex, UK

McGreevy, P.D., Grassi, T.D, & Harman, A.M. (2003) The visual field of dogs is influenced by skull shape. In Proceedings of the 4th International Veterinary Behavioural Meeting, 18th-20th August 2003, Caloundra, Australia, pp 47-50

McNaughton, N. & Corr, J. P. (2004) A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neuroscience and Biobehavioural Reviews*, **28**, 285-305

McPherson, C. (1998) Aetiology, characterization and prediction of separation-induced behaviour in the domestic dog. PhD-Thesis University of Southampton

Mech, L.D. (1970) The Wolf: the Ecology and Behavior of an Endangered Species. Natural History Press: New York

Melzack, R. (1954) The genesis of early experience on social behaviour: an experimental study of the dog. *Journal of comparative and physiological Psychology,* **47**, 166-168

Melzack, R. & Burns (1963) Neuropsychological effects of early sensory restriction. *Boln. Inst. F.stud. Mdd. Biol. Uni.Nac Mex.* **11**, 407-424

Melzack, R. & Scott, T.H. (1957) The effects of early experience on the response to pain. *Journal of Comparative Physiology and Psychology,* **50**,155-161

Melzack, R. & Thompson, W.R. (1956) Effects of early experience on social behaviour. *Canadian Journal of Psychology*, **10**, 82-92

Miller, P.E. & Murphy, C.J. (1995) Vision in dogs. *JAVMA*, **207**, 1623-1634

Miller, D., Staats, S., Partio, C. & Rada, K. (1996) Factors associated with the decision to surrender a pet to an animal shelter. *JAVMA*, **209**, 738-742

Mills, D.S. & Sheppard, G. (1999) An evolutionary approach to the analysis, assessment and treatment of behaviour problems in companion animals. In: Proceedings of the Second World Meeting on Ethology, pp 61-68

Mills, D.S. (2002) Considerations on the use of pheromonotherapy and pharmacotherapy for the treatment of companion animal behaviour problems. I: Proceedings of the 2002 CABTSG/AWSELVA Study Day, Birmingham, CABTSG-AWSELVA, pp 31-33

Murphree, O.D. & Dykman, F.A. (1965) Litter patterns in the offspring of nervous and stable dogs: 1 Behavioural tests. *Journal of Nervous & Mental Disease*, **141**, 321-322

Nack, R.A. (1999) Managing separation anxiety in a dog. *Veterinary Medicine*, **94**, 704-716

Neitz, J., Geitst, T., & Jacobs, G.H. (1989) Color vision in the dog. *Visual Neuroscience*, **3**, 119-125

Nelson, C.A. (1999) Change and continuity in neurobehavioral development: lessons from the study of neurobiology and neural plasticity. *Infant behaviour & development*, **22** (4), 415-429

Niebuhr, B.R., Levinson, M., Nobbe, D.E. & Tiller, J.E. (1980) Treatment of an incompletely socialized dog. In: Hart, B.L. (Ed.) Canine Behavior, CA. Veterinary Practice Publishing: Santa Barbara

Nobis, G. (1979) Der alteste Haushund lebte vor 14,000 Jahren. *UMSCHAU*, **19**, 610

Nott, H. (1992) Behavioural Development of the Dog. In: Thorne (Ed.). The Waltham Book of Dog & Cat Behaviour. Pergamon Press: Oxford, pp 65-78

O' Farrell, V. (1992) Manual of canine behaviour. Gloucestershire, British Small Animal Veterinary Association, 2 ed.

O'Kleefe, J. & Nadel L. (1978) The Hippocampus as a Cognitive Map. Claredon Press: Oxford

Overall, K. (1997) Clinical behavioural medicine for small animals. St. Louis: Mosby

Overall, K., Agulnick, L., Dunham., A., *et al* (1999) Qualitative and quantitative differences in vocalizations by dogs affected with separation anxiety and unaffected dogs using sonographic analysis. In: Proceedings of the Second World Meeting on Ethology, pp 108-113

Overall, K., Dunham, A. & Frank, D. (2001) Frequency of non-specific clinical signs in dogs with separation anxiety, thunderstorm phobia, and noise phobia, alone or in combination, *JAVMA*, **219**, 467-473

Owen, D., Andrews, M.H. & Matthews, S.G. (2005) Maternal adversity, glucocorticoids and programming of neuroendocrine function and behaviour. *Neuroscience and Biobehavioural Reviews*, **29**, 209-226

Pagani, M., Rimoldi, O., Pizzinelli, P. Furlan, R.L. & Crivellaro, W. (1991) Assesment of the neural control of the circulation during psychological stress. *Journal of the Autonomic Nervous System*, **35**, 33-42

Pageat, P. (1998) Pathologie du comportement du chien. 2eme edition, Editions du Point Veterinaire: Paris

Panksepp, J. (1998) Affective Neuroscience: The Foundation of Human and Animal Emotions. Oxford University Press: New York, USA

Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malec, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A. & Kruglyak, L. (2002) Genetic Structure of the Purebred Domestic Dog. *Sience*, **304**, 1160-1164

Paul, E.S., Harding, E.J. & Mendl, M. (2005) Measuring emotional processes in animals: the utility of a cognitive approach. *Neuroscience and Biobehavioural Reviews*, **29**, 469-491

Pavlov, I.P. (1927/1960) Conditioned Reflexes: An investigation of the Physiological Activity of the Cerebral Cortex, GV Anrep (Trans). New York:

Dover (reprint), cited in: Lindsay, S.R., Handbook of Applied Dog Behavior and Training: Adaptation and Learning. Vol. 1., Iowa State University Press: Iowa

Peichl, L. (1991) Morphological types of ganglion cells in the dog and wolf retina. *The Journal of Comparative Neurology,* **324**,590-602 cited in: Lindsay, S.R., Handbook of Applied Dog Behavior and Training: Adaptation and Learning. Vol. 1., Iowa State University Press: Iowa

Pettijohn, T.F., Wong T.W., Ebert P.D. *et al* (1977) Alleviation of Separation Distress in 3 Breeds of Young Dogs. *Developmental Psychobiology*, **10** (4) 373-381

Pfaffenberger, C.J. & Scott, J.P. (1959) The relationship between delayed socialisation and trainability in guide dogs. *Journal of Genetic Psycology,* **95**, 145-155

Pfaffenberger, C.J. & Scott, J.P. (1976) Early rearing and testing. In: Pfaffenberger, C.J., Scott, J.P., Fuller, J.L., Ginsburg B.E. & Bielfelt, S.W., (Eds.) Guide dogs for the Blind: Their Selection, Development and Training, 13-37, Elsevier: Amsterdam

Platt, D. M. & Novak, M. A. (1997) Video stimulation as enrichment for captive rhesus monkeys. *Applied Animal Behaviour Science*, **52**, 139-155

Pluijmakers, J.J.T.M., Appleby D.L. & Bradshaw, J.W.S. (2003) Sensitive periods in the development of behavioural organization in the dog and the role of emotional homeostasis. In: Proceedings of the 4th International Veterinary Behaviour Meeting at Caloundra, Australia 18-20 August, pp 119-126

Pluijmakers, J.J.T.M., Appleby, D.L., Bradshaw, J.W.S. (2005) 'Does exposing puppies to video images increase behavioural organisation and decrease the potential for the development of fear and inappropriate avoidance behaviour?' Proceedings of the Australian Veterinary Behaviour Interest Group, AVA conference, Gold Coast Australia, May 2005, pp133-143

Pluijmakers J.J.T.M., Appleby D.L., Bradshaw J.W.S. (2005) 'Does exposing puppies to video images increase behavioural organisation and decrease the potential for the development of fear and inappropriate avoidance behaviour?' Proceedings of the Eleventh annual meeting of the European Society of Veterinary Clinical Ethology, Marseille 8-9 October 2005, pp 137-146

Podberscek, A., Hsu, Y. & Serpell, J. (1999) Evaluation of clomipramine as an adjunct to behavioural therapy in the treatment of separation-related problems in dogs. *Veterinary Record,* **145**, 365-369

Powell, S.B., Geyer, M.A., Gallagher, D. & Paulus, M.P. (2004) The balance between approach and avoidance behaviours in a novel object exploration paradigm in mice. *Behavioural Brain Research*, **152**, 341-349

Prescott, M. J., Morton, D. B., Anderson, D., Buckwell, A., Heath, S., Hubrecht, R., Jennings, M., Robb, D., Ruane, B., Swallow, J. and Thompson, P. (2004) Refining dog husbandry and care. *Laboratory Animals*, ***38*** *(Suppl. 1)*, S1-S94

Rooney, N. J., Bradshaw, J. W. S. & Robinson, I. H. (2000) A comparison of dog-dog and dog-human play. *Applied Animal Behaviour Science*, **66**, 235-248

Roy, V. & Chapillon, P. (2004) Further evidences that risk assessment and object exploration behaviours are useful to evaluate emotional reactivity in rodents. *Behavioural Brain Research*, in press

Russell, P.A. (1973) Relationships between exploratory behaviour and fear: A review. *British Journal of Psychology*, **64**, 363-374

Savolainen, P., Zhang, Y., Luo, J., Lundeberg, J. & Leitner, T. (2002) Genetic Evidence for an East Asian Origin of Domestic Dogs. *Science*, **298**, 1610-1612

Schaffer, H.R. & Emmerson, P.E. (1964) The development of social attachments in infancy. Monographs of the Society for Research in Child Development, 29, cited in: Gross, R. (1996) Psychology: The Science of Mind and Behaviour, Hodder & Stoughton, London: third edition, 548-573

Schapiro, S.J. & Bloomsmith, M.A. (1995) Behavioral effects of enrichment on singly-housed, yearling rhesus monkeys: An analysis including three enrichment conditions and a control group. *American Journal of Primatology*, **35**, 89-101

Schilder, M.B.H. & Van der Borg, J.A.M. (2003) Training dogs with help of the shock collar: short and long term behavioural effects. *Applied Animal Behaviour Science*, **85**, 319-334

Schneirla, T. A. (1965) Behavioral development and comparative psychology. *Quarterly Review of Biology*, **41**, 283-302

Scott, J. P. (1958) Animal Behavior. The University of Chicago Press: Chicago, USA

Scott, J.P. (1962) Critical periods in behavioral development. *Science*, **138**, 949-958

Scott, J. P. (1963) The process of primary socialization in canine and human infants. *Monographs of the Society for Research in Child Development*, **28**, 1, 1-45

Scott, J. P. (1968) Early Experience and the Organization of Behavior. Wadsworth Publishing Company Inc: California, USA

Scott, J. P. (1992) The phenomenon of attachment in human-nonhuman relationships. In: Davis, H. & Balfour, D. (Eds.) The Inevitable Bond: examining scientist-animal interactions. pp 72-73. Cambridge University Press: Cambridge. UK

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

Scott, J.P. & Marston, M.V. (1950) Critical periods affecting the development of normal and mal-adjustive social behaviour of puppies. *The Journal of Genetic Psychology,* 77, 25-60

Scott, J.P., Stewart, J.M & DeGhett, V.J. (1974) Critical periods in the organization of systems. *Developmental Psychobiology.* 7. 489-513

Seksel, K., Coyle, W. & Chaseling, S. (2001) Stresses associated with moving house with pets. In: Proceedings of the Third International Congress on Veterinary Behavioural Medicine. pp 47-49

Seksel, K., Mazurski, E.J. & Taylor, A. (1999) Puppy socialisation programs: short and long term effects. *Applied Animal Behaviour Science,* **62**, 335-349

Serpell, J. & Jagoe, A. (1995) Early experience and the development of behaviour. In: Serpell, J. (Ed.) The Domestic Dog: its Evolution, Behaviour and Interactions with People. Cambridge University Press: Cambridge

Simmel, E.C. & Baker, E. (1980) The effect of early experiences on later behavior: a critical discussion. In: Simmel, E.C. (Ed.) Early Experiences and Early Behavior: Implications for Social Development, 3-13, Academic Press: New York

Simpson, B. (2000) Canine Separation Anxiety, Small Animal Exotics Compendium April 2000, pp 328-338

Slabbert, J.M. & Rasa, O.A.E. (1993) The effect of early separation from the mother on pups in bonding to humans and pup health. *Journal of the South African Veterinary Association,* **64**, 1, 4-8

Smith, P. K. (1979) The ontogeny of fear in children. In: Sluckin, W. (Ed.) Fear in Animals and Man pp 164-197. Van Nostrand Reinhold Company: Berkshire, UK

Solokov, E.N. (1960) Neuronal models and the orienting reflex. In: Brazier M.A.B. (Ed.) The central nervous system and behavior. Josiah Macy Foundation: New York

Solomon, R.L. & Corbit, J.D. (1974) An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychological Review,* **81**, 119-145

Spruijt, B. M. (2001) How the hierarchical organization of the brain and increasing cognitive abilities may result in consciousness. *Animal Welfare*, **10**, S77-S87

Stanley, W.C. & Elliot, O. (1962) Differential human handling as reinforcing events and as treatments influencing latter social behaviour in basenji puppies. *Psychological Reports*, **10**, 775-788. Cited in: Sautter, F.J. & Glover, J.A. (1978). Behaviour, Development and Training of the Dog. Arco Publishing Company Inc

Stone, C. P. (1921) Notes on light discrimination in the dog. *Journal of Comparative Psychology*, **1**, 413-431

Takeuchi, Y., Houpt, K.A. & Scarlett, J.M. (2000) Evaluation of treatments for separation anxiety in dogs. *Journal of the American Veterinary Medical Association*, **217**, 342-345

Thompson, W.R., Watson, J. & Charlesworth, W.R. (1962) The effects of prenatal stress on offspring behavior in rats. *Psychological Monographs*, **76**, 1-26

Toates, F. (1999) Stress. Cited in: McFarland, D., Animal behaviour: Psychobiology, ethiology and evolution.. Paerson Education Limited: Essex, 3 ed.

Tuber, D.S., Hothersall, D., Peters, M.F. (1982) Treatment of Fears and Phobias in Dogs. *Veterinary Clinics of North America: Small Animal Practice*, **12**, 4 , 607-623

Turner, D., Appleby, D.L. & Magnus, E. (2000) Annual review of cases 2000. *Association of Pet Behaviour Counsellors*, APBC, UK

Van Reenen, C.G., OÇonnell, N.E., Van der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B. & Blokhuis, H.J. (2005) Responses of calves to acute stress: Individual consistency and relations between behavioural and physiological measures. *Physiology & Behavior*, **85**, 557-570

Vinagradova, O.S. (1995) Expression, Control, And Probable Functional Significance of the Neuronal Theta-Rhythm. *Progress in Neurobiology*, **45**, 523-583

Vincent, J. D. (1986) Biologie des Passions. Odile Jacob: Paris, France

Voith, V.L. & Borchelt, P.L. (1985) Separation anxiety in dogs. *Compendium on Continuing Education for the Practicing Veterinarian*, **7**, 42-52

Wayne, R.K. & Vilá, C. (2001) Phylogeny and Origin of the Domestic Dog. In: Ruvinski, A., Sampson, J. (Eds.) The Genetics of the Dog, CAB I publishing, pp 1-13

Webster, S.D. (1997) Being sensitive to the sensitive period. In: Proceedings of the First International Conference on Veterinary Behavioural Medicine. pp 20-27 Universities Federation for Animal Welfare: Potters Bar, UK

Weerth de, C., Buitelaar, J.K. & Mulder, E.J.H. (2005) Prenatal programmming of behavior, physiology and cognition. *Neuroscience and Biobehavioural Reviews*, **29**, 207-208

Wells, D.L. (2004) A review of environmental enrichment for kenneled dogs, Canis familiaris. *Applied Animal Behaviour Science*, **85**, 307-317

Williams, J. M. G., Watts, N., MacLeod, C. & Mathews, A. (1997) Cognitive Psychology and Emotional Disorders. John Wiley & Sons Ltd: West Sussex, UK

Wolfle, T.L. (1990) Policy, program and people: the three P's to well-being. In: Mench, J.A. & Krulisch, L. (Eds.) Canine Research Environments, pp 41-47, Bethesda, MI, Scientist Center of Animal Welfare

Woolpy, J.H. (1968) Socialisation of Wolves. In: Masserman, J.H. (Ed.), Animal and Human, 82-94, Grune and Stratton

Woolpy, J.H. & Ginsburg, B.E. (1967) Wolf Socialization: A Study of Temperament in a Wild Social Species. *American Zoologist*, **7**, 357-363

Wright, J.C. (1983) The effects of differential rearing on exploratory behaviour in Puppies. *Applied Animal Ethology*, **10**, 27-34

Zimen, E. (1987) Ontogeny of approach and flight behaviour towards humans in wolves, poodles and wolf-poodle hybrids. In: Frank, H. (Ed) Man and Wolf: Advances, Issues and Problems in Captive Wolf Research. Dr W Junk: Boston

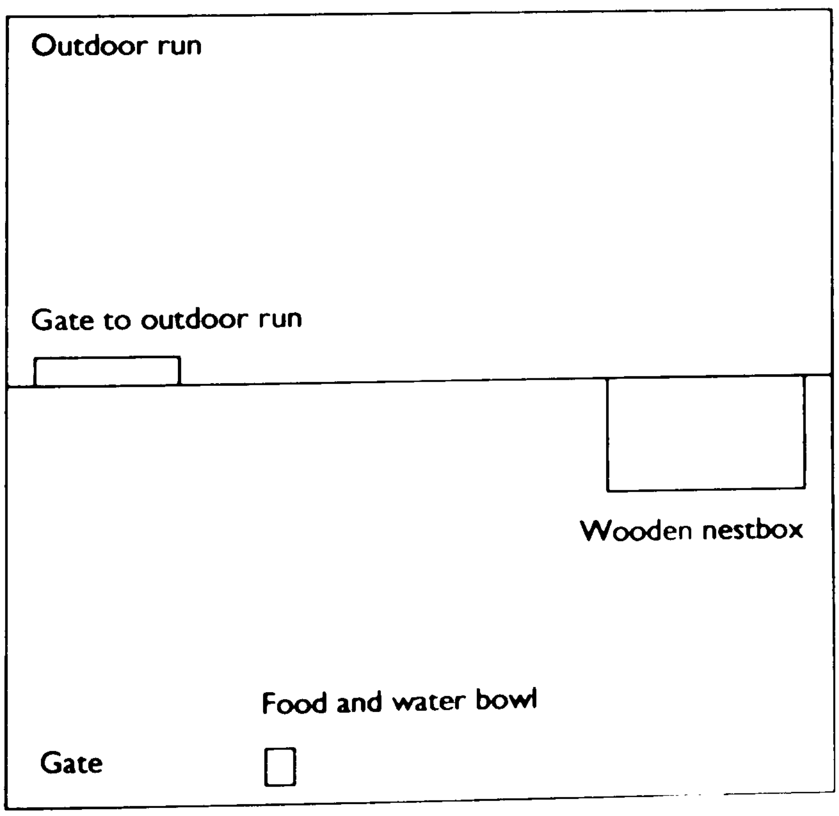
Zimmerman, A., Stauffacher, M., Langhans, W. & Wurbel, H. (2000) Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research*, **121**, 11-20

Appendix I

Living environment of the puppies (Chapters 4, 5, 6)

The dimensions of the kennel could vary between litters but were approximately 2 m x 3 m for indoor and 2 m x 3 m for the outdoor run. The puppies were housed with their littermates and the bitch and had access to the outdoor run through a gate in the wall. In each indoor kennel a wooden nestbox and a food and water bowl was placed. The walls were made out of stone, the front was made of railings and gave view to a corridor and the opposite kennels. The kennels were lightened with day light or artificial light.

The puppies were exposed to humans minimally twice a day during feeding routines in morning and afternoon, and during daily cleaning. Puppies might have received individual exposure to humans during inspections for physical health and/or socialisation sessions of a few minutes to the breeder's and/or visitors.



Appendix 2

Description of video images used (Chapters 4, 5, 6)

Table 1 Overview of stimuli displayed on videotape used during experiment one

Stimuli displayed on videotape used in experiment 1, Chapter 4
Different types of persons e.g. male, female, wearing glasses
Different breeds of dogs
Village
Cars
Buildings
Vacuum cleaner
Flowers
Scooter
Child toy
Roller skates
Trucks

Table 2 Overview of stimuli displayed on videotape used during experiment two and three

Stimuli displayed on videotape used in experiment 2 and 3 (Chapters 5, 6)
Different type of persons (e.g. wearing caps, glasses, dressed differently)
People (male, female, adult, children) playing or training their dogs
Dogs playing
Vacuum cleaner
Trucks
Garage
Brush

Office

Bucket

Scooter

Bicycle

Bicycle pump

Garden furniture

Dogs

Brush

Laundry hanging out

Kitchen

Washing machine

Kettle

Dustbin

Iron table

Living room

People walking through doors

Shopping centre

Scooter

Roller skaters

Children at school

Ball

Terrace

Market place

Hats

Toddlers

Cat

Motors

Toolkit

Prams

Walking sticks

Cows

Tractor

Description of one of the frames displayed on video tape:

- Close up of bicycle standing still
- Woman with dog approaches bicycle walks around the bicycle, rings bicycle bell
- Woman with dog walking in shopping centre passing parked bicycles
- Woman with dog walking in urban environment cyclist passing

Appendix 3

Questionnaire Owner survey (Chapter 6), translated from the Dutch original

This survey is designed to improve our understanding of the development of dogs. By taking the time to fill out this questionnaire you will be helping to improve our knowledge. The success of this survey will to a large extent be determined by the accuracy of the answers. Please answer each question in as much detail as possible.

FIRST SOME QUESTIONS ABOUT YOURSELF AND YOUR DOG

1 What is:
your surname: ....
....
your dog's name?.....................and
breed..

2 What is your dog's registration number?
....

3 What were your main reasons for acquiring this dog? *(Please tick all that apply.)*
- ❑ companionship
- ❑ protection
- ❑ exercise/recreation
- ❑ breeding
- ❑ other

....

4 Your family consists of: *(Please tick all that apply and explain with further detail.)*

❑ adults: number of males .... number of females ....
❑ children: number of children between 0 and 4 years ....
number of children between 5 and 12 years ....
number of children between 13 and 17 years ....
❑ other animals: number of dogs excluding this dog ....
number of cats ....
other species namely .... number.............

5 The area you live in is: *(Please tick one.)*
- ❑ rural
- ❑ urban (e.g. city center)
- ❑ semi-urban (e.g. suburb, village)

6 What kind of living situation do you have? *(Please tick one.)*

- ❑ apartment/flat without a garden
- ❑ small house with a garden
- ❑ large house with a garden
- ❑ farm
- ❑ other

....

7 How is your dog housed? *(Please tick one, and specify if necessary.)*

- ❑ in a living area of your house (i.e. rooms where people live)
- ❑ in a separate area of the house (i.e. rooms which people don't often go into)
- ❑ in an outside building, kennel, garage, barn or shed
- ❑ combination, namely ....If a combination what percentage of the day does your dog spend in a living area of your house? .... %

SOME QUESTIONS ABOUT YOUR DOG'S EARLY EXPERIENCES AND BEHAVIOUR UNTIL THE AGE OF 16 WEEKS

8 At what age did your dog receive its last vaccination? At ....weeks.

(*You can look this up in your dog's vaccination booklet if you are not sure.*)

9 Have you been advised to keep the puppy on your premises and not take it out to public places until it has been fully vaccinated?

- ❑ Yes, by *(Please tick all that apply.)*
  - ❑ the breeder
  - ❑ the veterinary surgeon
  - ❑ the trainer
  - ❑ other dog owners
  - ❑ other

  ....

- ❑ No

10 Did your puppy stay on: *(Please tick one.)*

- ❑ your premises all the time until his vaccination program was completed
- ❑ frequently visit several places away from the house before his vaccination program was completed.

11 How old was your puppy when you started to take it out to different public places (e.g. busy shopping street, places where people congregate) on a frequent basis? ....weeks.

12 Did you introduce your dog to the objects/persons or experiences listed below ?

Please first write down at what age the dog came in contact with the stimuli for the first time and than tick the frequency which applies most for each age group separately. An example: If the dog for the first time encountered traffic when it was 13 weeks old and was exposed to traffic once a day every subsequent week; then please write down in the column 'Age of first contact in weeks': 13. In the column 'From the moment you obtained the puppy until the age of 12 weeks' tick 'never' and in the column 'From 12 until 16 weeks ' tick 'once a day.'

	Age of first con-tact in weeks	frequency from the moment you obtained the puppy until 12 weeks of age						frequency from 12 weeks until 16 weeks of age					
stimuli		several times a day	once a day	several times a week	once a week	almost never	never	several times a day	once a day	several times a week	once a week	almost never	never
adult, female family members, at home													
adult, male family members, at home													
children belonging to your family, at home													
people, the dog has not met before, at home													
people, the dog has not met before, away from home													
children (older than 4 years) the dog has not met before													
babies and toddlers the dog has not met before													
dogs, the dog has not met before, at home													
dogs, the dog has not met before, away from home													
other pets													
livestock													
sights and sounds of domestic appliances (i.e. television, vacuum cleaner, washing machine)													
car travel													
traffic													
children's play area													
places where people congregate e.g. busy shopping street													
the countryside													
sudden loud noises													

13 Did you expose your puppy to the stimuli mentioned in question 12. *(Please tick all that apply.)*

- ❑ by interfering as little as possible
- ❑ by giving food to the puppy when introducing the stimuli
- ❑ by playing with the puppy when introducing the stimuli
- ❑ by petting and/or praising the puppy when introducing the stimuli

14 How often did you lift the puppy up or carried it around? *(Please tick one.)*

- ❑ often
- ❑ occasionally
- ❑ rarely
- ❑ never

15 From the moment you acquired the puppy until the age of 16 weeks, how long has your
puppy been left alone on an average working day? *(Please tick what applies most.)*

- ❑ never
- ❑ less than 2 hours a day
- ❑ between 2 and 4 hours a day
- ❑ more than 4 hours, but less than 6 hours a day
- ❑ between 6 and 8 hours a day
- ❑ other

....

How many days per week has your puppy been left alone for the mentioned period of time in an average working week? .... days per week

16 How often did you walk your dog and for how long?
.........times a day. For how long on average? *(Please describe the duration for every walk separately.)*

....

17 How often did you play with your dog whilst on walks? *(Please tick one.)*

- ❑ never
- ❑ occasionally
- ❑ on approximately half of the walks
- ❑ on most walks
- ❑ on every walk

18 On an average day how long in total did you and/or other members of your family actively interact (e.g. playing, caring, talking, training) with your dog, not including the time you walk the dog? *(Please tick what applies most.)*

- ❑ less than 15 minutes
- ❑ between 15 and 30 minutes
- ❑ more than 30 minutes but less than 1 hour

- ❑ between 1 and 2 hours
- ❑ other ....

19 What sort of games did you or other family members play with your dog. How often a week on average? *(Please tick all that apply and explain with further detail.)*

❑ chasing games	.... times a week
❑ rough-and-tumble	.... times a week
❑ hide and seek	........times a week
❑ search	........times a week
❑ tug of war	.... times a week
❑ fetch	.... times a week
❑ games with other dogs	.... times a week
❑ others	.... times a week
❑ none	

20 Did you go to a training class with the puppy? *(Please tick what applies and specify.)*

❑ Yes

- ❑ to a class with only puppies between the age of 8 to 16 weeks
- ❑ to a class with dogs of mixed ages

How old was your dog when you attended classes?
..........................weeks
What type of course was this (i.e. obedience, agility, hunting)?
....

❑ No

21 How did you punish your dog? *(Please tick all that apply and specify.)*

- ❑ physically namely
  ....
  ....
- ❑ by scolding the dog
- ❑ by a mixture of both physical and verbal punishment
- ❑ other namely
  ....
  ....

22 Did you play audio tapes or CDs to accustom your puppy to sounds, other than music or speech?

❑ Yes, from ....weeks until ...... weeks

- ❑ once a day
- ❑ several times a day
- ❑ once a week
- ❑ several times a week

❑ other namely

....

❑ No

23 In which situations is or has your dog *reacted apprehensively or fearfully* (e.g. by advancing and withdrawing from the stimuli with lowered ears and a lowered tail)? *(Please indicate how frequently your dog has demonstrated this behaviour for each stimulus. If it has never encountered a stimulus of this kind mark 'not applicable').*

stimuli which have elicited *apprehensive or fearful* behaviour	never	less than half the time	more than half the time	always	not appli-cable
adult, female family members					
adult, male family members					
children belonging to your family					
babies and toddlers					
unknown children visiting the home					
unknown children away from home					
strangers away from home					
strangers approaching/visiting the home					
other dogs, at home					
other dogs, away from home					
other pets *(Please specify.)*					
livestock *(Please specify.)*					
traffic *(Please specify.)*					
veterinary examination					
when handled or reached for in other situations *(Please specify.)*					
in restricted spaces e.g. small room					
when getting into your vehicle					
when walking away from home					
sights and sounds of household appliances					
sounds *(Please specify.)*					
other stimuli at home *(Please specify.)*					
other stimuli away from home *(Please specify.)*					

24 When did you first become aware that your dog showed some kind of *apprehensive / fearful* behaviour? *(Please tick one.)*

- ❑ as soon as I got it
- ❑ later on, when the puppy was at the age of about .... weeks

25 In which situations is or has your dog *reacted aggressively (e.g. barks, growls, lunges in an aggressive way or bites).* *(Please indicate how frequently your dog has demonstrated this behaviour for each stimulus.)*

stimuli which have elicited *aggressive* behaviour	never	less than half the time	more than half the time	Al-ways	not appli-cable
one sex of unknown dog *away* from home					
unknown dogs and bitches *away* from home					
opposite sex dog/bitch living *in* the home					
same sex dog/bitch living *in* the home					
known dogs when in possession of food					
other animals *away* from home					
other animals living *in* the home					
unknown people *away* from home					
unknown people coming *to* the home					
people the dog knows when in possession of items it steals					
people the dog knows when in possession of food					
people the dog knows when in possession of own items					
people the dog knows					
new baby or baby becoming independently mobile in the home					
children					
persons the owner interacts with away from home					
dogs or bitches the owner interacts with away from home					
in response to owner's control when reacting to other stimuli					
traffic					
veterinary examination					
when handled or reached for in other situations					
in restricted spaces e.g. small room					
other stimuli/situations than those listed separately *at* home *(Please specify.)*					
other stimuli/situations than those listed separately *away* from home *(Please specify.)*					

26 When did you first become aware that your dog showed some kind of *aggressive* behaviour? *(Please tick one.)*

- ❑ as soon as I got it
- ❑ later on, when the puppy was at the age of about .... weeks.

27 Has your dog ever had an unpleasant/traumatic experience which could explain his expression of fearful or aggressive behaviour? *(Please tick one and specify.)*

- ❑ Yes, with ....
  at the age of ....
- ❑ No

28 Did your dog until he was 16 weeks old develop an illness making it necessary to keep him on your premises? *(Please tick one and specify.)*

- ❑ Yes
  He was ill at the age of .... weeks until .....weeks for .... days
- ❑ No

29 Have you ever asked anyone for advice about or help with a problem of your dog's behaviour?

- ❑ Yes If yes, was it: *(Please tick all that apply.)*
  - ❑ a vet
  - ❑ a behaviourist
  - ❑ an instructor at a training class
  - ❑ a friend
  - ❑ other
    ....
- ❑ No

Describe the problem you asked advice about in your own words

....

....

....

THANK YOU VERY MUCH FOR YOUR COOPERATION. PLEASE RETURN THE FORM TO:

Jolanda Pluijmakers
Herendaal 21
6228 GV Maastricht
Tel.: 043 -3560623
Fax: 043 - 3560624
E-mail: hoc@cuci.nl

Appendix 4

Example letter

Example letter send to veterinary practices in the UK by DA to recruit control group described in Chapters 8 and 9.

Dear

I am in the process of collecting data for a paper on separation anxiety and I would be grateful for your help. The main part of the project concerns the correlation between the age and environment puppies are obtained from and the types of the behaviour problem they are referred to a pet behaviour counsellor for in later life. For one of the data sets I need information about the environments owners obtain their puppies from. To ensure that there is as little bias as possible I would like to collect this data from owners of dogs visiting veterinary practices. This is because it is assumed that most dog owners attend a practice after obtaining their puppy e.g. for vaccinations, and the samples will be representative. However to be able to collect the data in sufficient quantity I need the assistance of colleagues working in veterinary practices.

I would be very grateful if you could arrange for people working in reception to ask clients with dogs from 6 months of age up to 7 years of age to complete one of the short questionnaires enclosed while they are visiting your practice. I have enclosed a stamped addressed envelope for you to use when returning the forms to me. Ideally I need completed forms returned to me by the 18th of March. This may mean that it is not possible to have all the forms completed in that time. If you are in a position to have more questionnaires completed than I have sent you I would be happy if you would allow me to send you some more.

Thank you in anticipation

Yours sincerely

David Appleby Dip CABC

Appendix 5

Questionnaire control group

Questionnaire for control group described in Chapters 8 and 9.

Where Are Dogs Obtained From And Where Do They Live Now?

This survey is designed to improve our understanding of the environments puppies are housed in before moving to live with their owner, and the environments that dogs live in now. By taking just a few moments to answer these few questions about your dog you will be helping to improve the welfare of dogs in general. When you have finished please return this form to the person who gave it to you.

For the purposes of the survey only owners of dogs between 6 months and 8 years of age are being asked to take part.

Thank you for your assistance.

Other than the breeder are you the first owner of your dog? Please circle your answer.

Yes / No

What breed is your dog? Please circle your answer.

Pedigree / A cross between two pedigrees and you know for certain what they are / Mongrel

If your dog is a pedigree, what breed is it? ....

If a cross, write in the breeds of <u>both</u> parents ....

How old is your dog (in years and months)?

.... years and .... months

What sex is your dog? Please circle one answer.

Dog / Neutered dog / Bitch / Neutered bitch

Please circle how many weeks old your dog was when you obtained it?

0 */ 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12 / 13 / 14 / 15 / 16 / 17 / 18 / 19 / 20 / 21 / 22 / 23 / 24

*Bred by current owner

Which <u>one</u> of the following best describes where your puppy was housed before you obtained it? Please circle one answer.

Kennel / Out building / Barn / Shed / <u>Isolated</u> part of the breeder's home /

<u>Living area</u> of the breeder's home / Pet shop / Rescue society /

Did not see where it was housed / Other (please state where)...............................

Your family consists of how many adults (18 years or older)? Please circle answer.

1 / 2 / 3 / 4 / 5 / 6 / 7

Your family consists of how many children (younger than 18 years)? Please circle answer.

0 /1 / 2 / 3 / 4 / 5 / 6 / 7

How many other dogs do you have, *not* including this dog? Please circle answer.

0 /1 / 2 / 3 / 4 / 5 / 6 / 7

Which of the following options best describes your living situation? Please circle one answer.

Apartment, maisonette or flat / house in a street with other houses / house in a rural area (e.g. cottage or farm)

How is your dog housed? Please tick *one* answer.

- ❑ In a living area of your house (i.e. rooms where people live)
- ❑ In a separate area of the house (i.e. rooms that people don't often go into)
- ❑ in an outside building, e.g. kennel shed or garage

When your dog is left alone, does he regularly do one or more of the following? Please circle answer.

- barking Yes / No
- howling Yes / No
- destruction Yes / No
- scratching (e.g. at doorways, carpets) that results in damage Yes / No
- inappropriate defecation or urination in a dog that is otherwise housetrained Yes / No