Exploring domestic rabbit (*Oryctolagus cuniculus)* personality utilising behaviour coding, behaviour testing and a novel behaviour rating tool

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THESIS SUMMARY

The purpose of the study was to attempt to identify personality traits in domestic rabbits (*Oryctolagus cuniculus*) and to evaluate a range of tools, suitable for use in a shelter setting, that can be used to measure personality traits. A literature review highlighted limited evaluation of reliability and validity in rabbit personality research published to date. Additionally, there is a lack of clarity on what is being measured by some behaviour tests that are currently employed in animal personality research and there are limited tools available to measure domestic rabbit responses to humans.

Chapter three highlights several uses of rabbit behaviour and personality data in United Kingdom (UK) shelters. Shelter staff reported uses for understanding the behaviour of an individual rabbit to support the management of the individual while at the shelter and to match the rabbit to the most suitable future home. Challenges facing shelter staff to collect behavioural data for their rabbits centred around a lack of resources, specifically time available for collecting behavioural data. An additional challenge reported by shelter staff was inaccurate information being reported by the person handing the rabbit into the shelter. To ensure any personality assessment tool could be integrated into shelter routines, the tools would need to be relatively quick to complete and should ideally include a range of data collection methods so that a full picture can be available.

In Chapter four, the results of a behaviour rating survey that was distributed to a self-selected pool of rabbit owners or those that worked with rabbits, using social media are reported. The survey was also completed by animal care technicians for rabbits taking part in direct behavioural observations, including a suite of behaviour tests and observations within the home cage. The use of an online survey enabled a large number of participants to take part. Following examination of the reliability of the data

(interrater) and dimension reduction statistics, three components were retained that included 15 of the initial 47 items and accounted for 60.6% of the variance in the data (*n*=1,234). However, sufficient thresholds for inter-rater reliability were not achieved. As intended in the selection of survey items, the retained components accounted for intraspecific social behaviour, human-rabbit interactions (avoidance of humans) and boldness in relation to the environment. However, only the human-rabbit interaction component had sufficient distribution of scores across the sample population to consider this a personality trait.

Behavioural tests are commonly used as measures of an individual animal's personality; however, several tests have conflicting interpretations of the underlying traits that may drive behaviour in these tests. In Chapter 5, a suite of tests were used, reflecting three commonly used test paradigms for domestic rabbits; the open field test, novel object test and a new human interaction test. Five human-interaction items measured were reliable between raters and between tests and two items, location during subtest 3 where the handler was sat inside the door of the enclosure and a combined outcome score for subtest 3, 4 (stroke rabbit) and 5 (pick up rabbit) were retained to create component 2 on the final solution of the principal component analysis. From two variations of both the open field and novel object tests, two components were also derived, reflecting exploration and curiosity in rabbits. These three components were reliable between raters and between tests and accounted for 75.2% of the cumulative variance in the data. The component labelled 'exploration' comprising variables of activity in the open field tests were found to negatively correlate with component 2 from the behaviour rating scale, reflecting avoidance of humans. This is similar to past research in young rabbits where resistance to handling was correlated with activity in the open field.

The use of behavioural observations in the home cage environment is rarely performed for personality assessment in domestic animals due to how time consuming such observations can be. As a requirement for the tools was to be able to be utilised by shelter staff, where time constraints are an important factor, home cage behavioural observations were designed to be quick to complete. Following a pilot test including three hours of observations over the day, it was possible to determine the behaviours that could be observed using video cameras positioned adjacent to or above rabbit enclosures. Additionally, this pilot test revealed that within the times of day available for testing, none were preferable over any other in terms of the range of behaviours observed in 12 rabbits. The main study therefore utilised three five-minute sampling points across the day with the refined ethogram and 30 second focal sampling. It was not possible to complete dimension reductive statistics on the sample of 16 rabbits used for this part of the study, although the behaviours observed in the relatively short time frame did represent activity patterns observed in past research.

Two tools, the behaviour rating survey and suite of behaviour tests, are proposed to be retained for future examination of the utility of these tests in a shelter setting to measure rabbit behaviour and personality. These retained tests would provide information on an individual rabbit's social behaviour (intraspecific), response to humans, boldness in relation to the environment, exploration and curiosity. Future research is recommended to determine the suitability of these tests for use in shelters, and to understand the predictive validity of these tools. That is to understand the usefulness of rabbit personality assessments to identify aspects of behaviour that are stable between different environmental contexts, such as between a shelter setting and within a home following being rehomed.

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KEY TERMS

Animal personality research – refers to research conducted across a wide range of disciplines that study the characteristics of individual non-human animals.

Animals – non-human animals.

Behavioural coding – studies employing behavioural observations where behaviours are recorded, usually with the use of a pre-set ethogram.

Behavioural profile – a description of behaviour demonstrated by an individual at a specific point in time which may be determined through observations or ratings of the individual's behaviour in naturally occurring or test settings.

Behavioural rating – studies employing behavioural observations to rate responses seen based on pre-set criteria, such as Likert scale responses for increasing affective state response.

Behavioural syndrome – the statistical correlation between two traits at population level, within which each individual can be measured and allocated a behavioural type (Sih *et al.,* 2004: Bell, 2007).

Behavioural tests – Behaviour is coded or rated while the focal individual is within a test setting. The test may take place in the home environment or an alternative environment. Solicited response to the test paradigm are the focus of observations.

Boldness/shyness – A frequently studied personality trait in animals (Réale *et al.,* 2007), it may reflect risk taking behaviour at a super-trait level. Rödel and Monclús (2011) interpreted exploration, anxiety and vigilance responses of rabbits as lower order traits of the bold-shy continuum.

Character[istics] – also known as traits.

Dimension – Following reduction statistics, dimensions, also referred to as components or factors, represent clustered variables (items) that load together.

Latency to enter open field (LEOF) - Also known as an emergence test, involves the focal animal being placed in the open field (OF) in a shelter, i.e. box, and latency to enter the OF is scored. The LEOF is considered to be a measure of boldness-shyness (Rödel *et al*., 2006; Perals *et al*., 2017) or fearfulness (Carter *et al*., 2013).

Human interaction test (HIT) – Taking two forms, forced engagement (handling) and optional engagement (approach/intruder tests) the HIT is thought to measure boldness (Rödel *et al.,* 2015; Rödel *et al.,* 2017) as it assesses an animal's response to a potential threat.

Natural setting – is taken to mean the setting familiar to that individual, for example, in captive domestic animals, the natural setting may be the enclosure the animal spends most of its time in. For wild animals, the natural habitat is likely to be the natural setting.

Novel object test (NOT) – Exposure to a novel stimulus by adding an item that the animal has not encountered before to the animal's environment. May be presented in the animal's usual environment or within a novel environment.

Novel substrate test (NST) – An alternative to the novel object test, where an unfamiliar substrate is utilised.

Open field (OF) – Also referred to as the novel arena, the OF is used as the test environment for the OFT and may vary in shape and size but ordinarily has marked out areas that allow measures of activity to be recorded.

Open field test (OFT) – involves the animal being placed in the OF (see above for OF definition) and behaviour is monitored in relation to locomotion and other behaviours. The OFT is a behavioural test designed to measure explorative (Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; Rödel *et al.,* 2017) or anxious tendencies in animals (Gould *et al*., 2009).

Personality [field of research] – the study of the characteristics of an individual or population in relation to behaviour and cognitive processes (Burger, 2015).

Personality [unit of measurement] - "*consistent* [across situations] *and repeatable behaviour at the level of the individual"* (Carter *et al*., 2012a, p.153) where a population demonstrates between-individual variation (Stamps and Groothuis, 2010; Carter *et al.,* 2013).

Temperament – also known as personality (for discussion see section 1.2).

Trait – "*psychological structures*" influenced by biology (McCrae, 2004, p.4), incorporating "*specific aspect[s] of a behavioural repertoire that can be quantified and that shows between-individual variation and within-individual consistency*" (Carter *et al.,* 2013, p.467).

Shelter – Also known as animal rescue centres or rehoming centres, a shelter is any location where people take in, care for and in some cases, rehome pet animals given up by an owner, strayed/abandoned or confiscated by the authorities.

Super-trait – Within trait theory, traits may be studied in hierarchical levels. Super-traits (also known as higher-order traits) supersede lower order (basic) traits and should not correlate with other super-traits. Lower order traits may correlate with other lower order traits that converge on a super-trait.

Rabbit/s – refers to the species *Oryctolagus cuniculus*, wild or domestic breeds, unless stated otherwise.

ACRONYMS

HIT – Human interaction test

LEOF – Latency to enter open field

NOT – Novel object test (large items used in current study)

NST – Novel substrate tests

OF – Open field

OFT – Open field test

PCA – Principal Component Analysis.

RaBRT – Rabbit behaviour rating tool

RSPCA – Royal Society for the Prevention of Cruelty to Animals

RtR – Relinquishment to rehoming. The journey of an animal that enters a shelter from the point of entry to adoption.

RWAF - Rabbit Welfare Association (and Fund)

Chapter 1

Introduction and literature review

Presentations

- July 2016 International Society of Applied Ethology International Conference, Edinburgh, UK - *Why and how should we assess pet rabbit personality?* (poster)
- December 2015 Moulton College Postgraduate Research Symposium, Northamptonshire, UK - *Companion animal personality* (presentation)

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Personality research, the study of an individual's character, has increasingly been applied to the study of non-human animals (hereafter referred to as animals) over the past two decades (Figure 1). Domestic rabbits make an interesting species for personality studies as they have only been domesticated from their wild counterparts, the European rabbit (*O. cuniculus*), for a few hundred years (Vriends-Parent and Vriends, 1989; Irving-Pease *et al.* 2018). Additionally, recent research has highlighted welfare concerns for domestic rabbits in relation to the common use of solitary housing for this social species and handling practices that may cause stress (Rooney *et al.,* 2014; McBride, 2017; Oxley *et al.,* 2019). Research using a group of semi-wild European rabbits (wild rabbits that have lived in a confined area for many generations) has explored the development of personality in young rabbits, specifically looking at vigilance, exploration and boldness (Rödel *et al.,* 2006; Rödel and Monclus, 2011; Rödel *et al.,* 2015; Rödel *et al.,* 2017). This body of work provides a range of behaviour assessment tools that may be used to test personality in rabbits. However, to date only three studies have explored personality in adult rabbits (>1 year) with just one of these utilising behavioural tests that were scrutinised alongside other measures to determine if the tests were valid and reliable (Andersson *et al.,* 2014).

Trait theory provides a framework for the study of personality that accounts for genetic and environmental influences. Trait theory, heavily used within human personality research and more recently adopted by those studying animal personality, seeks to identify where any given individual lies along a continuum of a particular trait (Burger, 2015), in relation to all others within the population being measured. Personality traits are relatively stable over time and across situations for the individual, but individuals

within a population present a range of different characteristics (Réale *et al.,* 2007; Carter *et al.,* 2013). The field of animal personality research has grown over the past two decades and a number of researchers have attempted to provide methodological frameworks to new studies to enhance the quality and replicability of research (Taylor and Mills, 2006; Uher and Asendorpf, 2008; Weiss and Altschul, 2017).

Applied ethologists have adopted personality research methods to support selection decisions of individual animals best suited to functional roles in human society (Voisinet *et al.,* 1997; Svartberg and Forkman, 2002; Hausberger *et al*., 2004; Duffy and Serpell, 2012; King *et al.,* 2012; Foyer *et al*., 2014). However, no such tool is currently available for domestic rabbits (*Oryctolagus cuniculus*). Such a tool may be beneficial when specific traits are desirable for a specific role. For example, where a rabbit may be handled frequently, a bolder rabbit (in response to humans) may be better suited than a shy one (e.g. educational establishments using rabbits as training aides). Additionally, it may be possible to tailor the captive environment to best suit an individual rabbit. For example, if a pet rabbit demonstrates a shy personality during personality assessments conducted in a shelter, it may benefit from a quieter home once rehomed.

Commonly used tools in rabbit personality research include behaviour tests such as the open field test, human interaction tests and novel object tests, among others. Researchers have also utilised surveys that are completed by people knowledgeable about the animal's behaviour and behavioural coding where the animal's behaviour is measured in its natural setting (including the captive environment). The target trait being measured by the behaviour and survey tools have not been consistent between studies of rabbit personality to date (see section 1.6.5) and further research to understand the target traits and correlations between traits in adult rabbits is needed. The purpose of the current study was to attempt to identify personality traits in adult,

domestic rabbits and to evaluate a range of tools that can be used for measuring such traits.

1.2 Rabbits

1.2.1 Wild rabbit behaviour

The family *Leporidae* includes 10 genera of rabbits (24 species) and the true hares (Chapman and Flux, 1990). Although some rabbit species have been successful and have wide distributions, the European rabbit (*Oryctolagus cuniculus*) is relatively limited to only the Mediterranean region in its natural range (Chapman and Flux, 1990), however it has been introduced to other parts of the world by humans. Leporidae are prey species adapted for the detection and evasion of threats. Moveable and large ears help detection of acoustic signal threats and large, side-facing eyes enable the detection of visual threats (Chapman and Flux, 1990). The hind legs of all Leporidaes are elongated to support running. Living in colonies up to 20 adults (Mitchell-Jones *et al*., 1999) social groups demonstrate linear intrasexual rank hierarchies (Rödel *et al.,* 2009; von Holst *et al*., 1999; von Holst *et al*., 2002). However, the structure of these groups is fluid and varies throughout the year (von Holst *et al.,* 1999; von Holst *et al.,*2002).

Rabbits seek refuge in burrows to evade threats and these networks of tunnels and chambers also support females in rearing altricial kits for the first two to three weeks of life (Chapman and Flux, 1990; Rödel *et al.,* 2017). Wild rabbit pups typically leave the nest and start to explore at around 19 days (Rödel *et al.,* 2017) and are weaned from 25 days (Reyes-Meza *et al*., 2011). The juvenile period follows and can be defined as rabbits aged one to four months (Reyes-Meza *et al*., 2011). Rabbits are sub-adult up to the first spring after being born (up to one year of age, Rödel *et al*., 2015), therefore rabbits are considered adult from one year, which would typically align with the first breeding season in wild rabbits.

Rabbit behavioural development is influenced by environmental conditions as early as the second and third postnatal weeks (Kersten *et al.,* 1989) and continues into the juvenile stages (Rödel *et al.,* 2006), however pre-natal physiological factors (such as uterine position and number of litter mates) may influence physiology in a way that has behavioural consequences later in life (Hudson *et al.,* 2011). Links have also been identified between social behaviour in later life and litter size and body mass in semi-wild rabbit pups (Rödel *et al*., 2006; Rödel and von Holst, 2009). Domestic animal breeders, including rabbit breeders, may have an important role to play in the development of domestic animal personality during sensitive periods of development in animals (Hausberger *et al*., 2004; Foyer *et al*., 2014). For example, breeders can ensure optimal conditions are achieved to produce animals well adapted to the environment and experiences they will face in the future.

1.2.2. Rabbit domestication

All domesticated rabbits are thought to have originated from the European rabbit (*O. cunniculus)* (McNitt *et al.,* 2013). Irving-Pease *et al. (*2018) propose that domestication should be thought of as a process rather than a single event, and note that while the earliest records of rabbits being kept by humans dates to Roman times (100 BC), morphological (skeletal) changes only occur from the 1800s. Other sources cite rabbit domestication origins between 500AD and 1000AD, however, rabbits were likely kept in captive settings as a source of food and for their fur (Vriends-Parent, 1989; CAWC, 2006; Buseth and Saunders, 2015) but were not subject to intensive selective breeding until the 1800s (Irving-Pease *et al.,* 2018). During the 1800's it is also thought that rabbits were kept as pets (Vriends-Parent and Vriends, 1989; Carpenter, 2003; Irving-Pease *et al.,* 2018). Rabbits have therefore been domesticated more recently than other companion species. Additionally, unlike other domesticated companion animal species, such as the dog (*Canis lupus familiaris*), for which behavioural characteristics

were selected for during domestication (Clutton-Brock, 1995), morphological features were the focus of selection in the domestic rabbit (Buseth and Saunders, 2015). Specifically, fur type and colour, ear shape and size, and body size were selected for (CAWC, 2006).

The earliest records of distinct rabbit breeds are from 1850 (CAWC, 2006) and currently the British Rabbit Council recognise 80 rabbit breeds within four breed categories (British Rabbit Council, 2016). The breed categories reflect the history of the selection for fur types and colours and include 'fancy', 'rex', 'lop' and 'normal fur breeds'. Domestic rabbits are popularly kept as pets, with approximately 600,000 to 900,000 rabbits kept as pets in the United Kingdom in 2019 (PDSA, 2019; PFMA, 2019), adecrease from five years earlier when 1 million were reportedly kept (PFMA, 2014). Other contexts where domestic rabbits are used include for animal-assisted therapies (Nimer and Lundahl, 2007), breeding for showing and to supply other uses, farmed for meat and fur and within laboratories. Additionally, rabbits are housed within educational facilities as training aids for students taking animal management courses. While there is a body of literature exploring the behaviour of domestic rabbits, particularly in the laboratory setting, there is limited research looking at domestic rabbit behaviour in other settings.

1.2.3 Domestic rabbit behaviour and welfare

Past research has linked persistent hypothalamic–pituitary–adrenal (HPA) axis activation to poor health in captive animals (Ray and Saplosky, 1992). Domestic rabbits are subject to management practices that may put their welfare at risk due to increased HPA axis activity, such as inappropriate enclosure size (Cornale *et al*., 2016), transportation (Liste *et al*., 2008) and relocation to another enclosure (Peric *et al*., 2017). Handling can be an important aspect of husbandry when moving rabbits and conducting health checks (Oxley *et al.,* 2019). However, rabbit handling may also

present a welfare concern owing to their role as a prey species. Rabbits have evolved to interpret approaches from above as threats, for example, a human adult approaching a rabbit from above may be perceived as a threat (McBride, 2017). Exposure during early development (pre-weaning) plays a role in rabbit responsiveness to humans (Bilkó and Altbäcker, 2000; Csatadi *et al.,* 2005; and Dúcs *et al.,* 2009), however, responses of adult rabbits to handling is not well studied. Nor is it known if rabbits have consistent responses over time to being handled. If rabbit responses to humans are found to reflect a personality trait, measuring this trait may be beneficial to enable selection of rabbits best suited to specific situations (e.g. laboratories, pets and educational establishments using rabbits as training aids), supporting the refinement of management practices to the individual rabbit's needs (Krall *et al.,* 2019).

Wild rabbits live in complex social groups and also have a choice of movement to escape potential threats, such as a dominant conspecific. This choice of proximity to conspecifics may not always be available for captive rabbits (Szendro and McNitt, 2012; Valuska and Mench, 2013). Rooney *et al.* (2014) highlighted an increase in non-favourable behaviours, such as aggression towards other rabbits and avoidance of other rabbits),in socially housed pet rabbits. Similarly, Szendro and McNitt (2012) proposed that solitary housing may be preferable for laboratory rabbits, so long as the cage is large and well enriched. However, solitary housing has been reported to negatively impact the frequency of exercise, the range of behaviours observed (Whary *et al.,* 1993; Trocino *et al.,* 2014) and longevity in domestic rabbits (Schepers *et al.,* 2009). Unlike their wild counterparts, pet rabbits are often housed alone. Only 41.9% of pet rabbits were reported to be kept with a conspecific in a survey of pet owners in the United Kingdom (Rooney *et al*., 2014). Possible benefits of housing rabbits alone include a reduction in injuries and negative effects of group housing for subordinates

(von Holst *et al.,* 1999; Szendro and McNitt, 2012). Social grouping is complex in domestic rabbits and the development of tools that could support successful rabbit-rabbit matches would be beneficial. .

1.2.4 Pet rabbit relinquishment and rehoming personality assessments

Past research has identified welfare risks to pet rabbits due to lack of owner knowledge or lack of interest in the rabbit (Mullan and Main, 2006; Schepers *et al*., 2009; RSPCA, 2011; and PDSA, 2013). Studies of rabbit relinquishment have echoed this lack of investment in the rabbit (Ulfsdotter *et al*., 2016) and also owner issues (Ledger, 2010), such as housing issues (Cook and McCobb, 2012; Ellis *et al.,* 2017), as factors affecting the relinquishment of pet rabbits. The Companion Animal Welfare Council suggests that all animals in shelters (also referred to as rescue and rehoming centres) should be assessed for "*temperament [and] response to different environments and stimuli*" (CAWC, 2011, p.4) prior to rehoming, and that this information should be provided to prospective owners so that an informed decision can be made when selecting a pet. For pet dogs, in an attempt to reduce the likelihood of re-relinquishment to shelters, personality tests have been developed that provide a behavioural profile of the individual animal so that it can then be matched to the most suitable owner (Curb *et al.*, 2013). If pet rabbits are shown to have personality traits that can be reliably and accurately measured, it may be possible to implement a similar assessment at point of purchase (pet shop or shelter) that could potentially improve the owner's understanding of the individual animal's needs and their satisfaction as a pet rabbit owner. At present, no means of assessing personality in pet rabbits exists in a format that would be suitable for use in a shelter.

1.3 Definition of personality and associated terms

A number of papers have acknowledged the varied use of terminology within the field of animal personality research and attempted to standardise terminology and associated definitions (Réale *et al.,* 2007; Gosling, 2008; MacKay and Haskall, 2015; Weiss and Altschul, 2017). A search for research articles (conducted on 3rd July 2019 in Science Direct) using the terms, 'animal AND temperament', 'animal AND personality', and 'animal AND "behavioural syndrome" within the title, key words and abstracts, demonstrated that temperament was the most frequently used term for published research (Figure 1). However, the terms temperament and personality have been acknowledged to be used interchangeably across disciplines (Gosling, 2001; Bell, 2007; Réale *et al.,* 2007).

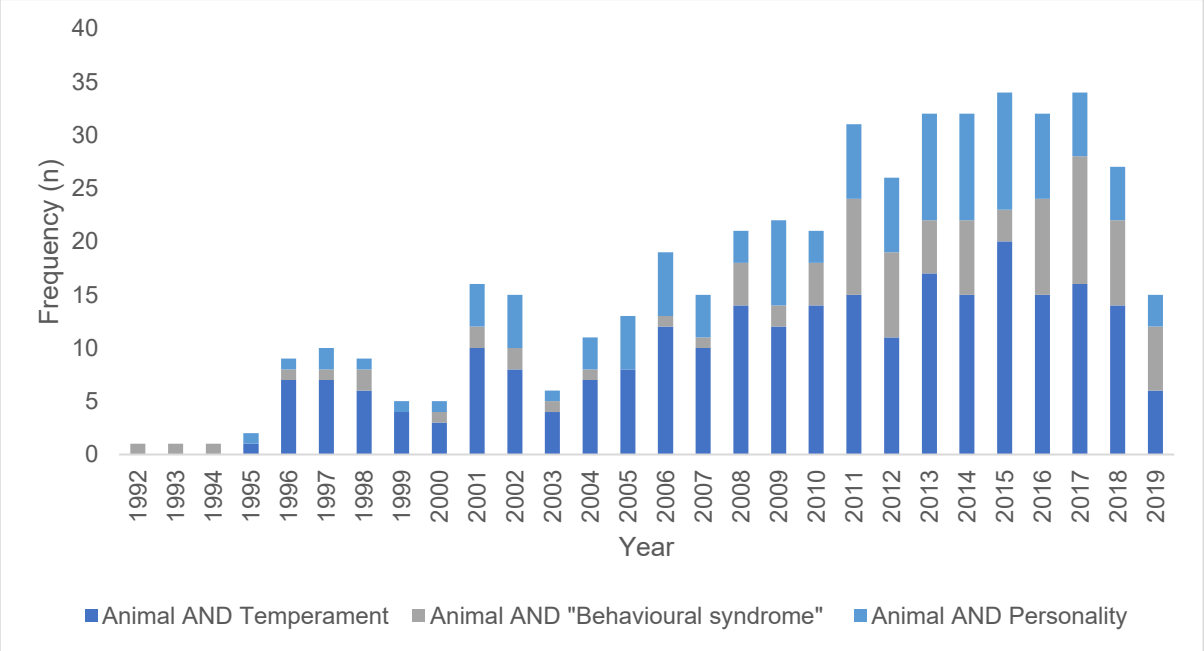


Figure 1 A ScienceDirect search for articles listing animal personality research in the title, key words and abstract for all research and data articles, shows the increase in published work on the topic since the early 1990's.

Personality as a unit of measurement, often assumes a wider definition than temperament (Table 1.1) and is considered in the current study to be the "*consistent* [across situations] *and repeatable behaviour at the level of the individual"* (Carter *et*

al., 2012a, p.153) where a population demonstrates between-individual variation (Stamps and Groothuis, 2010; Carter *et al.,* 2013). Personality is considered to represent the current characteristics of an individual and reflects the culmination of the individual's genetic expression and experiential development, at the point of assessment. The term temperament is used in psychology to refer to inherited, biological pre-dispositions towards certain characteristics (Burger, 2015). When describing temperament, many definitions refer to 'tendencies' to react to environmental stimuli in a specific way and suggest that such tendencies appear early in life (Table 1.1). The emphasis on the biological (inherited) basis for temperaments is explored in behavioural ecology studies seeking to understand the adaptive value and mechanistic features of temperament (Réale *et al*., 2010; Koski, 2014), where fixed behavioural responses to challenge may at first appear maladaptive in changing physical and social environments. MacKay and Haskell (2015) make the distinction that temperament can refer to a behavioural response in a single context, which reflects work in the field of behavioural ecology to develop operational definitions of specific temperament traits to aid the ecological validation of each trait (Sih *et al*., 2004; Réale *et al*., 2007). Weinstein *et al.* (2008) caution against the use of the term temperament over personality where there is no clear reason to differentiate. The authors go on to argue that assumptions of temperament traits being inherited and early appearing, as a comparison to personality, are flawed as both genetic and experiential factors contribute to both temperament and personality development. There appears to be no clear distinction between temperament and personality, therefore temperament is considered synonymous with personality and the term personality is used throughout the current study.

Research examining behavioural syndromes, a term used within behavioural ecology studies (Sih *et al.,* 2004; Réale *et al.,* 2007; Sih *et al.,* 2012.) when studying wild animal

populations (MacKay and Haskell, 2015), focuses on the ecological and evolutionary value of inter-individual differences in personality. It refers to the interplay of two traits, such as boldness and aggressiveness (Sih and Bell, 2008) and can be thought of as the statistical correlation between the two characteristics at population level, within which each individual can be measured and allocated a behavioural type (Sih *et al.,* 2004; Bell, 2007). Although analogous to personality (Bell, 2007), the study of behavioural syndromes is currently limited to wild animal populations to explore individual differences as they contribute to population level behaviour, and the investigation of traits that may have evolved together (Bell, 2007). Within this current study, behavioural syndromes are considered an operational definition for exploring the intercorrelation of two or more traits (Wilson *et al.,* 2019). In the study of animal personality (including temperament) and behavioural syndromes, researchers have used a trait approach to describe individuals in relation to the wider population, or sample of the population being studied.

As terminology is sometimes used inconsistently, approaches to animal personality research also differ, as do the statistical models used to scrutinise new construct development and the results from old and new constructs. A number of researchers have therefore attempted to provide frameworks for others working within this relatively new field of research (Taylor and Mills, 2006; Uher and Asendorpf, 2008; Gosling, 2008; Weiss and Altschul, 2017). The construct development process involving, data reduction statistics, and psychometric analysis procedure for the present study are outlined in Chapter 2.

1.4 Theoretical framework

Personality is considered to be the expression of underlying behavioural control mechanisms (Eysenck, 1967; Gray, 1970). Such control mechanisms are influenced by genetic and environmental factors (internal physiological, e.g. hormones, and

external factors, e.g. physical and social), including during pre and post-natal development (Krueger *et al*., 2006; Groothuis and Maestripieri, 2013; Briley and Tucker-Drob, 2014). Therefore, two animals from the same litter but raised in different environments may differ in their adult personality, and equally, due to the role of genetics, two non-identical individuals reared in the same environment may differ in their adult personality. Trait theory is used to identify an individual's position on a trait continuum (Burger, 2015) and is commonly utilised by those studying animal personality as researchers attempt to describe the traits of individuals in relation to the population level of that trait. As traits represent the behavioural manifestation of the underlying mechanisms that control behaviour (Eysenck, 1967; Gray, 1970) they can be studied through behavioural observations of the individual or population of interest. Réale *et al*. (2007) consider traits and characteristics to be synonymous and define a trait as *"a characteristic of an organism shared by all or some of the individuals of a species that can vary"* (p.293). A personality trait is "*a dimension of personality used to categorise [an individual] according to the degree to which they manifest a particular characteristic*" (Burger, 2015, p.153). Increasing evidence suggests that at least some traits are correlated with each other and between contexts (Bell, 2007).

Trait theory was therefore utilised in the current research as it allowed for the exploration of population level personality types, traits, and is already well established within animal behavioural literature for animal personality assessment. As such, behavioural observations and owner reports of a rabbit's behaviour, can be utilised to explore a range of personality traits in adult rabbits. This data can then be explored through psychometric analysis for reliability and validity, described further in chapter 2. The goal of which would be to identify personality traits that exist in the sample population of adult rabbits, and to test the reliability and validity of the different tools utilised.

Table 1.1. Terminology used in animal personality literature is not used consistently between studies or fields of study; however, the underlying meaning of the terms temperament and personality overlap significantly.

	Definition	Field of study	Reference
Personality	"*The combination of characteristics or qualities that form an individual's distinctive character."*	Common language	English Oxford Living Dictionary (2017)
	"The various aspects of a person's character that combine to make them different from other people."	Common language	Oxford Learner's Dictionary (2017)
	[concerns the way] *"individuals differ in their enduring emotional, interpersonal, experiential, attitudinal, and motivational styles*".	Human psychology personality	McCrae and John, 1992, p.175
	"…consistent behavior patterns and intrapersonal processes originating within the individual"	Human personality psychology	Burger, 2015, p.4
	"…*A specific aspect of a behavioural repertoire that can be quantified and that shows between-individual variation and within-individual consistency"*	Behavioural ecology	Carter *et al.,* 2013, p.467
	"*the phenomenon that individual behavioural differences are consistent over time and/or across situations*"	Behavioural ecology	Réale *et al.,* 2007, p.294
	"…a set of behaviours that are consistent over context and time"	Animal behaviour / ethology	Gartner, 2015, p.102
	"…the individual's behavioural variation in reference to the personality dimensions found in the population"	Animal behaviour / ethology	MacKay and Haskell, 2015, p.470

	Definition	Field of study	Reference
Temperament	*"Temperament: in human research... the inherited, early appearing tendencies that continue throughout life and serve as foundation to personality."*	Human psychology	Gosling, 2001, p.46
	"…aspects of an individual's personality that are often regarded as innate rather than learned"	Psychology / ethology	Weiss and Altschul, 2017, p.180
	"…the characteristic style of emotional and behavioural response of an individual in a variety of different situations that is often, but not invariably, demonstrated very early in life. It is the stance that an individual takes towards its environment across time and situations. It refers to styles of responsiveness and not to specific acts."	Behavioural ecology	Box,1999, p.34
	"…the animal's behavioural response in a single context measured on some biological scale"	Animal behaviour / ethology	MacKay and Haskell, 2015, p. 470
	"*…inherited, early appearing tendencies that continue throughout life and serve as the foundation for personality*"	Comparative (cross species) psychology	Gosling, 2008, p.986
	"…temperament, personality and individuality describe the phenomenon that individual behavioural differences are consistent over time and/or across situations"	Behavioural ecology	Réale *et al.,* 2007, p.294
Behavioural syndromes	*"…addresses the study of correlations at the population level either between the same behavioural trait in two different environmental contexts or between two distinct behavioural traits"*	Behavioural ecology	Réale *et al*., 2010, p.3938
	"Behavioural syndromes occur when _ individual differences are consistent across contexts and are analogous to 'personality' or 'temperament'"	Behavioural ecology	Bell, 2007, p.755

Definition	Field of study	Reference
"Suites of correlated behaviours expressed either within a given behavioural context or across different contexts"	Behavioural ecology	Gosling, 2008, p.986
"…correlations among traits"	Animal behaviour / ethology	Wilson *et al.,* 2019, p.3

1.5 Advances in animal personality research

Animal personality research tends to fall within three categories, with much overlap. Behavioural ecologists seek to explore questions of adaptive value of personality and function and comparative psychologists seek to compare personality between species, including humans, and may explore the origins of personality traits. Applied studies, may seek to explore personality constructs with a view to utilising these to address challenges, such as selecting the most suitable working animals (Sinn *et al*., 2010; Duffy and Serpell, 2012; Harvey *et al*., 2016), selecting suitable candidates for conservation reintroduction programmes (Bremner-Harrison, 2004; McDougall *et al*., 2006; Duckworth, 2008) or to support captive animal welfare management (Gold and Maple, 1994; Wielebnowski,' 1999; Watters and Meehan, 2007; Powell and Gartner, 2011; and Doane and Sarenbo, 2019) and selective breeding practices (Voisinet *et al.,* 1997; Kadel *et al.,* 2006; British Limousin Cattle Society, 2008). The work by behavioural ecologists has provided understanding of the adaptive value of personality in a range of animal species and the work by comparative psychologists has supported the development of methodologies (Uher and Asendorpf, 2008; Gosling, 2008; Weiss and Altschul, 2017). The latter of these lends insights from many decades of learning about human personality and the development of tools and statistical analysis to measure and interpret this latent phenomenon. These benefits can support applied ethologists and welfare scientists when seeking to understand and enhance animal welfare at an individual level.

The identification of personality types that are more likely to have elevated hypothalamic–pituitary–adrenal (HPA) hormone levels (i.e. cortisol and corticosterone) could enable proactive management of these individuals and the adoption of husbandry design and management practices that reduce stress, and therefore promote better health. Research exploring the link between physiological processes

and personality traits has identified a link with the HPA response, in baboons (*Papio anubis*) (Ray and Saplosky, 1992; Sapolsky, 1994), macaques (*Macaca mulatta*) (Capitanio *et al*., 1999 and Capitanio *et al*., 2004) and great tits (*Parus major*) (Carere *et al*., 2003). Circulating HPA hormone levels, such as corticosterone and cortisol, have been linked to personality characteristics such as excitability, confidence, aggression and caution in primate and bird species (Ray and Saplosky, 1992; Capitanio *et al.,* 1999; Capitanio *et al.,* 2004; Carere *et al.,* 2003), and the implications for poor health in species experiencing on-going HPA activation were highlighted as issues for animal health in captive animals (Ray and Saplosky, 1992). As the physiological mechanisms controlling HPA are genetically determined, there is scope for selectively breeding individuals with personalities best suited to the role of the animal in human society (Voisinet *et al*., 1997; Svartberg, and Forkman, 2002; Duffy and Serpell, 2012; King *et al.,* 2012).

While there is a growing interest and a range of uses for animal personality testing in domestic species, the development of valid and reliable assessments is time consuming. Some animal personality research to date has received criticism for the lack of reporting of critical validation and reliability data (Foyer *et al.,* 2013). The use of personality tests as a diagnostic tool in euthanasia decision making at shelters has also been critiqued (Patronek and Bradley, 2016; Patronek *et al*., 2019). While personality tests may have their place identifying the character of an individual in relation to the population as a whole, such tests are not designed to be diagnostic and no test to date has met the criteria for use to diagnose or predict specific traits (e.g. tests measuring food aggression in shelter dogs did not predict the behaviour in the home (Marder *et al*., 2013)). Marder *et al.* (2013) highlight that, although many tools have been developed that could potentially be used at shelters to identify personalities

in pets, issues with the application of such tests may make results invalid. See Chapter 2 for methodological framework and statistical analysis testing plan for this research.

1.6 Literature review – rabbit personality

1.6.1 Search criteria

To gain an understanding about the existence of personality traits in rabbits and methods used to measures these traits, a literature review was conducted. Three search engines were used to locate literature on the topic of rabbit personality (Google Scholar on 5th Jan 2018 (first 100 results were reviewed), Web of Science 13th March 2018, and ScienceDirect on 13th March 2018; all were re-run on 16th July 2019). Six different search terms were included to ensure all terminology in use in animal and psychology fields was reflected, and thus cast as wide a net as possible. The terms 'rabbit' and or 'Oryctolagus' were paired with each of the following; 'personality', 'temperament', 'behavio[u]ral syndromes', 'behavio[u]ral styles'. Rabbits could be wild or domestic but were always required to be of the species *Oryctolagus cuniculus*. When 50 consecutive items were not relevant, the search ended. This was only required for the Google Scholar and Science Direct searches.

Approximately, 71,000 items were identified by the literature search from both 2018 and 2019. A total of 29 relevant articles (59 prior to removing duplicates), determined by title, were screened. Screening included the examination of abstracts. Only research articles that measured rabbit behaviour with reference to stable behaviour patterns or owner report of personality, were retained, resulting in 16 articles used for qualitative synthesis. The majority of papers excluded were not specific to rabbit personality or behaviour assessment but may have referred to the topic (10) and the remaining three were purely examining physiological characteristics. Some of the retained articles did not explicitly state that they had researched rabbit personality,

however, they related to ontogenetic factors relating to behaviour in adult rabbits and so were retained and considered important to understand rabbit behavioural development in a range of situations.

Gartner (2015) found four rabbit personality research articles in her review of pet personality research, so the inclusion of the terms behavioural syndromes and behavioural styles has yielded additional work in the area of personality research in rabbits in this current review. The articles found in the present search were examined to understand the research question being explored by each paper, the methods used, the characteristics of the rabbits being studied, and the traits identified. Not all articles provided all of the information sought (five had missing information). The findings of the review are summarised in Table 1.2.

1.6.2 Literature findings

The papers had a range of research questions; however, one question was examined in seven papers which were seeking to understand the early development of personality traits in rabbits, in relation to ecologically relevant behavioural development (Table 1.2). Rödel and colleagues had authored five of these papers (five), studying a group of semi-wild rabbits housed at the University of Bayreuth in Germany. Eight papers were focused on domestic rabbits, and it could be assumed that a ninth paper (Gosling and Bonnenburg, 1998) also focused on domestic rabbits as rabbits owned by American pet owners participated in this study. Both male and female rabbits were studied in the majority of papers (68.8%, 11/16), with just three focusing on a single sex and two not reporting the sex of the rabbit. The three single sex rabbit papers were all conducted within a laboratory setting and two of the studies sought to explore underlying genetic mechanisms affecting personality development through selective breeding for personality traits.

Young rabbits (pup to sub-adult ages) have been studied more frequently in relation to personality than adult rabbits (75%, 12 of 16 studies only included rabbits <1yr old). As personality is still expected to be developing in young animals and may not be stable until the animal is mature (Koski, 2011), there is still a lack of research describing personality traits that exist in adult rabbits (1 year and over).

1.6.3 Tools used – Behaviour tests

The two most common methods of measuring animal personality include behavioural observations and survey ratings by people familiar with the animals (Gosling, 2008). This was found to be the case in the current literature review with all but one study utilising behaviour observations only or in combination with a survey. Behaviour observations mostly utilised a test paradigm that was expected to elicit specific responses in the rabbits relating to underlying mechanisms controlling these behaviours, such as the open field test (OFT, *n*=9). Other behavioural tests used included human interaction tests (HIT) (*n*=5), predator response tests (*n*=4), novel object tests (*n*=3), conspecific social tests (*n*=3) and a step-down test (*n*=2). Most of these behaviour tests lasted no longer than five minutes with two exceptions (Rödel *et al.,* 2006; Rödel and Monclús, 2011). The wide use of behaviour tests, demonstrates a focus in the literature on a limited number of traits in rabbits. Boldness, as a super trait, is commonly studied in rabbits however there is scope for wider exploration of other relevant traits to rabbits using a bottom-up approach.

In a previous review of behavioural tests used to assess personality in domestic dogs, it was determined that the biological relevance of these experimental tests is not always well supported for the target species (Forkman *et al.,* 2007). While the open field, predator response test and conspecific social test have clear ecological meaning, the other tests, the step-down,= and novel object tests (depending upon the object used)t may not have direct ecological relevance to rabbits. Several studies in the

current literature review sought to further understanding of the traits being measured and provide a basis for understanding how behaviour within some of these tests changes during early development. Each test type is described below, highlighting the biological relevance of each test for rabbits and the traits hypothesised to be measured.

1.6.3.1 Predator response test

Rabbits have been shown to increase vigilance behaviour around predator test models, including fox faeces (Monclús, *et al.,* 2005; and Monclús *et al*, 2006b) and a visual, aerial predator (Monclús and Rödel, 2009; Andersson *et al.,* 2014). However, Rödel *et al.* (2006) also identified that rabbits both investigated the fox odour and demonstrated a lack of HPA reactivity during this test. Andersson *et al.* (2014) also demonstrated an interplay between exploration of novel objects and response to an artificial aerial predator, not only in the clustered item loadings following factor analysis (which may be due to the premature use of a rotated factor analysis prior to determining the factors were divergent) but also in the form of a weak positive correlation (r=0.32, p=0.02) between the response of rabbits to novel objects in a novel environment and response to the artificial aerial predator. Therefore, it could be that the predator test models represented a novel stimulus but not a predatory threat to the rabbits. Alternatively, this result may be interpreted to suggest predator response and exploration of novel stimuli are controlled by the same underlying mechanism in rabbits.

In addition to presenting different predator stimuli (fox faeces or bird of prey model with recorded sounds) the studies also varied in the use of additional stimuli (water or food) placed in proximity to the predator faeces stimuli (Rödel *et al*., 2006; Reyes-Meza *et al.,* 2011; Rödel and Monclús, 2011). The presence of the second stimuli may affect the motivational state of the animal causing the lack of clarity in terms of what trait the test is measuring. These variations across studies also make cross study comparisons

difficult. Age and the presence and proximity of conspecifics also appears to affect scores on the predator response test (Monclús and Rödel, 2008) and consistency of responses over time for an individual has not been demonstrated for this test in the papers reviewed.

While having ecological relevance to rabbit personality, the relevance of predator threat stimuli in domestic rabbits, or the applied value of a tool to assess predator response in domestic rabbits, may be less important than responses of the rabbits to humans that they are likely to encounter through their life. Predator responses were not explored in the present study.

1.6.3.2 Jump-down test

In the jump-down test, the focal animal is placed on an elevated platform and the following measures are taken; latency to jump down from the platform and frequency of jumps down from the platform. The jump-down test is used in two studies located through the literature review. Reyes-Meza *et al.* (2011) reported a relationship between huddle position and results from a jump down test in rabbit pups. Rabbits occupying a peripheral huddle position had a higher frequency of jumps down and shorter latencies to jump down. However, the research group found the opposite to be true in a later study (Rödel *et al.,* 2017) where heavier pups (known to occupy a more central huddle position) were faster to jump down from the platform. While the earlier paper suggested the jump-down test had the ability to discriminate between individuals, in the later study the researchers surmised that developmental factors, particularly motor development, may affect scores to this test. Further work is needed to determine if the test is beneficial to detect individual differences in adult rabbits and to understand any underlying mechanisms, if any, that may control jump-down behaviour. It is unclear if the jump-down test measures a consistent behavioural response in rabbits and so it is not incorporated into the current study.

1.6.3.3 Human interaction tests (HIT)

Tests measuring responses of animals to humans come in many forms, including approach by a human intruder (where the animal may react by approaching, freezing or evading the person) or handling (where the animal is scored on their latency to struggle or the degree to which they struggle). The human-intruder test is thought to assess a response to a threatening situation (Kalin and Shelton, 1989; Gottlieb and Capitanio, 2013) with suggested underlying mechanisms driving responses to this paradigm including activity, emotionality, aggression and displacement (Gottlieb and Capitanio, 2013). As rabbits are a prey species, humans may be a stressor to domestic rabbits (McBride *et al.,* 2006; Bradbury and Dickens, 2016). Two of the rabbit personality papers measured responses of rabbit pups to being handled (Rödel *et al.,* 2015; Rödel *et al.,* 2017) and a third scored responses of adult rabbits to being handled by their owner (Mullan and Main, 2007). This latter paper, and the fourth paper that used HIT's, also measured rabbits' responses to an unfamiliar person, where the rabbit had the opportunity to approach or not (Mullan and Main, 2007; Heker and Lui, 2014).

Rödel *et al.* (2015; 2017) consider the handling test to be a measure of boldness and found that it was correlated with exploration but not consistently. While Rödel *et al.* (2015) found that fast explorers (in an open field test) were less likely to struggle during handling. Rödel *et al.* (2017) found the opposite, where fast explorers were faster to struggle during handling. Both studies utilised the same handling methods and rabbits were the same age during testing.

It is not clear what underlying mechanisms HIT's measure, however the applied value of understanding individual consistencies over time in response to humans may be of value for a range of human-rabbit contexts where rabbits are approached and handled by humans, e.g. pet, educational/training facility, shelter, farm, laboratory and animal assisted therapy. HIT's are explored in the current study to identify suitable measures

for measuring rabbit personality and to explore correlations between HIT scores and other behavioural measures to support the further understanding of relevance of the test for rabbit personality research.

1.6.3.4 Open field test (OFT)

The OFT (also referred to as a novel environment test), used in nine studies in this review, has been reported previously to elicit less activity (measured using a range of variables including: distance travelled, duration of time moving, rearing behaviour, entering the centre of the OF (Royce, 1977)) in more anxious animals and elicit more activity in less anxious animals (Gould *et al.,* 2009). However, recent research has demonstrated that locomotory behaviour in the OFT is more likely to be a measure of exploratory behaviour (Perals *et al*., 2017) including in rabbits (Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; Rödel *et al.,* 2017). The distinction here may relate to the point of entry to the OF, where animals being forced into the OF may be more fearful than those allowed to enter at will (Carter *et al.,* 2013). The OFT may involve placing the focal animal in an OF from which it cannot escape (Walsh and Cummins, 1976), forced entry, or placing the rabbit in a starting box, where the latency to exit the box can also be measured. A pitfall of starting the OFT with the focal animal in a starting box is the limit on the number of variables that can then be recorded should the animal choose to remain within the box.

Latency to enter the OF (LEOF, also known as an emergence test), is considered to be a measure of boldness-shyness (Rödel *et al.,* 2006; Perals *et al*., 2017) and fearfulness (Carter *et al.,* 2012b). Latency to enter the OF has also been correlated with other behavioural variables in young rabbits. Fast explorers of the OFT were less sociable (more likely to be aggressive to conspecifics) and bolder (struggled less in a human handling test) (Rödel *et al.,* 2015). These correlations between variables may reflect a behavioural syndrome, whereby the same underlying mechanism that controls

latency to enter the OF may also control aggressive behaviour and response to humans. Unfortunately, these studies only examined traits within young rabbits (less than 1 year old) and the relationship between traits and the validity of these tests as a measure of personality in mature rabbits, is still poorly understood. Further exploration of the relationship between the variables measured in the OFT is needed to advance understanding of the underlying mechanisms controlling these behaviours and to support the development of clear measures of specific personality traits in adult rabbits.

The lack of standardisation between studies has been criticised in relation to the physical and environmental set up of the OF and the effect of transportation from the home environment to the OF (Walsh and Cummins, 1976; Stanford, 2007). The size and shape of the OF have not been explored in detail to understand the impact that they have on the behaviour of rabbits during testing, however size has been demonstrated to impact activity in other species (Walsh and Cummins, 1976) and so cross study comparisons should be interpreted with caution. The home cage set up (substrate and stocking density) has also been shown to impact rabbit behavioural response in the OFT (Trocino *et al.,* 2004; Buijs and Tuyttens, 2015), which may be an important consideration when using the OFT across populations of rabbits maintained in different environments, such as pet animal studies (Andersson *et al.,* 2014).

Recent work to provide construct validity to the OFT as a measure of personality, and the various variables which can be measured in this test, have demonstrated habituation to the test in rabbits with repeated exposure in relation to distance travelled (decreases over exposure) (Daniewski and Jezierski 2003; repeated three times 14 days apart), latency to leave the start corner and latency to enter the centre (both increasing over exposure) (Buijs and Tuyttens, 2015; repeated over three consecutive days). These studies again use juvenile rabbits which are still developing physically, physiologically and behaviourally and so the effect of repeated exposure to the OFT in

adult rabbits is unknown. Additionally, these studies tested habituation in relatively short time frames. Personality tests should ideally repeat assessments over time, to determine the stability of the traits over time, and so the effect of longer lapses between testing needs to be understood.

1.6.3.5 Novel object tests (NOT)

Novel object tests (adding an object that the animal has not encountered before to the animal's environment) were used to assess neophobia (fear of novelty) in wild rabbits and demonstrated individual variation in response to such items (Sunnucks, 1998) but habituation with repeated exposure was also observed, as found in other species (Hemsworth *et al.,* 1996). In other species, NOT's have been used as a measure of boldness (Carter *et al.,* 2012b; Blaszczyk, 2017) but have also been described as tests of fear (neophobia) and exploration (Forkman *et al.,* 2007; Carter *et al.,* 2013; Buijs and Tuyttens, 2015). However, this supposed disagreement may be more reflective of differences in the level of study of each trait, as some researchers identify boldness as a super trait encompassing fear, anxiety and exploration (Rödel and Monclús, 2011). In any case, there is a need for clearer definitions of investigated traits in animal personality studies (Dingemanse *et al.,* 2007). Three rabbit studies utilised novel object tests and each offered a different underlying mechanism driving the behavioural response to novel objects (reactivity, defined as a scale of timidity to aggressiveness (Gacek *et al.,* 2012) which may be similar to boldness, boldness (Andersson *et al.,* 2014), and fear and anxiety (Buijs and Tuyttens, 2015)).

Novel objects may vary in form but also in presentation. Objects may be added in the animal's natural environment (home cage) or whilst the animal is in a novel environment, such as during an OFT (Andersson *et al.,* 2014). Objects may simply be placed on the ground (Andersson *et al.,* 2014) or suspended (Gacek, *et al.,* 2012; Buijs and Tuyttens, 2015). The presentation of the item may have consequences for how it

is perceived by rabbits, for example, an item that moves may elicit a stronger response in a prey species, than a stationary item.

The items used across the three rabbit studies under review included a suspended ribbon, a suspended plastic bottle, a rubber duck, a wooden pyramid and a ball. The latter three were all presented on the floor. None of the studies justified the selection of the specific items used, reasons for the presentation format used or the real-life relevance to rabbits of the sensory properties of these items, such as the size, shape, colour, movement or olfactory cues provided. Koski (2011) highlights the importance of assessing the real-life relevance of behaviour tests, however, in the present literature review, the NOT appears to have been used for construct validation or discrimination for other tests, as such the NOT has received little attention to understanding the ecological relevance to rabbits. Evidence to justify relevant novel objects and identify the underlying traits NOT may stimulate is still lacking. Novel objects may be more likely to be encountered by domestic rabbits living in artificial environments and therefore warrants further investigation to understand the underlying mechanisms that drive responses to novel objects, in addition to the development of clearer guidance on testing protocols.

Table 1.2: Rabbit personality literature search (*n*= 16) Three search engines were used; Google scholar on 5th Jan 2018 (first 100 entries reviewed), Web of Science 13th March 2018, and ScienceDirect on 13th March 2018, all were re-run on 16th July 2019

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
Behaviour natural setting	Monclús and Rödel, 2009.	Development of personality (a)	64	Semi-wild	<1yr juveniles (m/f) >1yr adult (f)	1	T1. Behaviour coding in semi-natural setting using continuous recording for scanning events while feeding.	2hrs and 18hrs / animal	N	Vigilance
	Eccard and Rödel, 2011	Development of personality (siblings) (a)	55	Semi-wild	5 - 22wks (m/f)	1	T1. Behaviour coding in semi-natural setting using focal animal sampling and continuous recording of chase behaviours.	11hrs avg. trial 1 9hrs avg. trial 2	Y	Aggression
Behavioural tests	Zworykina, Budaeu and Zworykin, 1997	Exploring the use of an operant task to measure individual differences. (b)	14	Chinchilla bred	3-4 months (m)	1	T1. Skinner box (operant task) test following training, repeated test in same test box. Recorded the number of lever presses, food items eaten, frequency of errors, frequency of checking for food behaviour, rearing, active and grooming behaviour.	60min	Y	Exploration

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
	Rafay and Fl'ak, 2010	Underlying mechanisms represented in the OFT (b)	40	NZW,wWild and wild x NZW	57-107 days (m/f)	1	T1. Open Field Test (OFT) individually, recording the number of line crossing with the OF.	5min	N	Reactivity
	Daniewski and Jezierski, 2003	Selective breeding for personality (b)	1,340	New Zealand White (NZW)	42 days - 20 weeks (for weighing) 2 months (at start) (m/f)	1	T1. OFT individually, including latency to exit start box and activity (number of zones entered of 20) in OF.	5min	Y	Activity
	Reyes-Meza *et al.,* 2011	Development of personality (siblings) (b)	12 litters	Chinchilla strain	birth to 120 days (m/f)	7	T1. Huddle position and corticosterone (urine) T2. OFT, including latency to exit start box and activity T3. Jump-down test (two heights). Latency to step down. T4. Social aggression test in novel arena with water bowl for one rabbit, entered as a group, following habituation and water deprivation. Latency to make contact and proximity to other rabbits. T5. Social choice maze. Latency to contact and proximity T6. Threat test. Response to sound of unknown rabbit screaming. Duration/frequency of thumping, rearing, escape attempts or freezing. T7. Predator response maze while water deprived, faeces of fox next to water. Latency to pass the faeces and make contact with the water dish, duration freezing and time spent at furthest point from faeces.	T1. 3,360 'frames' T2. 5min. T3. 3min. T4. 5min, T5. 5min T6. 2min 18 secs T7. 3min	Y	Bold, Proactive

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
	Rödel and Monclus, 2011	Development of personality (a)	15	Semi-wild	130 - 241 days (m/f)	2	T1. OFT, latency to start of activity and exploration. T2. Predator odour response. Duration out of burrow, toilet use, scanning while feeding behaviour.	T1. 24hrs T2. 48hrs	N	Exploration Anxiety Vigilance Boldness as super trait including three above
	Gacek *et al.*, 2012	Underlying mechanisms affecting personality development (genetic) (b)	252	NZW and Termond White	6m for sires (m) Not clear for offspring (m)	1	T1. Novel object in home cage. Categorical responses scored 1-5.	Not stated	N	Reactivity
	Heker and Lui, 2014	Development of personality (experiential) (d)	Not stated	Not stated	Birth - 30 days for pre-conditioning (u) <75 days T1 / T2 (u)	2	T1. Human interaction test (HIT) recording latency of the rabbit to approach the human and proximity to the human. T2. OFT, details not available.	Not stated	Y	Exploration and response to humans (inferred)
	Buijs and Tuyttens, 2015	Underlying mechanisms affecting personality	10	Hycole cross	12 – 15wks (f)	3	T1. OFT including latency to emerge from start corner, on 3 consecutive days. Frequency of rearing and grooming, total distance travelled, the latency to enter the central area and the time spent in centre.	T1. = 5min x 3 T2. 5min T3. 5min	Y	Exploration

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
		development (b)					T2. Novel object in OF arena, plastic bottle filled with water and suspended from a string. Approach latency and movement, rearing and grooming. T3. Social runway maze with 3x unfamiliar rabbits behind wire. Latency to emerge from the start area, the latency to reach the area closest to the unfamiliar rabbits and time spent there, and total distance travelled.			
	Rödel, *et al.*, 2017	Development of personality (physiology) (a)	11	Semi-wild	12 - 17 days (m/f)	3	T1. Handling-restraint test scruffed 50cm above the ground. Latency of struggling during handling. T2. OFT. Distance travelled. T3. Jump-down test. Latency to step down.	T1. 15secs T2. 5min T3. 90secs	N	Exploration Boldness
Survey	Gosling and Bonnenburg, 1998	Exploration of animal personality with established survey (c)	29	Not stated	Not stated	1	T1. Survey, abbreviated 5 factor model including 50 items on 9 point scale.	Not applicable	N	Openness Conscientiousness Extraversion Agreeableness Neuroticism

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
Combined: Behaviour natural setting, tests and survey	Mullan and Main, 2007	Describe personality in rabbits (owner perception) (c)	102	Various domestic	2.2yrs mean (m/f)	4	T1. Behaviour coding in home cage, 30sec instantaneous recording, broad behaviours. T2. Owner survey, one open question with description of personality (adjectives). T3. Response to unfamiliar person. Response from 1m and rating score for response to approach (3 categorical options for score) and during veterinary exam (4 categorical options for score). T4. Response to familiar human (owner). Escape from capture (4 categorical options for score).	T1. 10 min	N	Physically active, Antisocial and negative, Enjoys human company, Mentally alert, Self-assured, Quiet and placid
Combined: Behaviour natural setting and tests	Rödel *et al.,* 2006	Development of personality (social predictors of later behaviour and physiology) (a)	14 - 41	Semi-wild	6 - 13wks old (m/f)	3	T1. Behaviour coding in natural setting. Frequency in close proximity to conspecifics (one-zero sampling at 2 min intervals). T2. OFT and serum corticosterone. Latency to explore and duration moving and scanning. T3. Predator response, faeces next to food bowl. Behaviour coding and food eaten.	T1. 28hrs T2. 24hrs T3. 120hrs	N	Exploration Vigilance

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
Combined: Behaviour natural setting and tests	Rödel *et al.*, 2015	Fitness consequences of personality (a)	19 – 110	Semi-wild	12days – 3months (m/f)	5	T1. Behaviour coding in natural setting. Proximity to nest and emergence day. T2. Behaviour coding natural setting. Social behaviour from 3mths old. Offensive, included chase and displace behaviours. Positive a frequency of close proximity to another instantaneous recording (2min interval). T3. Home range information. Enclosure use grid scores. T4. OFT. Distance travelled, distance travelled in centre and time spent in centre. T5. Handling test scruffed 50cm above ground. Binary score, struggled or not.	T1. n/a T2. 8 – 16hrs T3. 8 – 16hrs T4. 5min T5. 5secs	N	Exploration Handling response Sociable

Type of test	Authors	Purpose and setting	*n*	Breed	Age /sex	No. of tests	Methodology	Duration of time observed (if behaviour)	Test / re-test	Traits investigated
Combined: Behaviour tests and survey	Andersson, Laikre, and Bergvall, 2014	Identify personality traits in domestic rabbits (c)	29 - 61	Seven domestic	3.5months - 7 years (m/f)	6	T1. Novel object (NO) in home cage (rubber duck and wooden pyramid 11.5cm high). Coded position (instantaneous recording 10 secs), frequency of object contacts, latency to the first contact, and handling intensity (1-4 categorical). T2. OFT (4 zones, 10s sampling of position. Scored position / number of observations to give proportion of time across four zones score. T3. NO in OF arena x 2 (rubber duck, different colour to home cage and ball 56cm circumference). Coded position (instantaneous recording 10 secs), number of object contacts, latency to the first contact, and handling intensity (1-4 categorical). T4. Social test in OF arena as a leashed trained rabbit walked external to the arena (0.5–1.5 m). Behavioural responses continuous recording and frequencies of 12 behaviours. T5. Predator response, moving cardboard bird four times over arena with falcon sounds on 1st and 3rd move. Frequencies and duration (%) of time performing 8 relevant behaviours. T6. Subjective questionnaire based on horse tool modified with an experienced rabbit breeder, 68 adjective items with behaviour description, rated on 7-point scale.	T1. 5min T2. 5min T3. 5min T4. 30secs to 3.4min T5. 30secs to 1.1min	N	Coding: Exploration; anxiety; boldness / Subjective Q: Confidence; Sociality; Human-directed agreeableness ; Control

Notes table 1.2:
Sample sizes (*n*) varied between trials and tests in some papers so the lowest and highest number of animals tested are given.
Setting; a captive wild, b laboratory, c pet, d not stated.
Sex, m male, f female, u not stated.

1.6.4. Tools used - Behavioural coding in the natural/home cage setting

Studies utilising behaviour coding in the natural setting (that is, the setting that the individual is normally exposed to which may include captive enclosures) (*n*=5) were predominantly coding for social behaviours (3/5 studies) observed in semi-wild rabbits (4/5 studies) (Table 1.2). Affiliative behaviours were recorded as proximity to a conspecific and aggressive behaviours recorded as chase behaviours in young rabbits. Behavioural coding in the natural setting employ pre-determined ethograms to quantify the frequency of target behaviours in the focal animal, using instantaneous or all occurrence recording (Martin and Bateson, 2007). Young rabbits were studied in four of the five studies and were observed for durations between 10 minutes and 28 hours. The only study to focus on adult rabbits contained just 10 minutes of observations per pet rabbit, five minutes after a handling test with an unfamiliar person present (Mullan and Main, 2007), which may have impacted the rabbit's behaviour. Additionally, this study, and three of the other studies exploring behaviour in a natural setting, only sampled behaviour at one point in time, and so there is a lack of evidence to support the traits explored being stable over time. There is a gap in knowledge of adult rabbit personality (wild or domestic) stemming from observations in a natural setting. The present study aims to address this gap.

1.6.5 Tools used - Surveys

Three studies reviewed utilised surveys to be completed by a person knowledgeable about the individual rabbit, all of which targeted pet rabbit owners. Two of these tools utilised adjective rating questionnaires with behavioural descriptors for each adjective scored on a Likert scale. These surveys utilised a top-down approach as they were initially designed for other species and modified for the target species. The tools used had been initially developed for other species including humans (Gosling and

Bonnenburg, 1998) and horses (Andersson *et al.,* 2014). The third study asked one open response question where owners provided adjectives to describe their rabbit's behaviour (Mullan and Main, 2007) (Table 1.2). While this approach may not reflect standard practices for measuring personality, the grouped descriptors, which weresubjectively grouped by the authors, do show some overlap with rabbit personality traits explored in other studies. For example, the authors identified collections of adjectives that grouped into activity, social behaviour, and self-assuredness categories, the latter of which may reflect boldness (\Mullan and Main, 2007).

While Gosling and Bonnenburg (1998) reported rabbit scores in relation to fixed traits expected to be extracted from the modified human five factor model (openness, conscientiousness, extraversion, agreeableness and neuroticism), Mullan and Main (2007) subjectively grouped their free choice adjectives into six groups. In contrast, Andersson *et al.* (2014) explored rabbit personality using data reduction techniques, to explore the latent properties between all measures, resulting in four traits being proposed (confidence, sociability, human-directed agreeableness and control).

Just one study utilised a rating survey alongside other assessment tools for validation. Andersson *et al.* (2014) utilised a suite of behaviour tests alongside the modified horse behaviour rating survey but did not find consensus between the two methods in terms of the factors derived following data reduction. This may in part be due to the decision to use a survey designed for another species. Additionally, the items on the adjective rating survey (with behavioural descriptors) tool used did not overlap with the behaviour tests items recorded (OFT, NOT, social test and predator response were not well represented in the survey items). This makes the examination of concurrent validity, testing if the novel tool is measuring what it is expected to measure, impossible. While Gosling (2008) highlights the value of owner rating surveys, Koski (2011) identifies conflicting findings from primate studies in relation to consensus

between behavioural and survey rating tools. Some items used in the Andersson *et al.* (2014) survey (for example, "*Helpful toward humans: Subject is willing to assist, accommodate, or cooperate with humans*" (supplementary material)) are difficult to contextualise in relation to rabbit behavioural biology, which were strongly represented in the behaviour tests employed and no human interaction test was utilised.

1.6.6 Confounding variables

Sex differences were not observed in OFT's (Kersten *et al.*, 1989; Rödel *et al.,* 2006) or predator response tests (Rödel *et al.,* 2006) for juvenile rabbits. While not reported in the personality research reviewed here, there may be sex differences in rabbit responses to humans (d'Ovidio *et al.,* 2016, owners reported females were more aggressive to strangers). There are limited studies exploring personality in adult rabbits of either sex or accounting for neutered status. Such variables will be investigated in the current study.

Animals may also habituate to the handling context (Heker and Lui, 2014) and the OFT when used in less than two-week intervals (Daniewski and Jezierski, 2003; Buijs and Tuyttens, 2015). No research was found to demonstrate habituation to exposure over longer durations.

1.7 Summary of literature review and rationale

Due to the various interpretations of the different behavioural test paradigms used in animal personality research, it is recommended to use multiple measures to assess personality across various contexts. This helps to avoid inaccurate conclusions about the underlying mechanisms driving behavioural measures commonly used to measure traits (Carter *et al*., 2012b). Several studies in the review utilised multiple behavioural tests but just four utilised multiple methodologies and these were rarely examining the same traits, as would be needed to demonstrate concurrent validity (see Chapter 2).

The existing body of literature on rabbit personality is also strongly biased towards young, semi-wild rabbits. Such work has provided evidence for the existence of traits in rabbits but the stability of such traits into adult life are not understood, nor are the effects of sex or domestication on the presentation of such traits. No studies were identified that attempt to describe personality in pet rabbits with a view to designing a tool that could be used for matching rabbits to suitable situations, e.g. pet or educational facilitation.

1.8 Research aim and objectives

The purpose of the current research was to attempt to identify personality traits in domestic rabbits (*Oryctolagus cuniculus*) and to evaluate a range of tools that can be used for measuring such traits. Using a combination of psychology and behavioural ecology approaches, both top-down and bottom-up methods were combined to provide practical tools to assess the personality of adult, domestic rabbits. A series of behavioural observations, within the home cage i.e. a natural setting, and in experimental settings, were conducted to explore the presence of consistent behavioural reactions within individuals over time (the basic components of personality) and a behaviour rating survey was designed specifically for rabbits, allowing cross tool validations across the three methods for a sample of rabbits. To support future refinement of the tools for use within a shelter setting, a survey was distributed to shelters that rehomed rabbits within the United Kingdom to understand current methods used to determine the personality of the rabbits and any challenges the centre staff might face in using personality assessment tools in practice.

Aim - To identify personality traits in domestic rabbits and develop tools that can be used to explore personality traits in adult domestic rabbits within a shelter setting.

- Objective 1: Explore information gathering activities at rabbit shelters in relation to the rabbit's behaviour, personality and rehoming procedure to understand challenges in collecting and using rabbit personality information in shelters and support the development of appropriate assessment tools.
- Objective 2: Investigate personality traits in adult, domestic rabbits through the development of personality assessment tools that could be used within applied settings.

    a) Development of a behaviour rating survey tool to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

    b) Development of a suite of behavioural tests to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

    c) Development of behaviour coding tool for use within the home cage to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

Chapter 2

Personality tool development framework and psychometric (validity and reliability) standards

CHAPTER 2: PERSONALITY TOOL DEVELOPMENT FRAMEWORK AND PSYCHOMETRIC (VALIDITY AND RELIABILITY) STANDARDS

2.1 Personality tool development framework

A combination of top-down (using pre-existing tools and with target traits in mind) and bottom-up (exploring traits of biological relevance to the animal being studied or casting a wide net to capture any possible traits) methodologies are recommended to explore personality in animals (Uher and Asendorpf, 2008). This is beneficial when seeking to understand what personality constructs exist in less studied species as it does not assume that constructs will be the same across species that have been subject to different selection pressures. This combined approach was utilised in the current study.

The test methods commonly employed in animal personality studies include survey rating tools (adjective or behaviour based) completed by a person familiar with the focal individual, behavioural tests (designed to explore a specific aspect of behaviour, e.g. open field test, see Chapter 1 for review of behavioural tests used in rabbit personality studies) and natural setting behavioural observations (which may include undisturbed behaviour in a captive setting, such as the home cage) (Weiss and Altschul, 2017). Studies comparing behavioural observations and survey rating methods for cross test reliability recommend that both methods be employed where possible (Gosling, 2008; Carter *et al.,* 2012a; Carter *et al.,* 2013). In the current study, all three methods (survey rating tool, behaviour tests and behavioural observations in a natural setting / home cage) were employed. The tests have been designed to be relatively quick to complete, so that the resulting tools may have practical application where time constraints maybe an issue (e.g. in rescue and rehoming shelters) (Mornement *et al.,* 2014).

In the current study, personality as a unit of measurement is defined as "*consistent* [across situations] *and repeatable behaviour at the level of the individual*" (Carter et al., 2012a, p.153) where a population demonstrates between-individual variation (Stamps and Groothuis, 2010; Carter *et al.,* 2013). The population must demonstrate variation, that is, a range of responses are observed across individuals being studied. The identified traits must demonstrate across-situation consistency, that is, scores should correlate across tests for traits that would be expected to manifest across a range of situations, such as activity levels in the home cage (situation 1) and in a novel environment (situation 2). To be repeatable at the individual level, there must be consistency of scores made over time (test – retest reliability). Psychometric measures have been developed for use within psychology to support the development of tests for measuring latent qualities, including personality, and are commonly used in animal studies to identify reliable and valid assessment tools.

2.2 Psychometric measures

As discussed above, terminology used within animal personality research varies, and the same is true for terminology relating to validation and reliability testing. The terminology used in the present study is defined and employed in past research (Taylor and Mills, 2006; Sinn *et al.,* 2010). While reliability is often considered the first step of psychometric testing, content validity must be addressed initially when designing tools for personality assessment, and so is addressed first in the current study, followed by reliability measures and remaining validity measures. These were conducted in the order provided in the table, with a few exceptions which are described in the results section of the relevant chapters. Table 2.1 outlines the psychometric criteria employed in the present study, including definitions for each criterion and the standards used for each of the three personality tools utilised in chapters 4, 5 and 6.

Development of each tool is described in the following Chapters, along with results of the analysis process outlined in Table 2.1.

Table 2.1. Psychometric criteria employed in the present study, including a definition for each criterion and the standards used for each of the three personality tools utilised. Terminology and definitions for the purpose of each test are based on Taylor and Mills (2006) and are widely used in other studies.

Criteria	Purpose	Behaviour rating tool	Behaviour tests	Home cage observations
Content validity	Ensuring that the tool has the scope to fully test what it is supposed to.	Item list developed from peer reviewed studies. Pilot test conducted and participants were asked to identify any behaviours they felt may be missing. Three pilot test participants had at least post-graduate animal behaviour qualifications and rabbit behaviour experience.	Validated tests utilised except for HIT which is developed from studies in other species.	Ethogram developed from peer reviewed studies and behaviours selected to represent specific behaviour categories under investigation.
Analysis of distribution	A range of scores to each variable are required within the population, to demonstrate inter-individual differences within a population	Items screened for distribution using frequencies of responses to each scale point (1-5) where any item with <1% of responses on two or more scale points were examined more closely, with the potential to exclude from further analysis.	Mean / median (as appropriate to data type) and minimum and maximum scores are examined.	
Inter-rater reliability (consensus)	Two testers' scores are reliable when scoring the same individual at the same point in time.	Intra-class correlation coefficient, two-way random effects model (ICC(3,f) for consistency, Single Measures form ICC(m,1) (one rater going forward). Criteria: > 0.5 (moderate upwards) retained (Koo and Li, 2016; Trevethan, 2017).		Single observer.

Criteria	Purpose	Behaviour rating tool	Behaviour tests	Home cage observations
Test re-test reliability (consistency)	The individual is scored the same way when tested at two separate time points.	No re-test	Kendall's tau-b (τ_b) correlation coefficient was used for dichotomous variables (Harvey *et al.,* 2016), and Spearman's rank correlation (*Rho*=) was used for ordinal and continuous data (Sinn *et al.,* 2010) where variables were not normally distributed Criteria: p<0.05, retained at *Rho*= >0.43 based on average achieved in meta-analysis across studies in adult dogs (Fratkin *et al.,* 2013), however >0.6 is preferable (Ley, McGreevy and Bennett, 2009b)	Pearson's or Spearman's rank correlations were used for parametric or non-parametric variables, respectively (Sinn *et al.,* 2010) Criteria: p<0.05, retained at *Rho*= >0.43 based on average achieved in meta-analysis across studies in adult dogs (Fratkin *et al.,* 2013), however >0.6 is preferable (Ley, McGreevy and Bennett, 2009b)
Data reduction	Identify grouped measures (items/behaviours) and remove any items that do not load at sufficient cut offs per component. Component (dimension) reduction criteria were pre-set prior to data analysis. Clark and Watson (1995) suggest that lower order (narrow) traits should contain 4 to 5 moderately correlated items and that broader	Stage 1 Exploratory Principal Components Analysis (PCA) - Correlation matrix as the scales for the variables differed. - No rotation (Foyer *et al.,* 2013) - Item loadings set to >0.4 (Clark and Watson, 1995) or >0.5 where sample size is <100 (Budaev, 2010) and communalities are presented. • Kaiser-Meyer-Olkin (KMO) >0.05, Bartlett's test of sphericity <0.05 (Budaev, 2010). • Components with eigenvalues >1.0 were retained (Foyer *et al.,* 2013) • Complex items were avoided, retaining the item on the component with the higher loading. • Where loadings were similar (within 0.05) across two or more components, the item was retained on the component that gave better face validity. • Trivial components (less than two items loading at >0.55) were removed (Comrey and Lee, 1992).		

Criteria	Purpose	Behaviour rating tool	Behaviour tests	Home cage observations
	trait dimensions ought to include about 35 items.	Stage 2 PCA • As above criteria for stage 1 PCA but with rotation and communalities are reported for review of the variance that can be explained for each item within the extracted components. • Rotated using both orthogonal rotation (varimax) and oblique rotation (Direct oblimin) since it was not yet known if the components were correlated or not. Both rotations were reviewed along with correlations between component scores (Bartlett's method) to retain the structure that gave better content (face) validity and selecting the rotation determined on the presence or absence of correlations between component scores. The orthogonal rotation assumes the components are independent and the oblique assumes the components are correlated (Kline, 1994) (Item loadings set to >0.4 (Clark and Watson, 1995)). • Retained items and components were examined for content validity and internal consistency.		• Not conducted due to sampling adequacy not suitable in stage 1 PCA
Content validity (Face validity)	See above	• Review of item loadings for each component to ensure items are loaded logically.		Not required as dimension reduction was not possible
Internal consistency (reliability)	Items in each component should all measure the same thing, so the items should be correlated with one another (inter-item correlation).	• Correlation matrices were examined (items correlating <0.15 were excluded, 0.15 – 0.50 for the majority of items (and the mean average) was considered ideal (Clark and Watson, 1995). • For the RaBRT only, Cronbach's coefficient alpha (criteria: > 0.8, Clark and Watson, 1995) • Items with non-ideal correlations were reviewed and items were removed if required, reporting the further mean inter-item correlation.		Not required as dimension reduction was not possible

Criteria	Purpose	Behaviour rating tool	Behaviour tests	Home cage observations
Final PCA	Final selection of items to retain and identification of other items they are linked to prior to construct and concurrent validation.	Stage 3 – Final PCA solution retained • The remaining items were run through a third PCA to extract the simple structure. • Rotated using both orthogonal rotation (varimax) and oblique rotation (Direct oblimin) since it was not yet known if the components were correlated or not. Both rotations were reviewed along with correlations between component scores (Bartlett's method) to retain the structure that gave better content (face) validity and selecting the rotation determined on the presence or absence of correlations between component scores. The orthogonal rotation assumes the components are independent and the oblique assumes the components are correlated (Kline, 1994) (Item loadings set to >0.4, or >0.5 where sample sizes were below 100 (Clark and Watson, 1995)).		Not required as initial dimension reduction was not possible
External (population) variation	Exploration of population level factors that may drive variation in test scores, such as age and sex.	• Mann-Whitney U tests were used to examine differences of component scores by sex • Kruskal-Wallis tests were used to compare component scores to the age of the rabbit and accommodation category assigned (confidence interval set at 95%, p value set at 0.05). • The PCA (using suitable rotation, determined as described above) was re-run for two groups, male and female, to explore sex differences in traits between the tools.	• Independent t-test or Mann-Whitney U tests were used to examine differences of component scores by sex. • One-way ANOVA with Tukey post hoc analysis and Kruskal-Wallis were used to determine if component scores differed by site tested for the behaviour tests. •	Not required as initial dimension reduction was not possible

Criteria		Purpose	Behaviour rating tool	Behaviour tests	Home cage observations
Construct validity (consistency or lack of)	**Discriminate and convergent**	Where a tool measures more than one trait, understanding if the dimensions represent the same or different underlying drivers can be explored through discriminate and convergent validity. • Discriminate validity assumes all items on a scale measure either one trait or another with no overlap. • Convergent validity allows for correlations between scales that are thought to measure related dimensions.	• Spearman's correlation coefficient was calculated between the scores of the retained components (>0.7) (Mirkó, *et al.,* 2012) • Component scores were calculated for each tool: o Behaviour tests – generated in SPSS using Bartlett's method (Yong and Pearce, 2013) o Rating survey - the total score for all items on each component was divided by the number of items multiplied by 5 (the max score per item), giving a score between 0 and 1 for each component (rating survey only) (Wright, Mills and Pollux, 2011) and each component was correlated against the internal validation subjective rating questions.		Not required as initial dimension reduction was not possible
	Concurrent validity (correspondence)	The extent to which the tool is connected to an external outcome, usually by comparing findings to a validated tool already in use.	Described in each Chapter and conducted at the component level for the behaviour rating tool and behaviour tests and at the item level (individual overserved behaviours) for the home cage observations, due to no component scores being generated. The OFT, NOT and LEOF tests may be considered validated tools for rabbit personality assessment, however all tests are compared to be thorough.		

Chapter 3

Information gathering process in rabbit rehoming shelters

CHAPTER 3: INFORMATION GATHERING PROCESS IN RABBIT REHOMING SHELTERS

3.0 Objectives

Objective 1: Explore information gathering activities at rabbit shelters in relation to the rabbit's behaviour, personality and rehoming procedure to understand challenges in collecting and using rabbit personality information in shelters and support the development of appropriate assessment tools.

3.1 Summary

There is a lack of research exploring pet rabbit relinquishment and the processes that support the rehoming of rabbits. To identify current industry practices around rabbit relinquishment and rehoming, a survey was distributed to UK rabbit rehomers and rehoming shelters to identify the information gathering processes used to collect specific information about each rabbit while at the shelter and the use of such information to support rehoming. Important factors affecting the collection of behavioural, including personality, information while at rehoming shelters included challenges with information provided by previous owners, where it may be unreliable or unavailable if the rabbit was strayed. Challenges with collecting information on site included the lack of time and physical resources. All participants reported uses for behavioural and temperament information, but no standardised tools were found to currently exist for use with domestic rabbits in the rehoming shelter setting.

3.2 Introduction

Pet rabbits are the third most popular pet in the UK, but unfortunately many are relinquished and pass through shelters (Ellis *et al.*, 2017) or are rehomed through online adverts (Neville *et al.,* 2018) each year. The Rabbit Welfare Association (and

Fund) (RWAF) suggested that approximately 67,000 rabbits were being relinquished through shelters each year (RWAF, 2012) and a recent study found over 200,000 online adverts for rabbits to be rehomed within a two-year period in the UK (Neville *et al.,* 2018). Despite the large number of rabbits being relinquished by their owners, no research has explored strategies used to support the rehoming process for this species.

Behavioural tools have been developed for use within the rehoming shelter setting for assessing domestic dog and cat personality. Such tests commonly require a knowledgeable person to complete a survey about their experience of the animal's behaviour (Mirkó *et al.,* 2012; Lee *et al*., 2007) or behavioural test scenarios that can be conducted on site (ASPCA®'s Meet Your Match® Weiss *et al.,* 2015; ASPCA®'s Feline-ality™ Slater *et al.,* 2013; Dowling-Guyer *et al.,* 2011; Siegford *et al.,* 2003; Svartberg and Forkman, 2002). Tests developed for assessing dog behaviour in this setting have historically focused on aggressive behaviours to ensure that the animal is safe to be rehomed. However, aggressive behaviour is likely to be less of a safety concern when keeping domestic rabbits which are less likely to inflict injuries on their owners than cats or dogs (Chan *et al.,* 2017). Other factors about a rabbit's behaviour (e.g. compatibility with other animals) and personality may be important considerations for shelter staff and for adopters (O'Connor *et al.,* 2017).

Shelters can take on many forms from home-based operations with just a few rabbits in the care of foster carers, to much larger operations that take in a much wider range of species. A recent survey of shelters in the United States of America identified differences between shelter types and how information on resident animals was managed (Vinic *et al.,* 2019). Additionally, the study highlighted that less than 75% of shelters kept records about resident animals' 'medical or behavioural history', although by grouping medical and behavioural information into one category it is unclear if both

or just one of these is collected at each shelter. In the same study, a much lower proportion of dog shelters (54.2%) reported keeping records of behaviour evaluation results (Vinic *et al.,* 2019). There is little research to date that explores the impact of shelter size and staffing levels at UK shelters on the type and quality of information that is obtained during the relinquishment-to-rehoming processes (RtR). Additionally, the nature of behavioural information collected, and its use, is rarely reported outside of research into the development of specific behaviour evaluation tools.

The aim of this study was to understand if and how behavioural, including personality, information is being obtained during the rabbit RtR process at UK shelters and how this information is used. By understanding current practices and challenges around behavioural information collection, the results will support the development of a rabbit personality assessment tool that may be used to support rabbit rehoming shelters during the RtR process.

3.3 Methods

An incentivised questionnaire survey was designed for completion by personnel at UK rabbit shelters. The survey focused on the shelter's human resources and the collection and use of rabbit behavioural and personality information.

3.3.1 Survey design

The first page of the survey contained a participant information sheet and voluntary opt in tick boxes. The survey was approved by the Postgraduate Ethics Committee at the University of Northampton on 9th November 2016. The survey was reviewed by two specialists in rabbit behaviour and familiar with rabbit RtR in the UK (Volunteer Coordinator and Fundraising Officer at the RWAF and RSPCA's Scientific Officer for Companion Animals). The specialists reviewed the survey to ensure that the language used was accessible to shelter staff and that the categories provided for multiple choice

questions were sufficiently broad, they could suggest additional or alternative choices if needed.

The survey (Appendix 1) consisted of 22 questions including a mix of open-ended responses and single choice selections with space to provide further details. Five questions were about the rehoming shelter and the person completing the survey, including: 1) the role of the person completing the survey, 2) shelter type (including; fosterers only, home-based, one site (not home-based), multiple sites as part of the same organisation), 3) number of volunteers / staff, 4) location of the shelter and 5) species rehomed at the shelter). Shelter names were also requested to avoid duplicating responses but were not used in data analysis. Two questions (opt in to prize draw and contact details) were solely for the purpose of the prize draw. The prizes consisted of two rabbit enrichment parcels and were used to provide an incentive for spending time completing the survey.

Three questions related to how behavioural and personality information was collected, what was collected and when and how it was used. Two questions asked about factors that affect the collection of behavioural and personality information and other types of information at different stages of the rehoming process (at point of relinquishment, while a rabbit is on site, during the rehoming process). The term ‘temperament’ was chosen over ‘personality’ for the survey to reflect the individual character of rabbits. This was done to avoid deterring any respondents that may not be comfortable applying the term personality to describe animals, as previously reported in animal personality research (Jones and Gosling, 2005). As discussed in Chapter 1, the two are considered synonymous in this study. Two questions explored how rabbits are matched to other rabbits and support available to potential owners for rabbit matching. Four questions asked about the type of information that is collected at three stages of rehoming (relinquishment, whilst on site and during rehoming) for various types of

rabbit related information and how this information is stored and used. Three open response questions also enabled other methods of collecting information about individual rabbits to be described and any further comments to the survey. All questions were mandatory except the final general comments box and the prize draw opt in.

3.3.2 Survey distribution

To reach as many rabbit shelters as possible, rabbit shelters within the United Kingdom were contacted via the RWAF directory representing over 470 rabbit rehoming shelters (email sent with link to survey from the Volunteer Coordinator and Fundraising Officer at the RWAF,18th April 2017). The survey was distributed via esurv.org and was live from 1st March to the 15th April 2017. Two rabbit enrichment incentive packs were offered and participants that fully completed the survey could opt into the prize draw to win one of the packs. Shelters had the option to request a paper copy of the survey by emailing the author. One participant requested the paper survey and the responses were added to the online survey.

3.3.3 Data analysis

Data wesre collected in Microsoft Excel and analysis (Pearson's Chi square) was conducted in IBM SPSS Statistics 24 to look for associations between shelter types and the reported use of behavioural information.

For open response questions, responses were analysed using a thematic approach, grouping all similar responses at a basic level initially (question 12 'Please describe any factors that affect whether you collect data about a rabbit's behaviour / temperament whilst at your centre', question 13 'Describe factors that affect your ability to collect information at the following stages of the relinquishment to rehoming process: as the rabbit arrives / is handed over; whilst the rabbit is on site; at the time of adoption (about the potential new home / owners)' and question 14 'If a rabbit is to go to a home with other rabbits, how is a suitable rabbit selected? i.e. what factors are used to

determine if a rabbit is suited to the pets currently owned?') and then further grouping to create overarching themes (Q12 and 13 only, Tables 3.2, 3.4 and 3.5). Coding was conducted by a single coder (author).

3.4 Results

Only participants that completed up to question 11 were included for analysis, which incorporated responses to the demographic questions and three questions relating to the collection of and use of behavioural information, the key purpose of the survey. Forty-three participants completed up to question 11 of the survey representing 43 unique UK rabbit shelters and incorporating all surveys that were started. Thirty-four participants fully completed the survey (22 questions answered).

3.4.1 Participating shelters

The majority of sites were based in England (83.7%, 36) with three from Wales, one from Northern Ireland, one from Scotland and two others (Guernsey and Isle of Man). A range of shelter types were represented in the sample (4.9% home based, 27.9% one shelter sites, 34.9% multi-site shelters as part of the same organisation and 2.3% other, one site was fosterer based only). It is not known how reflective this is of the shelters receiving rabbits in the UK, however this reflects a 9% response rate. Some participants had indicated that they were homebased or single sites for this question however they had also indicated that they were an RSPCA site, as such, these six shelters were re-designated as 'Multiple shelters as part of the same organisation' to reflect any possible support and materials that they may have had access to as an RSPCA branch or fosterer. A range of staff and volunteer numbers were reported, including 21% family run organisations, 23% run solely by volunteers, 21% employed less than 10 members of staff, 12% employed 10-20 staff members and 23% employed more than 20 staff members (Figure 3.1). Home based organisations predominantly

reported being family run or operated by volunteers only. Just one home-based organisation reported having paid staff members. Single shelter and multi-shelter sites varied in personnel from being run by volunteers to employing more than 20 members of paid staff. Of the forty-three shelters represented, just five held only rabbits and these were made up of home-based operations (*n*=3), one single site shelter and one multiple shelter site, with the other thirty-eight shelters holding multiple species.

3.4.2 Behavioural information collection methods

Most participants identified that they utilised informal, *ad hoc*, observations (e.g. whilst completing other care tasks, such as health checking) to record behaviour and/or temperament information about rabbits (81.4%, 35 shelters). Although five participants indicated that they used a 'formal observation tool that has been validated in a scientific study', the descriptions of these indicated the use of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) 'welfare and behaviour observation rabbit' (RSPCA, 2014) sheet by four shelters and the fifth indicated that veterinary health checks were completed, as such, no validated rabbit behaviour observation tool was reported. However, a range of spreadsheets and databases were recognised for their use in storing rabbit information.

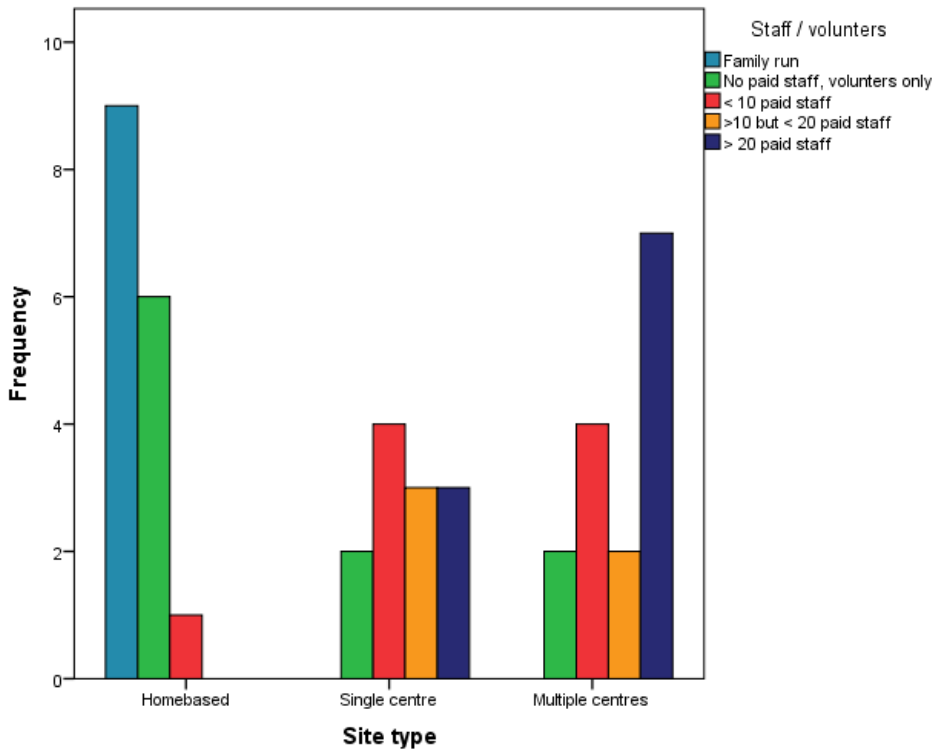


Figure 3.1 Rabbit rehoming shelters represented in the survey ranged from small home-based operations to multi-shelter organisations with more than twenty paid members of staff (*n*=43).

The majority of participants reported that behavioural and temperament information is collected from arrival and then as a continuous process throughout the rabbit's stay (63.9%, *n*=36 Table 3.1). Most information collected during the RtR process was stored on paper (52.8% of behavioural information (Table 3.1) and 43.6%, *n*=35, of all other records, Table 3.3) although electronic records were reportedly used by some shelters. Notes on a rabbit's physical health during its stay at the rehoming shelter and intended accommodation in a new home, were the only information that was always reported to be collected in a format that could be accessed by someone other than the observer (i.e. paper or computer-based records were kept) (Table 3.3).

3.4.3 Use of behavioural information throughout RtR process

The information gained from behaviour and temperament observations were used by a range of people at different sites. This ranged from just one person, that might not make any record of their observations, to a team of staff, volunteers and potential

adopters (Table 3.1). Two participants also indicated that they shared relevant behavioural and temperament information on their organisation's web or social media pages.

The most common uses for behavioural and temperament information collected by the represented shelters included 'to match a rabbit to another rabbit' (100% of participants) and 'to match a rabbit to an appropriate new owner' (100%, of participants). Nineteen unique responses were provided to question 14 "If a rabbit is to go to a home with other rabbits, how is a suitable rabbit selected? i.e. what factors are used to determine if a rabbit is suited to the pet/s currently owned?", with personality and temperament information being the most commonly reported consideration when matching a rabbit from the rehoming shelter to an established rabbit in a home (45.7%, *n*=35, Table 3.2). Additionally, the behaviour assessments completed by the shelters were also reported to be a useful source of information for the process of matching rabbits (11.4%, Table 3.2). Behaviour and temperament information collected while the rabbit was on site at the shelter was reported to be shared with potential/new owners by all 34 participants that completed the survey up to question 21 (100%). Temperament and behavioural information, as described by the previous owner, would be shared with a potential/new owner according to 79.4% and 82.4% of participants respectively (*n*=34).

Table 3.1 Question 10 responses to describe the timing of behavioural information collection, any tools used and access to the information generated. A total of 36 participants provided responses to this question but participants may not have answered all sub-questions. Multiple responses may also have been provided per sub-question.

Question 10 sub-questions	Frequency (%)
When are onsite behavioural / temperament assessments tests conducted?	
○ On arrival only	2 (5.5)
○ At arrival and continuous	23 (63.9)
○ A few days after arrival	3 (8.3)
○ At least one week after arrival	2 (5.5)
○ Formal assessment at/within 10 days	3 (8.3)
○ Two weeks after neutering	1 (2.8)
○ Once animal is settled (where no time frame is given. Many stated it depended on the animal settling)	1 (2.8)
○ Prior to rehoming	1 (2.8)
Name any software or tools used to write results of or store behavioural / temperament observation findings	
○ Paper-based (includes RSPCA Animal Welfare Observation Sheet)	19 (52.8)
○ Specific software listed: ○ Animal Shelter Manager (sheltermanager.com) (2), Anilog (2), VetRescue (1).	5 (13.9)
○ Spreadsheet / database	3 (8.3)
○ Animal file (not clear if electronic or paper)	1 (2.8)
○ None used	2 (5.5)
Who has access to the findings of these observations?	
○ Staff only	12 (33.3)
○ All staff / volunteers and potential adopters	5 (13.9)
○ Potential rabbit adopters (includes on website/social media) only	6 (16.7)
○ Survey participant only	3 (8.3)
○ Volunteer team only	1 (2.8)
○ Shelter owner / Trustees only	2 (5.5)

Table 3.2 Reported considerations for matching unfamiliar rabbits by rabbit shelter staff (*n*= 35). Nineteen unique responses were provided to question 14.

Shelter	Temperament / personality	Pair prior to rehome	New environment suitable	Age of rabbits	Sex of rabbits	Size of rabbits	Neutered status	Current rabbits experience with other rabbits	Try alternative rabbits	Adopter experience	Adopter expectations	Information about current rabbit	Health status (incl. vaccination status)	Own assessments used	other
1	Y	Y	Y			Y									
2				Y	Y	Y	Y					Y			
3			Y								Y				a
4		Y													
5		Y													
6			Y	Y				Y							
7	Y				Y	Y	Y			Y					
8											Y			Y	
9	Y			Y	Y	Y									
10		Y							Y						
11	Y								Y						b
12	Y			Y		Y									
13	Y		Y		Y			Y							
14		Y							Y						
15	Y			Y	Y	Y	Y	Y			Y	Y	Y		
16	Y	Y	Y					Y		Y		Y			
17		Y	Y												
18															c
19	Y		Y	Y	Y	Y	Y			Y	Y		Y		
20			Y				Y			Y			Y		
21			Y		Y		Y								
22															d

23		Y						Y							
24	Y		Y	Y		Y	Y						Y		e
25	Y				Y		Y	Y							
26	Y	Y												Y	
27		Y	Y						Y		Y				
28	Y			Y		Y	Y					Y			
29		Y													
30	Y			Y	Y	Y									
31	Y			Y	Y	Y									
32		Y													
33	Y			Y				Y		Y					
34		Y	Y					Y	Y						
35		Y		Y	Y		Y	Y				Y			
Number of shelters (%)	**16 (45.7%)**	**14 (40.0%)**	**12 (34.3%)**	**12 (34.3%)**	**11 (31.4%)**	**11 (31.4%)**	**10 (28.6%)**	**9 (25.7%)**	**5 (14.3%)**	**5 (14.3%)**	**5 (14.3%)**	**5 (14.3%)**	**4 (11.4%)**	**4 (11.4%)**	**5 - 14.30 %**

Others: a) rabbits can be 'fussy'; b) support for 'bonding process'; c) history of rabbit (reason for being at shelter); d) rabbit returned if do not get along; and e) behavioural issues.

Table 3.3 Format of information collected during the RtR process at 35 rabbit rehoming shelters.

	Information is NOT collected Frequency (%)	Collected but not written or typed (verbal communication only) Frequency (%)	Paper based (e.g. paper records, door information sheets, notice boards) Frequency (%)	Computer based or online (e.g. spreadsheet, database or specialist software) Frequency (%)
The reason the rabbit is entering the shelter (e.g. intake route, stray / relinquished pet, reason for relinquishment of pet)	0	1 (2.9)	15 (42.9)	19 (54.3)
The rabbit's previous daily care (e.g. handling, grooming, bedding / substrate used).	2 (5.7)	9 (25.7)	17 (48.6)	6 (17.1)
The rabbit's previous accommodation (e.g. type of accommodation, where the accommodation is kept).	1 (2.9)	10 (28.6)	16 (45.7)	8 (22.9)
The rabbit's previous social opportunities (e.g. frequency / type of interactions with other rabbits, other species, human caregivers).	1 (2.9)	8 (22.9)	19 (54.3)	7 (20.0)
The rabbit's health history.	0	1 (2.9)	21 (60.0)	12 (34.3)
The rabbits neutered status.	0	1 (2.9)	17 (48.6)	16 (45.7)
The rabbit's diet during its stay on site.	4 (11.4)	5 (14.3)	20 (57.1)	4 (11.4)
The rabbit's health during its stay on site.	0	0	21 (60.0)	14 (40.0)
The potential new rabbit owner's knowledge / experience of pet rabbits.	1 (2.9)	10 (28.6)	12 (34.3)	10 (28.6)
The potential new rabbit owner's home environment (e.g. how many people live there, what type of home?).	0	3 (8.6)	15 (42.9)	12 (34.3)
The age of children living with the potential new rabbit owner.	1 (2.9)	6 (17.1)	15 (42.9)	11 (31.4)
The potential new rabbit owner's current pets (NOT RABBITS) kept.	1 (2.9)	7 (20.0)	15 (42.9)	10 (28.6)

	Information is NOT collected Frequency (%)	Collected but not written or typed (verbal communication only) Frequency (%)	Paper based (e.g. paper records, door information sheets, notice boards) Frequency (%)	Computer based or online (e.g. spreadsheet, database or specialist software) Frequency (%)
The potential new rabbit owner's current rabbits kept if any (e.g. sex, neutered status, past experience with other rabbits).	0	5 (14.3)	18 (51.4)	10 (28.6)
The potential new rabbit owner's plan to accommodate the rabbit (e.g. type of accommodation).	0	0	3 (8.6)	17 (48.6)
The potential new rabbit owner's current pet rabbit's behaviour and temperament (e.g. how it responds to people, other animals or its surroundings, it's general character).	2 (5.7)	14 (40.0)	10 (28.6)	8 (22.9)
Information about a potential new owners' expectations of a new pet rabbit?	3 (8.6)	14 (40.0)	10 (28.6)	7 (20.0)
Total number of sites utilising each information storage method. **Frequency (%)**	**0 (0%)**	**22 (62.9%)**	**26 (74.3%)**	**20 (57.1%)**

A majority of participants used behavioural and temperament information to inform husbandry decisions while the rabbit is on site also, including ‘to identify any potential underlying medical conditions’ (93%) and 83.7% of participants reported using behavioural information ‘to enable staff to manage the rabbit whilst on site’. ‘To generate a training / socialisation plan’ and ‘to determine which accommodation to keep the rabbit in whilst on site’ were also common uses of behavioural and temperament information (55.8% and 44.2% respectively). Two participants indicated that they did not collect behavioural or temperament information however each provided three uses for the information (match to another rabbit, match to a new owner, identify medical issues), perhaps indicating that they saw a use for the information despite not considering that it was collected.

The use of behavioural and temperament information was similar across all shelter types (Table 3.4), however there was a significant difference between the shelter types in the use of behavioural information to determine which accommodation to keep a rabbit in (X^2 6.422, df=2, p=0.045). Home based sites reported using the behavioural information for accommodation selection decisions more so than the other shelter types (Table 3.4).

Table 3.4. Uses of behaviour and temperament information collected on site by three different rabbit shelter types (*n*= 43). The single site that was identified as a fosterer is described within the 'home based' shelter category.

Centre type	To match a rabbit to another rabbit	To match a rabbit to an appropriate new owner	To enable staff to manage the rabbit whilst on site	To determine which accommodation to keep the rabbit in whilst on site	To generate a training / socialisation plan	To identify any potential underlying medical conditions
Home based (n=16)	16	16	11	11	10	15
% of home based shelters	100	100	68.8	68.75	62.5	93.8
One centre (n=12)	12	12	12	3	5	10
% of one centre shelters	100	100	100	25	41.7	83.3
Multiple centres as part of the same organisation (n=15)	15	15	13	5	9	15
% of multi-centre shelters	100	100	86.7	33.3	60	100
TOTAL	**43**	**43**	**36**	**19**	**24**	**40**
% of all shelters	**100**	**100**	**83.7**	**44.2**	**55.8**	**93.0**

3.4.4 Challenges to collecting behavioural / temperament information

Resources, including time, office equipment, and staff or volunteer knowledge/experience, were the most commonly reported barriers that affected if and how behavioural and temperament information was collected (Table 3.5). Responses to question 12 also highlighted some positive perceptions of rabbit behaviour and temperament information collection and other considerations that affect the collection of this information. For example, rabbit behaviour information was noted as being important for potential adopters and for pairing rabbits. Rabbits that were due to be paired were a priority for behaviour assessment according to one participant and another participant noted that fosterers make the behavioural observations and report these back to the primary person rehoming the rabbits.

When asked to describe factors that limit a shelter's ability to collect behavioural and temperament information about individual rabbits at entry to the shelter (question 13), the most commonly reported response related to incorrect or misleading information being provided by the relinquishing owner (57.8%). A lack of information available for stray rabbits, or those that have not been handed over by an owner, e.g. through RSPCA inspectors, was also commonly reported. While on site, the impact of a change of environment was thought to cause the rabbit to be scared or stressed, affecting behaviour and resulting in the rabbit needing time to settle prior to conducting behavioural assessments. A lack of time was again reported as a challenge for rabbit carers to collect information, as was the inability to test for all environmental stimuli that may be relevant for rehoming (Table 3.6).

Table 3.5 Responses to Question 12 'Describe factors that affect if and how you collect information about a rabbit's behaviour / temperament whilst at your shelter'. Thirty-eight participants made 65 unique comments, broadly categorised into 22 categories.

		Frequency (%)
Benefits of testing	○ Behaviour assessment are conducted / part of daily routine	9 (23.6)
	○ Useful for pairing rabbits	2 (5.3)
	○ Information of the rabbit's socialisation is important to new owners	2 (5.3)
	○ Behaviour observations are important for health indicators	1 (2.6)
	○ Supports daily management of the rabbit	1 (2.6)
	○ Good for continuity between staff	1 (2.6)
Challenges	○ Time constraints	16 (42.1)
	○ Lack of resources e.g. Lack of IT so all paper-based, lack of space	5 (13.2)
	○ Knowledge / experience of staff / volunteers	5 (13.2)
	○ Owner providing untruthful behavioural information to secure rabbit a place at the shelter	3 (7.9)
	○ Rabbits stressed when they arrive / may take longer to complete if rabbit not settled	3 (7.9)
	○ Strays/abandoned have no information	3 (7.9)
	○ Owner observations incorrect	1 (2.6)
	○ Medical issues or neutering may result in postponed behaviour assessment	1 (2.6)
	○ Reaction to other rabbits	1 (2.6)
	○ If paired on arrival, will not test with other rabbits	1 (2.6)
	○ Rabbits prioritised for assessment and intervention if demonstrating behaviours of concern	4 (10.5)
Other considerations	○ Observations by foster carers reported back to primary rehomer	1 (2.6)
	○ Rabbits prioritised if to be paired to another rabbit	1 (2.6)
	○ Rabbits prior experience	1 (2.6)
	○ Weather	1 (2.6)
	○ Do not record behaviour observations but do record medical / health issues	1 (2.6)
	○ Not always necessary	1 (2.6)

Table 3.6. Challenges collecting information about a rabbit's behaviour and temperament at entry to shelter, whilst on site and during the rehoming process, as described by 26 participants from rabbit rehoming shelters in the United Kingdom.

Themes	Example responses	Frequency (%)
Owner providing unreliable information or missing information	"reported as being friendly and easy to handle when they are handed in. This is often not the case." "previous history that we are told by owners which can sometimes not be the correct information"	16 (61.5%)
No information available when arrives e.g. stray	"If a rabbit is brought in as a stray we have no data at all."	8 (30.8%)
Environmental setting change affects rabbits behaviour	"You never know how the rabbit will react in a new environment." "The rabbit has travelled and change in environment" "When the rabbit is handed over it may be quite scared, so acting unusually" "rabbits are sensitive" "it takes time for the rabbit to show their character"	12 (46.2%)
Lack of time for shelter workers to collect the data	"sometimes [there are many] rabbits to get to know and socialise" "site staff will have a limited time to spend with each animal"	7 (26.9%)
Unable to replicate all potential environmental stimuli in shelter setting	"difficult to assess how a rabbit will be behave in all potential rehoming scenarios due to limitations of our rescue accommodation" "difficult to guarantee how a rabbit will behave if ... it is staying in [an] outdoor hutch/run with us"	6 (23.1%)
Changes in behaviour following neutering	"many come in un-neutered which will affect behaviour greatly"	3 (11.5%)

Themes	Example responses	Frequency (%)
Others (each reported once):		
	“health issues may affect behaviour”,	
	“behaviour issues affect ability to interact”,	
	“different staff or volunteers each day”,	
	and “limited time for potential adopter to get to know rabbit”.	

3.5 Discussion

3.5.1 Participant demographics

Participants from a range of UK shelter types described their experiences with collecting information around rabbit relinquishment and rehoming. The shelters represented in this study vary from homebased, family run, rabbit only organisations to multi-site, multi-species and greater than twenty paid staff operations. As no previous studies have attempted to understand the demographics of rabbit rehoming shelters in the United Kingdom, it is impossible to know how representative this sample is of all UK rabbit rehoming shelters. However, based on past research (Ellis *et al.,* 2017), and data provided to CE by the RSPCA indicating the number of rabbits that entered the 12 RSPCA National Centres for the same time period (RSPCA, 2013), 1,207 rabbits entered 14 UK shelters in 2013. This is equal to each shelter receiving 86 rabbits each that year. The two non-RSPCA sites, one a home-based operation taking only rabbits and the other a larger organisation that also took in dogs and cats received the highest (153) and the smallest (52) number of rabbits that year, respectively (Ellis *et al.,* 2017). These figures indicate the complex interaction between shelter resources, in terms of space for rabbits and staff or volunteer levels, and the number of rabbits that pass through these centres each year. That is, a home-based, family-run operation was found to receive more rabbits than any of the RSPCA National Centres in 2013. This may link to the resources at the other sites being diverted to other commonly relinquished species such as cats and dogs (Casey, *et al*,. 2009; Diesel, *et al.,* 2010).

3.5.2 Behavioural information collection methods

Participants reported collecting and using behavioural and personality information collected from previous owners and during the rabbit's time with the shelter. Whereas

some participants reported that they retain the information in their own head, and inform other people if needed, other sites had a structured recording and reporting system, including the RSPCA's 'Welfare and behaviour observations rabbit' information sheet (RSPCA, 2017). The RSPCA's 'Welfare and behaviour observations rabbit' information sheet incorporates space for selecting from a choice of rabbit responses to a range of contexts, predominantly around human interactions and one section for onsite intraspecific interactions, and a comment section for each scenario.

The RSPCA's form requires the behavioural elements to be completed at regular intervals throughout a rabbit's stay at the shelter and incorporates historical information, such as, if the rabbit has previously lived with other rabbits, other animal species or children. By providing categories of possible behavioural responses to different scenarios, along with behavioural descriptors for the majority of these behaviours, it ensures that less experienced observers can make more reliable judgements. However, the scenarios and behavioural response options provided have not been tested for face or construct validity, nor has the tool been tested for inter-rater reliability. The tool and any behaviours represented within it have also not been tested for predictive validity to understand if they are stable over time or across contexts (shelter to home setting). No standardised tool for making assessments of a rabbit's behaviour or personality was reported within the survey responses.

Many shelters utilised paper-based information recording tools but electronic records were also used to store rabbit information at over a quarter of shelters, suggesting that any future tools developed to assess rabbit behaviour ought to be able to be stored in paper-based and electronic formats. Most shelters reported collecting behavioural and temperament information throughout a rabbit stay at the shelter, indicating that it is currently part of the daily routine for many shelters. However, as time constraints were

reported as a challenge for many participants, any new tools developed to assess rabbit behaviour should be time efficient to support uptake.

3.5.3 Use of behavioural information throughout RtR process

The most commonly reported use for behavioural and temperament information was to match rabbits to new owners and to conspecifics they could be housed with. During pairing activities, information about the rabbit's character (the words 'personality' and 'temperament' were used by participants) was the most frequently reported factor to consider. Additional feedback from rabbit rehomers would be beneficial to understand what elements of the rabbit's behaviour are considered important for matching a rabbit to a new owner or another rabbit and to understand their interpretation of the terms temperament and personality.

To date, there are no assessment tools available to record personality in rabbits to support the adoption process in shelters. Friendly behaviour towards a potential adopter (Gourkow and Fraser, 2006; Southland, Dowling-Guyer and McCobb, 2019) and the animal's personality (Weiss *et al.,* 2012) are commonly reported reasons for the adoption of a specific animal. However, behaviour in shelters may not reflect the animal's behaviour in the home and so reports from relinquishing owners (Duffy *et al.,* 2014) may provide additional information to potential adopters to support adoption decisions.

Rabbits are considered a social species that require intraspecific companionship although challenges with matching rabbits are commonly reported (Mullan and Main, 2006; Bourne, 2011; Stapleton, 2016). Assessing the personalities of rabbits housed successfully together could help to identify successful pairings. Where possible, future research could also explore any identified traits in successful and unsuccessful rabbit pairings, in relation to other variables such as the rabbit's sex and age.

In addition to behavioural information being considered important to the participants of the current study for understanding how a rabbit may respond in a new home or with new conspecifics, more practical elements were highlighted, including indicators of health issues and managing the rabbit while at the shelter. Incorporating practical outcomes (e.g. suggested socialisation training plans) to any behavioural information collection tools may promote buy-in from shelters, where time or resources may otherwise impair their ability to commit to data collection (Vinic *et al.,* 2019).

3.5.4 Challenges to collecting behavioural / temperament information

Shelter reported challenges in collecting behavioural and temperament information for rabbits were strongly affected by the ability to collect reliable information from a knowledgeable person, such as the previous owner. Where previous owner information was collected, rabbit carers report that such information was not always reliable. Three participants felt that previous owner information may be intentionally untruthful. Inaccurate accounts by relinquishing pet owners has been reported in past research (Segurson *et al.,* 2005). Sergurson *et al*. (2015) demonstrated that owners relinquishing pets were more likely to reveal behaviour problems when they thought the information would be held confidentially from the shelter held data. A recent study by Stavisky *et al*., (2017) surveyed 661 UK animal shelter staff about their perceptions of issues affecting the industry they worked in. The animal shelter staff in that study also highlighted a lack of knowledge about the animals on the part of relinquishing owners as a detrimental factor affecting their work activities. Further exploration of this issue warrants investigation to understand if the perceived unreliable information provided by a relinquishing owner is intentionally deceptive or reflects a misunderstanding or lack of knowledge on the part of the previous owner. The use of a structured previous owner report, designed for use at the point of relinquishment to

gather context specific behavioural information, may be beneficial to improve the reliability of behavioural information in relation to the home environment.

Where no knowledgeable person is present, tools developed to collect historical behavioural information may be redundant. Ellis *et al.* (2017) reported that approximately a quarter of rabbits entering two rehoming shelters in the United Kingdom in 2013 were strays or abandoned. Data from 12 RSPCA sites during 2013 (unpublished data) also suggests that approximately a quarter of rabbits enter shelters as strays or abandoned. For stray or abandoned rabbits, accounts from knowledgeable persons may not be available. The use of behaviour tests while on site at the shelter has been adopted for use with dogs and cats in shelters with varying results (Poulsen, Lisle and Phillips, 2010; Dowling-Guyer *et al.,* 2011; Marder *et al*., 2013; Slater *et al.,* 2013; Weiss *et al*., 2015) and could prove a useful supplement to behavioural and personality information collected for rabbits at shelters.

In relation to on-site behavioural information collection, staff or volunteer time was frequently identified as a constraint affecting the ability of shelters to collect this information. Behavioural and personality assessments will need to be time effective in their completion and also in any administrative activities (i.e. adding results to animal's file) that accompany the tests. Resources, including office equipment, were reported as a challenge in collecting and recording rabbit information. Stavisky *et al*., (2017) also reported resources and funds for resources as limiting factors affecting the UK animal shelter industry. The development of new tools for behaviour assessment at rehoming shelters needs to be deliverable in a paper-based format to ensure it can be used across sites, to avoid the need to buy additional equipment. The information gathered from the data collection activities can be easily uploaded into electronic data files for the shelters that use these.

The impact of the rehoming shelter environment was considered to affect behaviour and hinder the collection of behavioural and temperament information for rabbits. The effect of a changing environment (accommodation and technicians) on rabbits has been reported to cause a prolonged rise in glucocorticoids that have been associated with negative welfare states in animals and may last for up to three months (Peric *et al.,* 2017). While Peric *et al*. (2017) used laboratory rabbits for their study, it would be premature to rule out a similar effect in pet rabbits experiencing a changing environment. The domestic environments of pet rabbits may be equally restrictive in terms of the variety of sensory stimuli they are exposed to and the range of intra- and interspecific social opportunities available to them (Rooney *et al*., 2014, Edgar and Mullan, 2011; Mullan and Main, 2006). The effect of the current environment, i.e. the rehoming shelter, and length of time the rabbit has been in that environment need to be considered when collecting behavioural and temperament information. To ensure an accurate interpretation of a rabbit's personality, behaviour information should be collected at various time points throughout the rabbit's stay at a shelter. Behavioural tool development should focus on appropriate contexts that are relevant to the real world situation that the rabbit will live in and behavioural indicators that demonstrate stability over time.

Patronek and Bradley (2016) critiqued the reliance and emphasis placed on behavioural information collected at kennels when making decisions around euthanasia in shelter dogs, as some behaviours monitored (e.g. aggression) may be heightened within the rehoming shelter environment providing unreliable information that may result in euthanasia decisions. While rabbits may reflect less of a safety concern than pet dogs and as such, aggressive behaviour may be less of a concern for rehoming shelter personnel, it is still important for behavioural information to be reliable and demonstrate predictive validity. The predictive value of behavioural

information collection at rehoming shelters requires investigation for any such tools that are developed. That is, any tool used within a rehoming shelter should predict real life outcomes.

3.6 Conclusions – tool development

- While it was reported that behavioural and personality information is being utilised throughout the RtR process, most shelters are conducting informal observations as part of other daily activities and no standardised tool is being used.
- Despite the results indicating that no validated or standardised behavioural or temperament tools are currently used to support rehoming shelter workers to understanding a rabbit's behaviour or temperament, several benefits of collecting such information were reported and there is a clear function for such information as it is currently collated.
- Behavioural information collected while the rabbit is on site and relinquishing owner reports may both have their place to support shelter staff understanding a rabbit's behaviour during the RtR process.
- The information provided in response to the survey supported the development of behavioural tests and a behaviour rating tool developed to assess rabbit personality in Chapters 4 and 5, to ensure the practical challenges faced by the potential end users could be addressed.

Chapter 4

Rabbit behaviour rating personality survey

Presentations

- October 2019 Royal Society for the Prevention of Cruelty to Animals and United Federation for Animal Welfare Rodent and Rabbit Welfare Group meeting, London, UK. *Rabbit personality* (presentation)
- March 2020 International Society for Applied Ethology UK Regional meeting, University of Nottingham, UK.

CHAPTER 4: RABBIT BEHAVIOUR RATING PERSONALITY SURVEY

4.0 Objectives

Objective 2: Investigate personality traits in adult, domestic rabbits through the development of personality assessment tools that could be used within applied settings.

a) Development of a behaviour rating survey tool to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

4.1 Summary

While behaviour tests are an attractive solution to quickly understand an animal's personality, many factors will determine the animal's behaviour in any given situation and so a range of information sources will ideally be used to develop a picture of the individual. A behaviour rating questionnaire tool was developed to determine adult rabbit personality traits using ratings by a person knowledgeable about the focal rabbit (*n*=1,234), such as a pet owner or person that works with rabbits. Tests for validity indicate that a three-component solution (containing 15 items) should be retained, reflecting aspects of intraspecific interactions, avoidance of humans and boldness in relation to environmental stimuli. The latter two may be sub-traits of a higher order trait reflecting boldness. However, inter-rater reliability requires further investigating with individuals that have experience of rabbits in a pet setting, as threshold standards were not met in the current study. Additionally, where the rater has not experienced the rabbit in intraspecific situations, the component assessing intraspecific interactions will be redundant.

4.2 Introduction

The two most common methods of measuring an animal's personality include behavioural observations and questionnaire rating tools completed by individuals that are familiar with the focal animal, for example, pet owners (Gosling, 2008). However, the selection and development of appropriate tools for animal personality assessment will need to appreciate the contextual factors that may affect the quality (validity and reliability) of the information obtained. Relying solely on behavioural information obtained during the rabbit's stay at a shelter may also be problematic. Behaviour expressed in the shelter setting may not always represent the animal's behaviour within a home setting due to the sudden change of environment (Peric *et al.*, 2017). There may also be potential stressors in shelters where they may be housed close to other species, e.g. dogs, that may affect a rabbit's behaviour. Therefore, it may be difficult for shelter staff to get a clear idea of the rabbit's behaviour to match it to an optimal future home or owner. Previous studies that have compared the two methods, knowledgeable rater report versus behaviour observations, for cross test reliability recommend that both methods be employed where possible (Gosling, 2008; Carter *et al.*, 2012a; Carter *et al.*, 2013).

Questionnaire rating tools (also referred to as subjective ratings or adjective trait ratings) used in animal personality research comprise of a range of questions (items) that describe aspects of personality that are rated by a knowledgeable person. Such tools use adjective or behaviour rating, or a combination of adjectives with behavioural descriptors. A number of questionnaire rating tools have been developed for use with domestic animals, where the focal animal is rated for likeness to behaviours or adjectives on a Likert scale (Gosling and Bonnenburg,1998; Jones, 2008; Mirkó *et al*., 2012; Andersson, 2014; Chopik and Weaver, 2019).

Gosling (2008) acknowledged that there is reluctance among biologists studying animal personality to use questionnaire rating tools amid concerns of the subjective nature of rating personality in this way. However, such tools have been shown to be reliable and are advocated for wider use due to the reported benefits of such tools (Vazire *et al.,* 2007). A benefit of utilising rating questionnaires, which entail reflection of past experiences with an individual, is that the rater is making the measurement based on a period of interactions with the individual, not simply based on one observation at one point in time (Uher and Visalberghi, 2016). Questionnaire assessments also benefit from being less time-intensive, requiring fewer resources than direct behavioural testing (Wiener and Haskell, 2016) and so may be beneficial in shelter settings where resources may be restricted (Stavisky, *et al.,* 2017; Vinic *et al.,* 2019, and see Chapter 3.5.4). However, the caveat of questionnaire ratings is the requirement for a knowledgeable person to complete the questionnaire, which may not always be possible if the rater is not familiar with the animal, for example, where it has just arrived at the shelter or where many people have frequent short bursts of contact with the rabbits.

A number of tools have been adapted from human personality questionnaire tools for use with pet animals (Gosling and Bonnenburg,1998; Gosling *et al.,* 2003; Kubinyi, Turcsán and Miklósi, 2009; Nose and Kakinuma, 2019). Such tools have benefits for cross species comparative studies, however they may miss ecologically relevant traits for the focal species. A further drawback to questionnaire tools includes rater biases in relation to their expectations of the individual or possibly their expectations of the species or breed (Uher, 2013). Several dog personality studies have demonstrated a link between the rater's personality and the focal dog's personality (Kis *et al.,* 2012; Turcsán *et al.,* 2012; Konok *et al.,* 2015). The extent of the effect of this correlation in relation to describing dog personality is still poorly understood but may represent a

bias on the part of the rater. To overcome issues of biases on the part of the rater, Uher (2013) proposes that questionnaire items are derived from the "*behavioural-ecological systems of a population*" (p.36) with careful attention to language use that does not imply causal motivation. Such an approach would select for questionnaire items that reflect behaviours with ecological relevance to the target species. For example, the social structure of a species may be considered when determining suitable behaviours to include to determine individuals with a more or less sociable personality in that species and the behaviours selected may differ somewhat between gregarious and more solitary species.

Two questionnaire rating tools have been developed for use with domestic dogs and were designed specifically for the focal species. The Canine Behavioural Assessment and Research Questionnaire (C-BARQ) and modified C-BARQ (Starling *et al.,* 2013; Lofgren *et al.,* 2014) have been widely used in research on pet dogs, having been initially developed to focus on behavioural problems (Serpell and Hsu, 2001; Hsu and Serpell, 2003). In addition to studies exploring breed differences in personality in dogs (Starling *et al.,* 2013), the shortened C-BARQ has also been shown to have value for use in shelters, as results from the assessment demonstrated predictive validity for behaviours observed in the dog following adoption (Duffy *et al.,* 2014). The Monash Canine Personality Questionnaire Revised (MCPQ-R) has also been developed for describing personality traits in pet dogs (Ley *et al.,* 2008; Ley *et al*., 2009a; Ley *et al.,* 2009b) and more recently has been used to describe dog personality in relation to factors affecting the dog-pet owner dynamic (Ottenheimer-Carrier *et al.,* 2013; Schöberl *et al.,* 2016). These tools have been designed to capture traits relevant to dogs and were tailored to measure the relevant context of interest. For example, describing domestic pet and working dog personality broadly with the use of owner adjective ratings, developed from a human personality assessment tool but adapted

by dog behaviour specialists to identify extraversion, neuroticism, self-assuredness, training focus and amicability in dogs (Ley, Bennett and Coleman, 2008; Ley, Bennett and Coleman, 2009a; Ley, Bennett and Coleman, 2009b). Alternatively, some tools have been designed to measure behavioural problems that would exclude young dogs from becoming guide dogs with the use of owner rating surveys that describe human directed fear and aggression along with trainability and non-human directed characteristics (Serpell, and Hsu, 2001; Hsu and Serpell, 2003).

Questionnaire rating tools used for rabbits to date have utilised a top-down approach, whereby a tool already in use for another species has been adapted for use on rabbits (Gosling and Bonnenburg, 1998; Andersson *et al.,* 2014). In Gosling and Bonnenburg's (1998) study, 29 rabbits (along with five other animal species) were rated by their owners on 50 adjective items. These items were a sub-set of the five-factor model designed for use in humans to measure openness, conscientiousness, extraversion, agreeableness and neuroticism. The authors acknowledged the limitations of utilising the tool designed for humans, as vital aspects of personality for each of the species studied may be missed. They also justify the use of just the adjective for each item, rather than using a version of the survey that included descriptors for each, stating that such adjectives may have different meanings for each species in this study of six animal species. As such, the findings of this study in relation to rabbit personality are restricted to demonstrating the capacity and willingness of pet owners to describe their pet's personality in a format familiar to science and psychology at that time. However, Gosling and Bonnenburg (1998) demonstrated the benefits of using the internet for seeking participants to such questionnaires, where they achieved 1,640 responses across the six species they studied.

Andersson *et al*., (2014) adapted (with the support of a rabbit breeder) a questionnaire rating tool for use with rabbits from a questionnaire initially designed for use with

horses. Similarly to Gosling and Bonnenburg's (1998) study, the questionnaire utilised adjective rating, but also included descriptors for each adjective. Inter-rater agreement was achieved for 24 of the original 68 items, however the authors used a generous intra-class correlation coefficient cut off of >0.3. A stricter cut off >0.5, as proposed by Koo and Li (2016) and Trevethan (2017), would have resulted in retaining 17 items. Four factors were retained and labelled as 'confidence', 'sociability', 'human-directed agreeableness' and 'control', however examination of the items in each factor raises concern regarding the content validity of the three factors. The item 'predictable' loads on 'sociability' and 'dominant' loads on 'human-directed agreeableness'. For the 'confidence' factor, 'active' and 'lazy' load alongside 'timid', 'submissive' and 'inventive'. The authors found no correlations between the scores derived from the questionnaire ratings and a suite of behavioural tests also conducted with these rabbits (Andersson *et al.*, 2014), identifying the different focus of the behavioural tests in comparison to the questionnaire rating tool. While Andersson *et al.*'s (2014) study is the first to utilise a wide scoping tool to measure personality traits, as described by knowledgeable persons, in domestic rabbits, the use of a tool initially designed for another species limits the utility of this tool to describe traits relevant to domestic rabbits.

In the current study, behaviour descriptors were selected based on a review of literature on rabbit behaviour, which was refined through selection of behaviours that may represent traits likely to be relevant to the pet rabbit setting, including reactions to environmental stimuli or situations likely to be encountered as a pet / educational rabbit, social behaviour around other rabbits and social behaviour around humans. These broad categories have also been selected to reflect traits that can be measured using the standardised behavioural tests reported in Chapter 5 (such as exposure to novel objects and novel environments) and behaviours recorded in the home cage

observations (Chapter 6), enabling cross-tool validation. Additionally, they are thought to be relevant to the key uses of behavioural and temperament information, as identified by staff at 43 UK rabbit shelters (Chapter 3.4.3). Intraspecific pairings were the most commonly reported use for behavioural information at the shelters, followed by matching the rabbit to a new owner, therefore, understanding a rabbit's personality in relation to conspecifics and humans was considered important. The rabbit behaviour rating tool, RaBRT) used in the current study was designed to be completed by an individual knowledgeable about a focal rabbit in either a pet or work context. Responses from pet rabbit owners and individuals that work with rabbits, enabled exploration of the items of the RaBRT to identify a subset of items to retain for the final RaBRT tool, where acceptable reliability and validity criteria are achieved.

4.3 Methods

Rabbit owners and knowledgeable rabbit caretakers (including animal care technicians based at four land-based colleges that cared for the rabbits used in Chapters 5 and 6), were invited to complete a survey (containing the RaBRT and demographic information) that incorporated a behaviour rating tool, subjective rating on four traits and descriptive information about the rabbit they were completing the survey for. The survey contained two parts, with part two relating to experience with and perceptions of rabbits, which is not included in the following analysis.

4.3.1 Survey development

The items on the RaBRT were developed from a literature search in Google Scholar on 29th March 2017 using the search terms 'rabbit behaviour' and 'companion rabbit behaviour' yielding 155,376 and 3,140 results respectively. The first 100 returned items for each search were reviewed and articles were included if they were about rabbit

behaviour, in any context (laboratory, farm, pet or wild). This selection process yielded 24 and 9 articles (respectively for each term searched) for further review. An additional 12 articles were also added to this as they had been previously known to the author, giving 35 articles reviewed for the development of the rabbit behaviour terms. A list of 107 behaviours were generated initially. Following removal of duplicates and any behaviours that represented abnormal behaviours (as described by the authors in each paper), 95 items remained. To ensure that the survey tool allowed for cross tool comparison with the suite of behavioural tests being conducted (Chapter 5) only behaviours that reflected boldness or shyness, in relation to environmental situations, intra-specific behaviours and responses to humans were retained, excluding a further 48 behaviours. Each behaviour was given context, for example, for social behaviours a measure including feeding close to other rabbits and so this behaviour item became, 'feeds when close to another rabbit'. A final list of 47 behaviours were retained and agreed amongst the author and supervisory team (Appendix 3). All 47 items were rated on a five-point Likert scale (1= Never, 2= Rarely, 3= Occasionally, 4= Quite often, 5= Very frequently) with a sixth option of 'no opportunity to observe this behaviour' (added following pilot testing, see below). Four items were added to reflect the broad traits being explored and included 1) confident, 2) nervous (included as common language terms to indicate bold and shy individuals), 3) sociable with other rabbits, 4) sociable with people. These four items were used as validation questions, however as it is not known how each term is interpreted by the participants, they are explored in relation to the component scores generated (see concurrent validity below). Participants could also opt to indicate their gender and country of residence.

4.3.2 Behaviour rating procedure

The survey instructed participants to select one rabbit to report on. If they owned more than one rabbit, they were asked to select the rabbit that was closest to two years of

age. Adult rabbits were required to be over 1 year in age. In the first section, fourteen questions explored the individual rabbit's characteristics and ownership history, including age, breed, colour, if housed with other rabbits, length of ownership, hours a week spent with rabbits, type of accommodation, current health status, and number of adults and children that currently live in the home where the rabbit lives. An open response question asked for a description of the rabbit's character (subjective character descriptor) in two or three sentences. Following the 47 items (rated on a five-point Likert scale), an additional four questions required participants to score the rabbit on a visual analogue scale (100 points) for its likeness to four adjective items that represented the broad categories of behaviours of interest; 1) confident, 2) nervous, 3) sociable with other rabbits, 4) sociable with people.

A pilot group of five individuals were asked to complete the survey for a rabbit they knew and provide feedback on any questions that were not clear or suggest any other behaviours that may be relevant to rabbits that may have been missed. The pilot test participants included two non-rabbit specialists with no experience of studying animal behaviour and three behaviour specialists with experience of rabbit behaviour, all with at least postgraduate level qualifications in animal studies. Following suggestions from the pilot test, an additional option to add 'no opportunity to observe this behaviour' was added to all behaviour items. All pilot test responses were removed prior to analysis.

4.3.3 Survey distribution

To reach as many rabbit owners and individuals that work with rabbits as possible an online incentivised survey was used. The survey was live on www.esurv.org site from 10th February 2018 to the 30th April 2018 and was distributed via an advertising poster containing the weblink through social media platforms, including Twitter and Facebook. Additionally, it was shared by several animal welfare organisations on their own social media or webpages, including the Royal Society for the Prevention of Cruelty to

Animals (RSPCA) and the Rabbit Welfare Association (RWAF), both based in the UK. Such sampling methods are hindered by self-selection bias, where individuals choose to take part in a survey of interest to them (Bethlehem, 2010), which is likely to have influenced the demographic of participants in this study. As the survey contained two parts (the second part is not included for analysis here) and was estimated to take approximately 20 minutes to complete, an incentive (£30 Amazon.co.uk gift voucher) was incorporated to encourage full completion of both parts and was only available to individuals that had answered all compulsory questions and opted in to the prize draw.

The survey was advertised as open to participants from all countries but was only available in English. It was also available to rabbit technician staff at land-based colleges taking part in the behaviour study (Chapters 5 and 6, Appendix 2) and a minimum of two staff were asked to complete the survey for each rabbit that had been included in the behaviour testing, to explore cross test reliability. Two sites were able to provide responses from two raters for each rabbit . The survey was approved by the University of Northampton's Postgraduate Ethics board on 18th May 2017 (Land-based College staff version) and 15th January 2018 (online public version).

4.3.4 Data screening and analysis

Data wesre collated in Microsoft Office 16 Excel and IBM SPSS Statistics Version 22 was used for data analysis. Only participants that had answered all questions about a healthy rabbit and provided a response other than 'no opportunity to observe this behaviour' to the 47 behaviour items, were included in the analysis.

4.3.4.1 Inter-rater reliability

Two raters at site 1 rated the same 11 rabbits and two raters at site 2 rated another 9 rabbits, within 4 weeks of each other, to enable inter-rater reliability to be tested. Raters were asked to not discuss their responses and complete the surveys independently.

The raters had known the rabbits for a minimum of 2 and a maximum of 9 years (Mean 4.5 SE 0.9, median 4).

4.3.4.2 Dimension reduction, validity and reliability measures

Each of the 47 items were screened for distribution using frequencies of responses to each scale point (1-5) where any item with <1% of responses on two or more scale points were examined more closely, with the potential to exclude from further analysis. The process for the component reduction of the behaviour rating items, and validity and reliability testing, are described in Chapter 2 table 2.1 and were conducted in the order shown.

4.4 Results

4.4.1 Data screening

The public version of the survey was started by 4,120 participants. Eliminating responses where the participant answered ‘no’ to any of the four consent questions resulted in 4,088 responses and following screening for incomplete responses (where participants did not answer up to the end of the RaBRT survey) 3,402 responses were retained. A further 370 participants had indicated that their rabbit had a health issue and so these rabbits were removed at this stage, resulting in 3,032 responses from the public. A further 35 rabbits were also included from a population of rabbits at three land-based colleges, taking part in the behavioural testing elements (Chapters 5 and 6), resulting in an initial sample size of 3,067.

4.4.2 Analysis of distribution

Of the 47 items four had two response categories achieving <1% of responses. These four items appeared to overlap and so were correlated and the item with the optimal

distribution of response rates was retained. Two items explored the frequency of the rabbit urine spraying on a same sex or opposite sex intraspecific ('urine sprays other rabbits of the same sex' was retained, p<0.001, *Rho*= 0.725) and two items explored the frequency of urine spraying behaviour on a particular or any person ('urine sprays on a particular person' was retained, p<0.001, *Rho*= 0.705). Forty-five items were retained for initial analysis.

For inclusion in the initial data analysis, each rabbit represented required a valid response to all 45 remaining items (excluding all 'no opportunity to observe this behaviour' responses) giving a sample size of 1,234, including 12 for rabbits based at the land-based colleges utilised in Chapters 5 and 6). Some items resulted in a much higher frequency of 'no opportunity to observe this behaviour' response than others. Items with more than 10% responses (303 out of 3,032 responses for healthy rabbits) indicating that the behaviour could not be observed are listed in Table 4.1.

Table 4.1 Challenging items that were commonly scored as 'no opportunity to observe this behaviour' on the 47-item rabbit behaviour rating tool (RaBRT) including *n*= 3,032 responses relating to healthy rabbits from online participants.

Context	Item descriptor	*Frequency*
Interactions with other rabbits	Chases any other rabbits to make them move (displaces)	1145
	Explores the environment and new toys while close (within one body length) to other rabbits.	1158
	Grooms / washes other rabbits	1153
	Attacks any other rabbit (includes kicking, biting and persistent chasing)	1093
	Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits	1112
	Rests close (within one body length) of another rabbit / other rabbits	1112
	Urine sprays other rabbits of the same gender	1440
	Urine sprays other rabbits of the opposite gender	1327
	Feeds close (within one body length) of another rabbit / other rabbits	1129
	Keeps body crouched and head low when being approached by another rabbit	1150

4.4.3 Participant and rabbit demographic information

Of the 933 participants that answered the optional demographic questions, 93.4% were female. The majority lived in the UK (74.8%). Other participants lived in the United States of America (USA) (13.2%), Australia (4.1%), European countries (other than UK) (3%), Canada (2.3%) and other international countries (2.7%).

A range of rabbits were represented in this study (Table 4.2). The majority were neutered, and a larger proportion were males. House rabbits made up over half of the sample population (59.9%, 734 rabbits) and a range of housing types were used, Rabbit breed data (open response question) demonstrated that many rabbits were of mixed breed and the most commonly reported breed group were lops (Figure 4.1). Breeds were only reported where at least 10 rabbits of that breed were reported. The 'Other breeds' category represent breeds with less than 10 rabbits being reported in the survey population. Participants that answered the survey for a rabbit they owned (97.8%) had known the rabbit for a mean average of three years (± 2.6 years) and spent an average of 35.7 hours a week with rabbits (± 34.4 hours). Animal care technicians answering about the rabbits included in the behavioural tests used in Chapters 5 and 6, had known the rabbits for a mean average of 5.2 years (± 2.2) but only spent a mean average of 8.4 hours (± 6.7 hours) a week with rabbits. The implications for the length of time participants spent each week with rabbits are an important consideration in terms of their ability to reliably rate a rabbit using a survey designed for completion by a knowledgeable person (see discussion 4.5.1 relating to inter-rater reliability).

4.4.4 Subjective character descriptors

Participants used over 56 terms to describe their rabbits' character. Terms used for more than 1% of rabbits are shown in Table 4.3. The most commonly used terms were friendly/friend, loveable/loving/lovely, play/playful and affection/affectionate.

4.4.5 Inter-rater reliability

Ten items demonstrated moderate (ICC >0.5) to high (ICC >0.7) inter-rater agreement, however these were not the same items between test groups except for item Q23 'takes food by hand from an unfamiliar person' (Table 4.4). Inter-rater reliability statistics were not used to exclude any items from the dataset at this stage due to the specific context of the interactions the technicians have with the rabbits. This is considered to differ from the nature of the interactions a pet animal owner may have with a rabbit. As the majority of the participants were answering for a rabbit that they owned as a pet, all items were retained at this stage. The implications for the nature of specific rabbit-human interactions in relation to rating survey design and use are reviewed in the discussion (4.5.1).

Table 4.2 Demographic data of 1,234 healthy rabbits represented in the rabbit behaviour survey.

Sex	**Freq. (%)**
Male	670 (54.3)
Female	564 (45.7)

Neutered status	**Freq. (%)**
Neutered	1,066 (86.4)
Not neutered	159 (12.9)
Unknown	9 (0.7)

Age (years)	**Freq. (%)**
1	185 (15.0)
2	252 (20.4)
3	238 (19.3)
4	144 (11.7)
5	127 (10.3)
6	92 (7.5)
7	68 (5.5)
8	43 (3.5)
9+	56 (4.5)
Unknown	29 (2.4)

Accommodation type*	**Freq.**
Hutch/cage (single story)	146
Hutch / cage multi-story	270
Hutch with a run attached	309
Shed / outbuilding	177
Outdoor only	113
College	12
Other	65

Lives with other rabbits currently	**Freq. (%)**
Yes	994 (80.6)
No	240 (19.4)

*Housing type allowed multiple reponses.

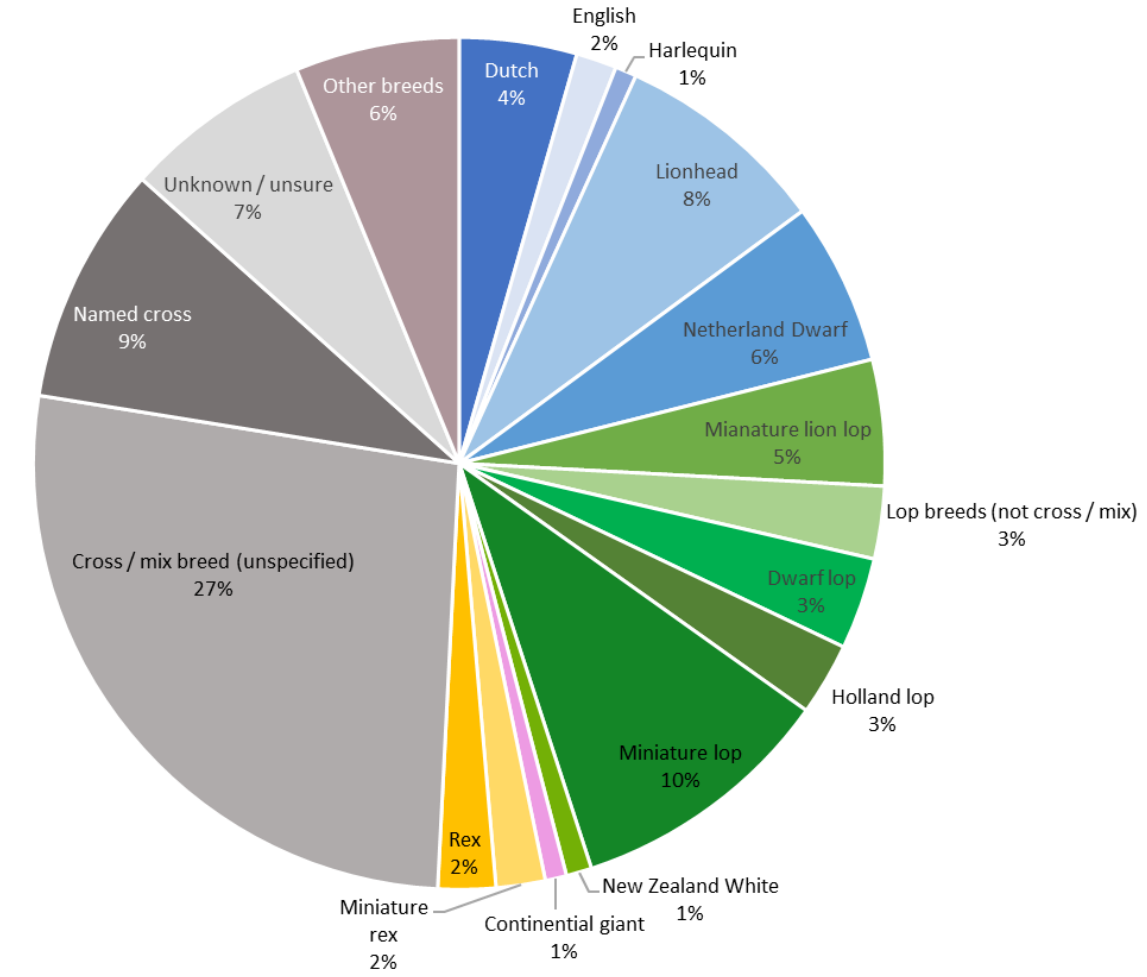


Figure 4.1 Reported breed types from the rabbit behaviour rating survey (*n*= 1,234), colour coded by breed group (Fancy = blue, Lop = green, Normal = pink, Rex = yellow).

Table 4.3 Subjective descriptive terms used to describe a rabbit's character by 1,234 participants completing the RaBRT.

Frequency	Descriptive terms	Frequency	Descriptive terms
278	**Friend** **Friendly**	27	Naughty
203	**Lovable** **Lovely** **Loving**	27	Territorial
163	**Play** **Playful**	26	Free
162	**Affection** **Affectionate**	**26**	**Timid**
134	**Inquisitive**	**25**	**Lazy**
128	Curious	24	Clever
125	**Cheeky**	24	Scared
125	Happy	**23**	**Funny**
82	**Confident**	23	Smart
68	**Shy**	22	Give
67	**Independent**	22	Protective
62	**Gentle**	21	Stubborn
57	**Mischief** **Mischievous**	19	Demanding
56	**Calm**	**19**	**Outgoing**
51	**Relaxed**	18	Comfortable
49	**Boss** **Bossy**	18	Keen
49	Explore Exploring	17	Chilled
45	**Dominant**	**17**	**Quiet**
40	**Intelligent**	15	Greedy
39	**Grumpy**	**14**	**Strong**
37	Sociable	13	Excited
34	Energetic	13	Nosey
33	**Nervous**	**13**	**Skittish**
31	Adventurous	13	Submissive
31	**Aggressive**	**13**	**Sweet**
30	**Cuddly**	12	Diva
28	Feisty	12	Interested
28	Sassy	12	Trouble

Note: Adjectives were selected where at least 1% of the sample population had used the term.
Items in **bold** are adjectives reported in Mullan and Main's 2007 study of 102 pet rabbit owners that were asked to describe their rabbit's personality.

Table 4.4 Inter-rater reliability of items on the rabbit behaviour rating tool. Intra-class correlation coefficients (ICC) (Two raters per site, 11 rabbits site 1, 8 rabbits site two). Items/raters achieving acceptable correlations are in bold.

		95% Confidence Interval				95% Confidence Interval		
Item	**Site 1 ICC**	**Lower bound**	**Upper bound**	**F (df)**	**Site 2 ICC**	**Lower bound**	**Upper bound**	**F (df)**
Q1 Rests in a shelter (e.g. box, house), if available (e.g. box, house), if available	**0.771****	0.402	0.940	8.714 (10)	-0.267			
Q5 Explores new items / toys confidently	0.375				**0.793***	**-0.061**	**0.913**	**4.421 (7)**
Q3 Runs where space allows	0.119				**0.631****	**0.268**	**0.955**	**8.652 (7)**
Q9 Struggles when being held or restrained	0.422				**0.747***	**0.161**	**0.944**	**6.909 (7)**
Q14 Explores new places confidently	-0.050				**0.542**	**-0.194**	**0.888**	**3.370 (7)**
Q22 Readily approaches unfamiliar people	0.455				**0.505**	**0.244**	**0.876**	**3.036 (7)**
Q23 Takes food by hand from an unfamiliar person	**0.508***	**-0.038**	**0.855**	**3.444 (10)**	**0.585***	**-0.133**	**0.900**	**3.821 (7)**
Q27 Attempts to bite unfamiliar people	**0.860*****	0.566	0.961	13.400 (10)	-0.172			
Q28 Runs away to avoid being touched by an unfamiliar person	**0.784****	0.379	0.937	8.250 (10)	0.336			
Q32 Attempts to bite familiar people	**0.792****	0.392	0.939	8.250	-0.144			

*p<0.05
**p<0.01
***p<0.001

4.4.6 Dimension reduction

For inclusion in the initial exploratory PCA, each rabbit represented required a valid response to all 45 remaining items (excluding all 'no opportunity to observe this behaviour' responses) giving a sample size of 1,234.

4.4.6.1 Stage 1 exploratory PCA

Sampling adequacy was good (KMO 0.846, Bartlett's Test of Sphericity $p<0.001$). Thirteen components had eigenvalues >1, accounting for 63.3% of cumulative variance (Table 4.5), however following the exclusion of trivial components (<2 items loaded at >0.55 on a component), components 3-13 (and the items loading on these components) were excluded, retaining 18 items for further exploration.

4.4.6.2 Stage 2 (rotated PCA)

Since it was not known if the components were independent, the PCA was rerun with the 18 retained items using both orthogonal (Varimax) and oblique (Direct oblimin) rotations. Exploration of the face validity of each solution along with discriminate validity analysis was used to select the correct solution. Sampling adequacy was good (KMO 0.873, Bartlett's Test of Sphericity $p<0.001$) and four components had eigenvalues >1.0, accounting for 63.0% of the cumulative variance.

Items loaded in groups of the same items for both orthogonal and oblique rotations. However, it was demonstrated through examination of the correlation between factor scores (Barlett's method) that the components were not correlated ($p>0.05$, *Rho*= <0.1 in all cases) therefore the orthogonal solution was retained as the components were discriminate (Table 4.6). Oblique rotation item loadings are provided in Appendix 4.

Table 4.5 Stage 1 PCA (no rotation) solution of 47 item RaBRT (*n*=1,234). Item loadings in bold were acceptable at this stage and retained for stage 2 analysis following removal of trivial components and complex items.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC9	PC11	PC12	PC13
Q28 Runs away to avoid being touched by an unfamiliar person	**-.680**										
Q31 Runs away to avoid being touched by a familiar person	**-.660**										
Q22 Readily approaches unfamiliar people	**.656**										
Q8 Evades handling by moving away when approached	**-.646**										
Q36 Attempts to hide (in a shelter of some sort) when being approached by a familiar person	**-.634**										
Q24 Attempts to hide when being approached by an unfamiliar person	**-.616**										
Q11 Rests close to people	**.574**										
Q23 Takes food by hand from an unfamiliar person	**.555**										
Q33 Readily approaches familiar people	**.536**										
Q14 Explores new places confidently	**.505**										
Q5 Explores new items / toys confidently	**.498**										
Q26 Thumps the ground when approached by an unfamiliar person	**-.433**										
Q13 Rests in the open	**.419**										
Q42 Rests close (within one body length) of another rabbit / other rabbits		**.840**									
Q44 Feeds close (within one body length) of another rabbit / other rabbits		**.829**									
Q41 Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits		**.824**									
Q39 Grooms / washes other rabbits		**.781**									
Q38 Explores the environment and new toys while close (within one body length) to other rabbits.		**.667**									
Q16 Runs and jumps in the air, turns mid-air and kicks legs out before landing (binkies)			.489								
Q15 Stands on back legs looking around in response to a loud noise			.462								
Q20 Rubs underside of chin on items in the environment			.409								
Q19 Explores new places cautiously (slow approach, and body may be lowered to the ground)	-.408			.471							
Q45 Keeps body crouched and head low when being approached by another rabbit				.440							
Q7 Explores new items / toys cautiously (slow approach, and body may be lowered to the ground)	-.425			.433							
Q32 Attempts to bite familiar people					.509						
Q27 Attempts to bite unfamiliar people					.457		-.454				
Q37 Chases any other rabbits to make them move (displaces)					.421						
Q35 Rubs underside of chin on familiar people			.434			-.487					
Q25 Rubs underside of chin on unfamiliar people	.454					-.472					
Urine sprays other rabbits of the same gender							.511				
Q2 Ignores new toys / items								.413			
Q12 Sprays urine on a particular person									.492		
Q29 Stands still with ears alert when a familiar person approaches									-.422	.401	
Q9 Struggles when being held or restrained									.412		
Q1 Rests in a shelter (e.g. box, house), if available										.524	
Q4 Rests with legs outstretched and belly to the floor or on its side											-.416
Variance	**15.4%**	**9.4%**	**6.9%**	**4.7%**	**4.3%**	**3.7%**	**3.1%**	**2.8%**	**2.5%**	**2.4%**	**2.3%**

Note: Nine items have been removed from the table as they did not contain any loadings >0.4.

Table 4.6 Stage 2 rotated PCA (Varimax with Kaiser Normalization) solution of 18 behaviour rating items (*n*=1,234). Four components were retained, demonstrating eigenvalues >1. Item loadings and communalities for each item are provided.

	PC1	PC2	PC3	PC4	h^2
Q42 Rests close (within one body length) of another rabbit / other rabbits	.934				.873
Q44 Feeds close (within one body length) of another rabbit / other rabbits	.919				.847
Q41 Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits	.917				.845
Q39 Grooms / washes other rabbits	.838				.707
Q38 Explores the environment and new toys while close (within one body length) to other rabbits.	.698				.543
Q31 Runs away to avoid being touched by a familiar person		.849			.766
Q8 Evades handling by moving away when approached		.779			.680
Q36 Attempts to hide (in a shelter of some sort) when being approached by a familiar person		.734			.601
Q33 Readily approaches familiar people		-.505			.412
Q23 Takes food by hand from an unfamiliar person			-.763		.652
Q24 Attempts to hide when being approached by an unfamiliar person			.709		.640
Q26 Thumps the ground when approached by an unfamiliar person			.681		.489
Q22 Readily approaches unfamiliar people			-.652		.635
Q28 Runs away to avoid being touched by an unfamiliar person		.541	.634		.707
Q14 Explores new places confidently				.749	.583
Q5 Explores new items / toys confidently				.660	.474
Q13 Rests in the open				.588	.381
Q11 Rests close to people		-.482		.509	.497
Variance	**27.4%**	**21.3%**	**7.5%**	**6.8%**	

***h^2* = communalties, defined as the proportion of each variable's variance that can be explained by the extracted components.**

4.4.7 Internal consistency

Correlation matrices and Cronbach's coefficient alpha were examined separately for the four retained components. PC1 had strong internal consistency (Cronbach's alpha 0.911) but may include redundant items (mean average correlation 0.686) (Table 4.7). PC2 had low consistency (Cronbach's alpha 0.408) and item Q33 had negative

relationship across the other three items. Removing item Q33 produced strong internal consistency (Cronbach's alpha 0.810) but higher than desirable inter-item correlations for item Q31 (mean average 0.590) (Table 4.7). PC3 had negative average correlation among items (-0.426), specifically items Q22 and Q23, and so was rerun excluding these items to give an improved Cronbach's alpha (0.729) and acceptable inter-item correlations (mean average 0.471) (Table 4.7). PC4 had moderate internal consistency (Cronbach's alpha 0.628) however inter-item correlations were ideal (mean average 0.229).

Table 4.7 Inter-item correlations of the four components following stage 2 (rotated) PCA of 15 retained items RaBRT data. Item Q33 was removed from PC2 and items Q22 and Q23 were removed from PC3 and are not included here.

PC1

	Q38	**Q39**	**Q41**	**Q42**	**Q44**
Q38	1.000				
Q39	.506	1.000			
Q41	.579	.694	1.000		
Q42	.560	.726	.847	1.000	
Q44	.556	.699	.824	.865	1.000

PC3

	Q24	**Q26**	**Q28**
Q24	1.000		
Q26	.390	1.000	
Q28	.636	.386	1.000

PC2

	Q8	**Q31**	**Q36**
Q8	1.000		
Q31	.683	1.000	
Q36	.493	.593	1.000

PC4

	Q5	**Q11**	**Q13**	**Q14**
Q5	1.000			
Q11	.234	1.000		
Q13	.188	.372	1.000	
Q14	.441	.263	.296	1.000

4.4.7.1 Stage 3 PCA final solution retained

Items Q22, Q23, Q33 were removed from the final solution and a rotated (Varimax) PCA conducted to explore the effect of removal of these three items on the structure of the final solution. The three-component solution had good sampling adequacy (KMO 0.853, Bartlett's Test of Sphericity $p<0.001$) and accounted for 60.6% of the cumulative

variance (Table 4.8). The three-component solution had improved content validity from the previous four factor solution as items relating to human interactions were now

Table 4.8 Stage 3 PCA. Item loadings for the behaviour rating survey following the final (retained) rotated (Varimax) PCA (*n*=1,234). The solution shown includes 15 retained items loading across 3 components, accounting for 60.6% of the cumulative variance.

	PC1	PC2	PC3	h^2
Q42Rests close (within one body length) of another rabbit / other rabbits	.934			.873
Q44Feeds close (within one body length) of another rabbit / other rabbits	.920			.847
Q41Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits	.918			.845
Q39Grooms / washes other rabbits	.835			.699
Q38Explores the environment and new toys while close (within one body length) to other rabbits.	.701			.541
Q28Runs away to avoid being touched by an unfamiliar person		.820		.694
Q31Runs away to avoid being touched by a familiar person		.781		.643
Q8Evades handling by moving away when approached		.759		.612
Q24Attempts to hide when being approached by an unfamiliar person		.710		.534
Q36Attempts to hide (in a shelter of some sort) when being approached by a familiar person		.698		.542
Q26Thumps the ground when approached by an unfamiliar person		.555		.321
Q14Explores new places confidently			.772	.605
Q5Explores new items / toys confidently			.693	.510
Q13Rests in the open			.608	.393
Q11Rests close to people			.535	.430
Variance	**26.4%**	**25.4%**	**8.7%**	

***h^2* = communalties, defined as the proportion of each variable's variance that can be explained by the extracted components.**

merged into one component. Communalities were more favourable (closer to 1.0) for items in PC1 but three items, one on PC2 and one on PC3, had communalities below 0.5, indicating these items may not be well explained by the component they are loaded on. Internal consistency on PC1 was very strong (Cronbach's alpha 0.911, mean average inter-item correlation 0.686). PC2 demonstrated strong internal consistency (Cronbach's alpha 0.832) and ideal inter-item correlations (mean average 0.347). PC3

had moderate internal consistency (Cronbach's alpha 0.628) and ideal inter-item correlations (mean average 0.299).

4.4.8 External (population) variation

Rotated (Varimax) PCA's were run separately for females (*n*=564) and males (*n*=670) rabbits using the 15 retained items. Both datasets had good sampling adequacy (KMO 0.844 females and 0.849 males, Bartlett's Test of Sphericity p<0.001 for both). The extracted three components were made up of the same items as the entire sample population (both female and male), although item loadings varied slightly within components (Appendix 5). Additionally, examination of component scores (see below) showed that male and female rabbits did not differ in their scores on any of the three components (Mann-Whitney U, p>0.05). Component scores also did not differ between rabbit age categories (PC1 $\chi 2$ =13.58, PC2 $\chi 2$ = 9.70, PC3 $\chi 2$ = 11.46, p>0.05 and df=9 for all),

PC2 scores were coded into groups (categorised as 'low' (less than one SD below the mean), 'average' (+/- one SD of the mean) and 'high' (greater than one SD over the mean)) to enable comparisons (Fisher's exact test) between neutered status and housing condition (outdoor rabbit, house rabbit) variables. However, the mean scores were high (>0.8) for PC's 1 and 3 (Figure 4.3) and so it would not be possible to achieve three distinct categories for these two components. Rabbits categorised by participants as either 'house rabbits or 'outdoor rabbits' did not differ in their PC2 score categories (p>0.05 in all cases). Neutered status was associated with PC2 categorised scores (p=0.49, Fisher's exact) (Figure 4.2).

An additional rotated (Varimax) PCA was run for a larger sample of responses from the public survey, retaining all responses from the initial pool of 4,120 rabbits where the 15 retained items had been answered with any response other than 'no opportunity

to observe this behaviour'. The sample contained 1,866 rabbits (1,664 reported as healthy and the remaining with a health issues reported) and achieved good sampling adequacy (KMO 0.850, Bartlett's Test of Sphericity p<0.001). A three-component solution was extracted (eigenvalues greater than one) and accounted for 60.3% of the variance and the fifteen items loaded on the same components as they did in the final rotated solution (Table 4.8). The final solution was therefore also retained after adding a further 652 rabbits to the sample.

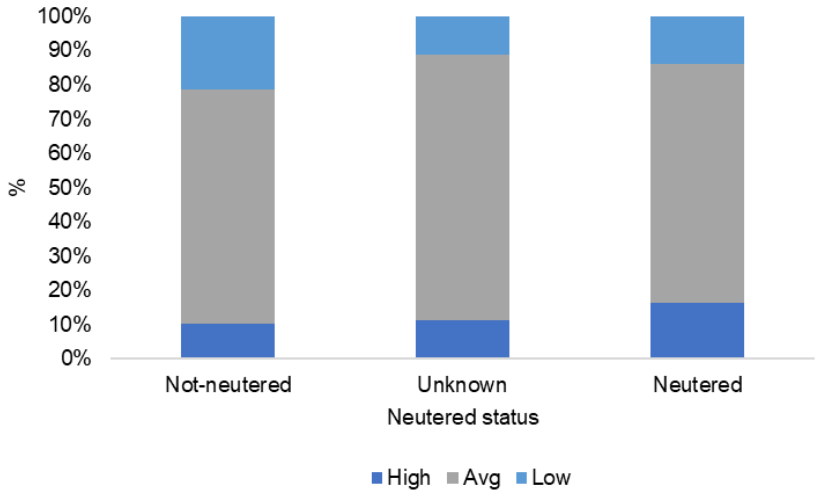


Figure 4.2 Neutered status was associated with categorised scores (low, average, high) for PC2 (p=0.49 Fisher's exact) for 1,234 rabbits rated by a knowledgeable person using the RaBRT. Neutered rabbits accounted for 1,066 of sampled rabbits, 9 had unknown neutered status.

4.4.9 Construct validity

Scores were generated for each rabbit (*n*=1,234) per component by dividing the sum of scores for all items (no items negatively loaded, and so no reverse scoring as required) in each component by the number of items in each component multiplied by 5 (max score per item). Each rabbit gained a score per item from 0 – 1. PC1 and PC3 scores were skewed towards higher scores (PC1 min 0.2, max 1, median 0.92; PC3 min 0.2, max 1, median 0.85) (Figure 4.3). PC2 scores demonstrated better distribution

(0.17 to 0.86, median 0.43). As the component scores were not normally distributed (p<0.001 in all cases), Spearman rank correlations were used to identify if the three retained components were discriminate or convergent. PC3 correlated weakly with PC1 and PC2 (*Rho*= 0.232, p<0.001 and *Rho*= -0.410, p<.001 respectively) (Figure 4.4). PC1 and PC2 were not correlated (*Rho*= .002, p>0.05).

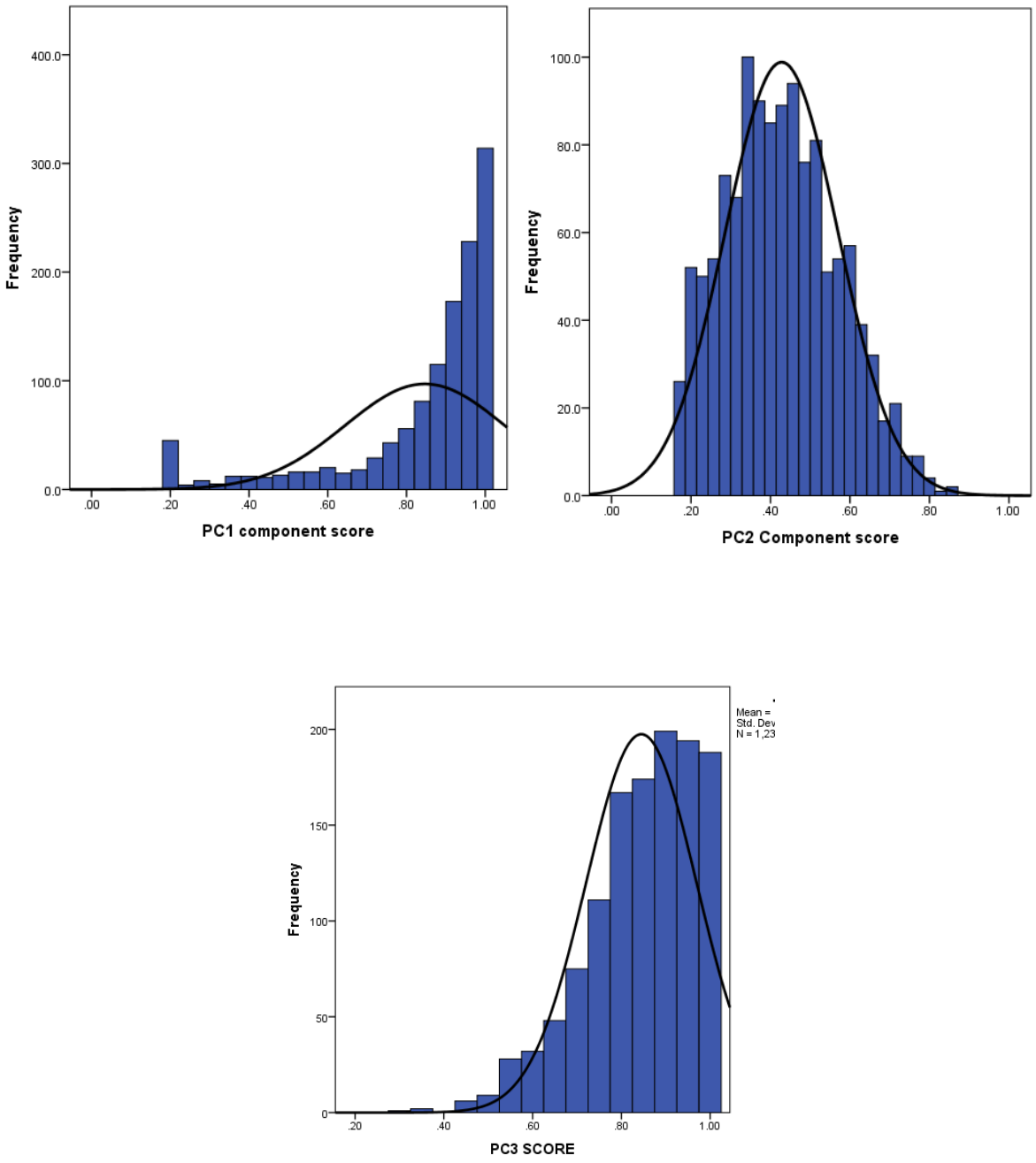
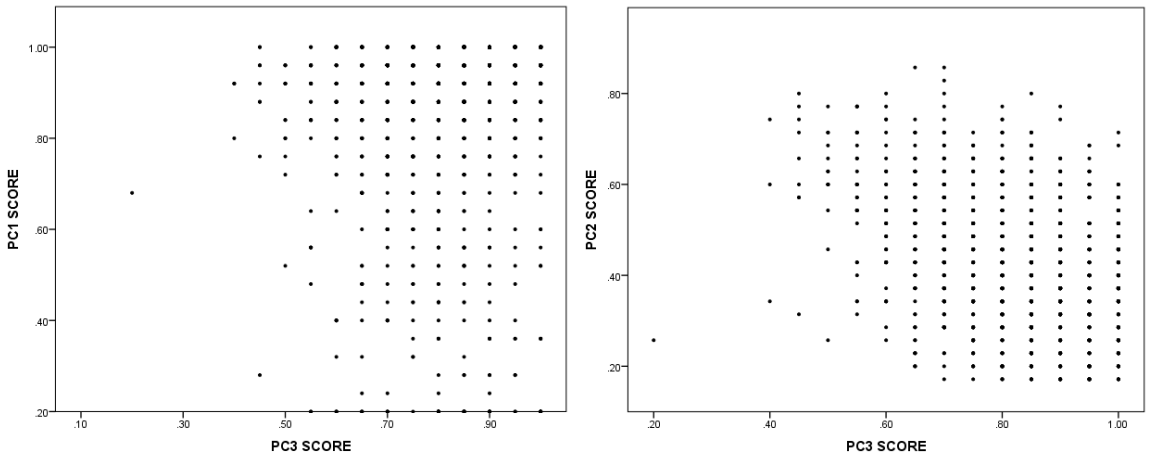


Figure 4.3 Component scores for the three retained components on the RaBRT (*n*=1,234) were not normally distributed (p<0.001 in all cases). PC1 mean 0.847 SD 1.05, PC2 mean 0.428 SD 0.57, and PC3 mean 0.816 SD 0.94.



a) b)

Figure 4.4 RaBRT PC3 scores were weakly correlated with scores for PC1 and PC2 (*Rho*= 0.232, p<0.001 and *Rho*= -0.410, p<.001 respectively).

4.4.10 Concurrent validity

The four within test validation questions were not normally distributed and so Spearman rank correlations were computed to explore associations between the within test validation question and between the within test validation questions and the scores generated for the three retained components. Scores for 'Confident' were moderately and positively correlated with scores for 'sociable with people' (*Rho*= 0.563, p<0.001) and negatively correlated with scores for 'nervous' (*Rho*= -0.694, p<0.001). 'Sociable with people' was also negatively correlated with 'nervous' (*Rho*= -0.503, p<0.001) (Table 4.9). 'Sociable with other rabbits' was not correlated with any other within test validation questions.

A weak positive correlation was observed in rabbits scored as 'sociable with other rabbits' and PC1 (*Rho*= 0.536, p<0.001). Scores on PC2 were moderately and negatively correlated with 'confident' (*Rho*= -0.463, p<0.001) and were positively correlated with 'nervous' (*Rho*= 0.512, p<0.001) (Figure 4.5). PC2 scores were also moderately and negatively correlated with 'sociable with people' scores (*Rho*= -0.616,

p<0.001) from the within test validation questions. PC3 also demonstrated a weak, positive correlation with scores on ‘confident’, ‘nervous’ and ‘sociable with people’ (Table 4.9).

Table 4.9 Within test validation questions on the RaBRT (*n*=1,234) show moderate positive correlations between rabbits scored as ‘sociable with people’ and ‘confident’ and moderate negative correlations between rabbits as ‘sociable with people’ and ‘nervous’. Scores of ‘confident’ were moderately and negatively correlated with ‘nervous’.

Rho=	**Confident**	**Nervous**	**Sociable with other rabbits**	**Sociable with people**	**PC1**	**PC2**	**PC3**
Confident	1.000				.082**	-.463***	.505***
Nervous	-.694***	1.000			-.048	.512***	-.470***
Sociable with rabbits	.152***	-.117***	1.000		.536***	-.065*	.169***
Sociable with people	.563***	-.503***	.216***	1.000	.068*	-.616***	.449***

*** p<0.001,
**. p<0.01,
* p<0.05

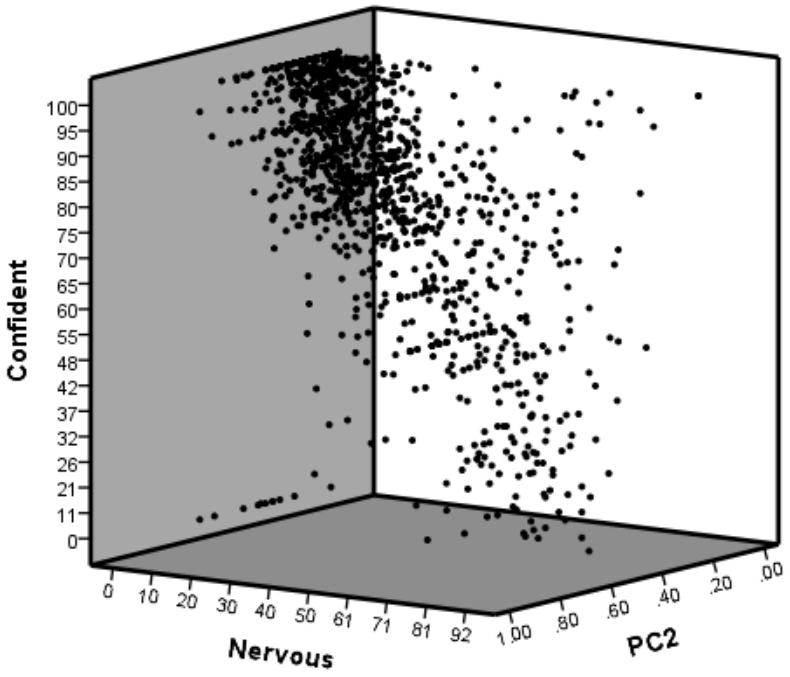


Figure 4.5 Interactions between scores for validation question responses ‘this rabbit is confident’ and ‘this rabbit is nervous’ and scores for PC2 on the RaBRT (*n*=1,234).

4.5 Discussion

Over-representation of female participants was reported in past surveys of rabbit owners (Oxley *et al*., 2018; Harvey *et al.,* 2019) and was also found in the current study. Details about the represented rabbits and their living conditions are reflected on from literature from a range of countries, where information was available, however, three quarters of the rabbits described were owned by residents in the UK.

The population sampled using the rabbit behaviour rating tool were predominantly pet rabbits that were neutered and lived with other rabbits. Both sexes were represented, although more were male (8.6% more males than females), as reported in a previous survey of 1,254 UK rabbit owners where 17.6% more males were reported (Rooney *et al.,* 2014) and a study of 93 Australian (AUS) pet rabbit owners (14% more males (Howell *et al.,* 2015)). A range of breeds were represented in RaBRT population, with those falling into the 'Lop' breed group (British Rabbit Council, 2016) being the most commonly reported pure breeds. Lop breeds have been reported to be the most commonly kept in two past studies that surveyed UK pet owners (Mullan and Main, 2006 and Rooney *et al.,* 2014). However, cross breeds were the most commonly reported type of rabbit (35.8% were known or unspecified/unknown cross breeds). A much lower proportion of cross breed rabbits were reported in Rooney *et al.* (2014) (14.9%). It is unclear if this reflects trends in the popularity of cross breeds, in the UK at least, the availability of cross breeds, or potentially is due to the current study sampling participants from outside of the UK also. A larger proportion of rabbits (59.9) were reported to be house rabbits in the current results, 30% more than reported in Rooney *et al.* (2014) reporting UK pet rabbit housing and just 1.5% more than rabbits described in Mayer *et al*'s., (2017) study of pet rabbit owners in the United States of

America (USA). Rabbits in the current research were less likely to be housed alone (19.4% housed alone) than past research (63% of AUS owners had only one rabbit (Howell *et al.,* 2015) and 32.9% of USA pet rabbit owners (Mayer et al., 2017)). This most likely can be explained due to the requirement for owners to answer the RaBRT for rabbits that have had experience living with another rabbit, to enable full completion of the social behaviour items. Lone housed rabbits may have been excluded more frequently when completing data screening and reviewing 'challenging items' (see 4.4.2).

The behaviour rating items generated through a literature search resulted in a three-component solution being retained reflecting the target traits of interest, including, intraspecific sociability (PC1), avoidance of humans (PC2) and a third component that may represent boldness in relation to the environment and environmental stimuli (PC3). Component two (avoidance of humans) demonstrated a preferable distribution of scores, that is the rabbits sampled showed a range of scores from those available, in comparison to PC1 and PC3 which were both skewed towards higher scores (Figure 4.3). Therefore, component two demonstrates inter-individual differences within the rabbit population sampled, as required by the definition of personality utilised in this study and defined in Chapter 1. Components one and three would benefit from further exploration in other populations of domestic rabbits to understand if they represent individual differences (personality traits) or a species level generic response. The RaBRT was only conducted at one point in time and so it is not possible to determine if the traits are stable over time. As such, the results from the RaBRT should be considered as a representation of rabbit behaviour profiles rather than stable personality traits.

From the initial 47 items selected from the literature review, fifteen were retained. A number of items, particularly those relating to inter-specific interactions, could not be

answered by a large number of participants (Table 4.1) which likely biased the final population of rabbits studied to include a larger proportion of socially housed rabbits (80.6%) than found in past research of UK pet rabbit demographics (41.9% housed with other rabbits in Rooney *et al*., 2014). This bias in the sample, towards rabbits kept in social conditions, can also be observed in the scores obtained for PC1 (intraspecific sociability) which skews towards higher scores (Figure 4.), more sociable rabbits. Rooney *et al.* (2014) also reported positive interactions being reported by owners more frequently than negative interactions in socially housed pet rabbits, which supports the idea that rabbits housed socially are more likely to demonstrate pro-sociable behaviours than negative social behaviours. It is not clear if this enhanced pro-social behaviour is observed as a result of these rabbits being more sociable in personality or because less sociable rabbits may be separated rather than kept in social housing. Additionally, the nature of the housing and space available to the rabbits may be a factor in the social behaviour observed (DiVincenti and Rehrig, 2017). Utilising the RaBRT in a laboratory setting may be beneficial to further explore any associations between pro-social behaviour and housing conditions, where these may be more fixed than in a pet scenario and will allow further exploration of criterion validity for the RaBRT.

Age, neutered status and sex have been associated with personality traits ascribed to domestic dogs (see Wiener and Haskell, 2016 for an overview), however male and female rabbits in the current study did not differ in scores achieved from the RaBRT. Differences between PC1 and PC2 scores and rabbit age were identified but the interaction is not clear. Scores to PC1 appear to peak in rabbits aged three to four years old and PC3 scores appear to be lower in rabbits at six years old, however they do then increase towards eight years old. Scores to PC2 differed with neutered status where entire rabbits may be more likely to achieve lower scores on this component

(avoidance of humans). The majority of rabbits in this study were neutered and the majority achieved 'average' scores for PC2 so this interaction between neutered status and human avoidance behaviour would benefit from further exploration.

The retained components reflect the three components extracted in Andersson *et al.'s* (2014) study using an adjective rating tool which also retained components for sociability (intraspecific), human-directed behaviour and confidence in relation to the environment. The component labelled 'confidence' in Andersson *et al.,* included the adjectives inventive, dependent / follower, submissive, timid, active, decisive, and lazy (Andersson *et al.,* 2014 supplementary material). These may be interpreted as overlapping with PC3 in the current study that reflects a rabbit's confident reactions to new environments and toys, and a rabbit that is more likely to sleep in the open or close to a human. While the nature of the RaBRT and Andersson *et al.'s* (2014) adjective rating tool vary, both extracted three components with similar representations in relation to domestic rabbit behaviour.

4.5.1 Reliability measures

The inter-rater reliability testing from the technician data resulted in ten items achieving moderate to strong agreement among raters, however only three of these items were retained to the final solution (Q5 and Q14 from PC3 and Q28 from PC2). As inter-rater reliability testing could only be conducted with a small number of raters representing only individuals that worked with these rabbits, the results of the inter-rater reliability testing should be reviewed with caution and consideration of possible contextual or length of experience factors that may affect the nature of observations of each rabbit in this setting. None of the intraspecific behaviours from PC1 achieved acceptable inter-rater agreement, which may be explained by the nature of the interactions and observation of the rabbits within the work environment which could cause a disruption to behaviour patterns. The nature of a rater's interaction with a focal animal has been

reported to be an important variable in other studies. Highfill *et al.* (2010) suggested that the specific nature of any interactions (i.e. handling for unpleasant procedures) and the possibility that the animal associated that specific human with unpleasant experiences, may affect the animal's behaviour to a degree that biases the rater's judgement of that animal. In the current study, the raters at each college had slightly different interactions with the rabbits they scored. At both sites, one person was considered the main technician that knew and cared for the rabbits. At site 2, one rater was the primary technician for the rabbits (60% of working week with rabbits) and invested time in training individuals, with the second rater being the cover for days off and spent 30% of working week with the rabbits. In contrast, at site 1, both raters spent just 5% of their working week with the rabbits, which affected the nature of their interactions with the animals, restricted to core activities such as feeding, cleaning (when no students were on-site) and health checks (Personal Comms. with technicians at site 1). As found by Andersson *et al.*, (2014) the specific nature of the rater's experience with the rabbits (farmed rabbits) was a potential factor affecting the scoring on the questionnaire rating tool, as the raters may have restricted experience with the rabbits in relation to some of the target traits being explored. It is also possible that the items on the RaBRT are not reliable measures of the retained components. Inter-rater reliability for the RaBRT needs to be explored further with different rabbit caregivers, e.g. pet owners who in the current study spent more time each week with rabbits. No guidance is available within the literature in relation to the level of knowledge needed to answer a personality survey rating tool about another individual. Rating by the centre staff was not reliable in the current study from most survey item, which may suggest that the RaBRT is not suitable for use by people that known the rabbit through a working role as an animal care technician. However, this would benefit from further testing with a larger population of raters and rabbits. To understand the utility of the RaBRT for use with pet rabbit owners (e.g. to be completed by pet owners relinquishing

a pet rabbit to shelter) or individuals working with rabbits in other contexts, e.g. animal assisted therapies, further testing is needed.

4.5.2 Internal consistency

The items retained on PC1 and PC2 reflected clear and distinct situations. PC1 contained only behaviours describing intra-specific interactions and PC2 described only human avoidance behaviours, and as expected, there was high internal consistency for these two components ($\alpha > 0.8$). PC3 contained items that may reflect boldness in relation to an environmental situation and one item that may reflect interactions with humans 'rests close to people', therefore a slightly lower alpha ($\alpha > 0.6$) may have been expected.

Cronbach's alpha for PC1 and PC2 are similar to scores reported by Gosling *et al*., (2003) study of dog personality rated by owners. However, in the current study PC3 is below the 0.7 threshold for acceptable alpha (Taylor and Mills, 2006) although previous studies have suggested that a lower alpha of 0.6 is also acceptable (Svartberg *et al.,* 2005). However, Clark and Watson (1995) argue that examination of the inter-item correlations is preferable to the Cronbach's alpha, to achieve inter-item correlations between 0.15-0.50 which they recommend demonstrates unidimensionality. Both were examined in the current study, showing that PC2 and PC3 had ideal inter-item correlations, that is, the items on each component can be said to be measuring the same underlying construct. PC1 had a very strong alpha but also high inter-item correlations (0.686) suggesting that there may be repetition in what is being scored by the five items on this component. Therefore, there is room for improving the efficiency of the tool, in relation to the time needed to complete the survey, by reducing items on PC1 (intraspecific social) going forward.

4.5.3 Construct validity

Components one and two were shown to be divergent and can be considered to represent different behavioural traits, however, PC3 may be convergent with both PC1 and PC2 as the component scores had weak correlations. The weak correlation between scores for PC1 (intraspecific) and PC3 (boldness in environment) may be explained by the low distribution of scores on both components. A weak negative correlation (-0.306) was observed between PC2 and PC3, indicating that a rabbit scored as bolder in relation to environmental stimuli (PC3) may be more likely to score low on avoidance of humans (PC2). While a low score on PC2 is not equal to a rabbit that is bolder around humans, there may be some interplay between boldness in relation to humans and the environment. That said, item Q11 on PC3 relates to the rabbit resting close to humans, which may account for the weak negative correlation with PC2 (avoidance of humans).

4.5.4 Concurrent validity

All four within test validation questions (four adjective ratings) correlated with each other (weak to moderate correlations). ‘Sociable with rabbits’ had weak positive correlations with ‘confident’ and ‘sociable with people’ (0.117, 0.216 respectively) and a weak negative correlation with ‘nervous’ (-0.117). ‘Sociable with people’ and ‘confident’ may be measuring the same underlying quality (moderate, positive correlations with each other) and both moderately and negatively correlated with ratings for ‘nervous’. It may be concluded that some participants considered a rabbit to be confident or nervous based on how they perceived its interactions with humans, rather than other rabbits or its engagement with the physical environment.

When exploring interactions between the adjective validation questions and the component scores, PC1 (intraspecific social) has some level of agreement (moderate,

positive correlation) with rater's perceptions of the rabbit in relation to its interactions with other rabbits. Additionally, PC2 has some agreement (moderate, negative correlation) with rater's perceptions of the rabbit in relation to its interactions with humans. PC2 was also negatively correlated with scores of 'confident' whereby a lower confident score gave a higher PC2 score and PC2 was positively correlated with 'nervous', that is, a higher 'nervous' score may also gain a higher PC2 score (Figure 4.5).

As noted above (concurrent validity), PC2 and PC3 are weakly, negatively correlated and a similar interplay was also observed between PC2, PC3 and scores for 'confident' (Table 4.9). As such, components two and three may be considered to represent lower order traits of a broader dimension relating to boldness.

4.5.5 External variation

Male and female rabbits did not differ in their allocated scores for the three components and following data reduction for male and female rabbits as separate groups, the items loaded on to the components in the same way, with some differences in item loadings. It can be concluded that the population of male and female rabbits assessed did not differ in their behavioural profiles, however as the majority of rabbits were neutered, the effect of neutering should be considered. Entire rabbits appeared to be more likely to be rated as low on PC2 (avoidance of humans) and neutered rabbits were more likely to be rated as high on PC2 (Figure 4.2), although the majority of rabbits across neutered statuses scored as average on PC2 (n=861 scored 'average' on PC2). Further exploration of the impact neutering may have on rabbit behaviour is necessary, in the current population neutered rabbits were more likely to be perceived to avoid humans than entire rabbits, when assessed with the RaBRT. As the population of rabbits tested during the behavioural tests (Chapter 5) were all neutered, it was not

possible to explore this phenomenon further in the current study through concurrent validation tests.

Past research has indicated that owners of house rabbits may feel more confident in handling rabbits and did so more frequently (Mullan and Main, 2007), which could suggest that house rabbits are more confident in human interactions. This would suggest that housing conditions, rather than stable personality traits, drive rabbit reactions towards humans. However, in the present study, housing condition (house rabbits or outdoor only rabbits) did not result in different scores for PC2 (avoidance of humans). Further research would be beneficial to understand how stable this trait may be over a life time and between different housing conditions.

4.6 Conclusions

- Three components containing 15 items of the RaBRT were retained which may represent two behavioural traits, sociability with rabbits (PC1) and boldness/shyness in relation to interactions with humans and the environment (PC2 and PC3). However, further testing with other domestic rabbit populations would be beneficial as the current population were largely skewed towards rabbits kept in successful social groups, which may have impacted the distribution of scores achieved for PC1, intraspecific sociability. Concurrent validity (correspondence) is further examined in Chapter 6. Additionally, as the stability of component scores was not tested over time, it would be more prudent to interpret the RaBRT scores as the current behavioural profile of each rabbit sampled, rather than stable personality traits at this time.
- Challenges with the nature of experience the rater (knowledgeable person) has had with the rabbit and the experiences the rabbit has been exposed to while

known to the knowledgeable person, may limit the study of some traits and other methods, direct observations of behaviour in test settings, may prove beneficial. At this stage, the RaBRT components generated are not considered reliable for use by animal care technicians and it is recommended that inter-rater reliability testing is completed with pet rabbit owners and those working with rabbits in other situations, e.g. animal assisted therapies.

- Owner ratings of confidence and nervousness may reflect representations of the rabbit's engagement with humans, more so than intraspecific interactions or engagement with the physical environment.
- Male and female, neutered rabbits do not differ in their behavioural profile as perceived by knowledgeable persons completing the RaBRT, however the impact of neutering needs consideration as entire rabbits were more likely to get low scores on PC2 (avoidance of humans).
- Further reliability testing would be advantageous in terms of inter-rater reliability testing with other populations of rabbit owners / care givers and predictive validity testing to demonstrate the application of the tool to real-life outcomes for the rabbits, for example, successful groupings. This would give further support for the use of the RaBRT within applied settings such as shelters and selection of individual rabbits for use in animal-assisted therapies.

Chapter 5

Development and validation of a suite of behaviour tests to assess domestic rabbit behaviour and personality

Presentations

- October 2019 Royal Society for the Prevention of Cruelty to Animals and United Federation for Animal Welfare Rodent and Rabbit Welfare Group meeting, London, UK. *Rabbit personality* (presentation)
- September 2017 University of Northampton Postgraduate Research Conference, Northamptonshire, UK – *Consistencies in rabbit responses to human interaction* (presentation)
- December 2016 Moulton College Postgraduate Research Symposium, Northamptonshire, UK – *Do domestic rabbits (Oryctolagus cuniculus) show individual consistency in their response to being handled?* (presentation)

CHAPTER 5: DEVELOPMENT AND VALIDATION OF A SUITE OF BEHAVIOUR TESTS TO ASSESS DOMESTIC RABBIT BEHAVIOUR AND PERSONALITY

5.0 Objectives

Objective 2: Investigate personality traits in adult, domestic rabbits through the development of personality assessment tools that could be used within applied settings.

b) Development of a suite of behavioural tests to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

5.1 Summary

While there has been growing interest in exploring and describing companion dog and cat personality, only limited studies have explored personality and individual behavioural profiles for domestic rabbits. Personality profiles of rabbits could have application as part of the rehoming process for relinquished rabbits to match the rabbit to a new home environment that it is not strongly aversive to, or to match a rabbit's personality to the potential new owner's requirements and wishes. Two commonly used test paradigms, the open field and novel objects, were used alongside a newly developed human-interaction test designed to be conducted in the home cage. Along with detecting inter-individual variation and stability across situations for individuals, the tests were conducted at two time points to determine if responses were stable over time. Three retained components (PC1 exploration, PC2 boldness related to human approach and PC2 curiosity) were shown to be reliable in terms of inter-observer and test -retest reliability. Neither component differed based on the sex of the rabbits or the site at which the rabbits lived. PC1 exploration reflects past research findings in relation to the underlying trait reflected within the open field test. Two items from the novel

human interaction test loaded onto PC2, however this may be interpreted as ease of handling, rather than boldness personality traits. Rabbit scores for ability to be picked up correlated with the study sites independant assessments of a rabbit's ease of handling. Just two items loaded on PC3 which reflected interactions with novel substrates in the home cage which has not been studied in rabbits before, therefore further work is needed to fully understand what this component reflects. Scores from the three retained components were not correlated to any of the three component scores generated through the behaviour rating tool developed in Chapter 5.

5.2 Introduction

Personality manifests in the behavioural repertoire of individuals and so can be measured using appropriate behavioural tests. In an attempt to reduce the likelihood of pet dogs being re-relinquished to rescue centres, personality tests have been developed that provide a behavioural profile of the individual animal so that it can be matched to the most suitable owner swiftly (Curb *et al.*, 2013), thus reducing the length of stay they have in an animal shelter and reducing the chances of the animal being re-relinquished. Additionally, these behaviour tests can help shelter staff to provide suitable care and a training plan for the animal whilst it is at the centre (Newbury, *et al.,* 2010), a commonly reported use of such information by rabbit shelter staff in the UK (Chapter 3). The Companion Animal Welfare Council suggest that all animals from rescue centres should be assessed for "*temperament [and] response to different environments and stimuli*" (CAWC, 2011, p.4) prior to rehoming, and that this information should be provided to prospective owners so that an informed decision can be made when selecting a pet. At present, no means of assessing personality or individual behavioural profiles in pet rabbits exists in a format that would be suitable for use in an animal rescue centre.

Rabbits being rehomed, particularly through rehoming centres, may lack any background information about their behaviour if they entered the centre as a stray or abandoned animal (stray/abandoned rabbits represent over a quarter of rabbits that entered two UK shelters in 2013 (Ellis *et al*., 2017)). Therefore, relying on owner reports through subjective ratings alone may result in information being unavailable for some rabbits and it may be difficult for shelter staff to get a clear idea of the rabbit's behaviour to match it to an optimal future home or owner.

In order to identify personality traits in rabbits, the majority of studies (Chapter 1.6) have utilised behavioural tests. Such tests have the capacity to differentiate between individuals within a population and can be utilised over time and across rabbit life stages, to determine if behavioural responses are stable over time. The third criteria required to meet the current study's definition of personality (Chapter 1.2) is to demonstrate consistency of behaviour across situations. The consistency of behaviours across situations is perhaps the most challenging aspect of behavioural tests developed to assess personality in animals, as many studies offer contradictory theories of the underlying factors that drive behavioural responses to the commonly used test paradigms (human interaction tests (HIT): Rödel, *et al*., 2015 and Rödel, *et al*., 2017; open field tests (OFT): Gould, Dao and Kovacsics, 2009; Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; and Rödel *et al.,* 2017; novel object test (NOT): Gacek *et al.,* 2012; Andersson *et al.,* 2014 and Buijs and Tuyttens, 2015), making cross situation consistency difficult to assess where different tests are conducted in an attempt to measure the same underlying construct.

Following a review of tests used to assess rabbit personality (Chapter 1.6.3), three test paradigms, including the open field, novel object and human interaction tests, were selected for use in the current study, incorporating nine subtests. These tests were selected to offer measures of boldness and fearfulness in relation to environmental

stimuli (Réale, 2007) and human interaction, which are considered to be highly relevant to the pet and educational setting for domestic rabbits. Since the majority of the rabbits being tested were socially housed (Appendix 2), intraspecific social behaviour was recorded during the human-interaction tests based on proximity to a conspecific. Solitary housed rabbits were not tested for social behaviours.

The latency to enter the open field (LEOF) test was added as an additional and separate test to the open field test (OFT), where rabbits are placed in the centre of the OF. While the OFT is expected to be a measure of exploratory behaviour in past research in rabbits (Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; Rödel *et al.,* 2017), the LEOF is considered a measure of boldness (Rödel *et al.,* 2006). Utilising both versions of the open field paradigm (start in the centre of the OF and latency to enter OF from a starting box) allows for further exploration of the underlying mechanisms that drive behaviour in this context and should support future research using these two variations of this test.

Novel object tests (NOT) conducted in rabbits have been considered to measure reactivity, boldness, fear and anxiety (Gacek *et al.,* 2012; Andersson *et al.,* 2014 and Buijs and Tuyttens, 2015). In past rabbit research (Chapter 1.6.3) NOT's have been utilised to assess the validity of other test paradigms and so have received very little research exploring the underlying mechanisms affecting response to this test specifically. In the current study, two forms of novel objects have been selected for use within the home cage. The first novel object was a large object (NOT) approximately the size of the rabbits being tested and the second was a novel substrate (NST). While the large object may not reflect ecologically relevant novelty experienced by wild rabbits, man-made objects are relevant to the domestic rabbit situation. Novel substrates are ecologically relevant to rabbits in that when foraging in the wild they may come across new substrates and will be required to make a decision to avoid it or

walk over it. Novel substrate (also referred to as novel surface) tests are thought to measure fearfulness in donkeys and horses (Lansade, *et al*., 2016 and Gonzalez-D, *et al.,* 2017) but have not been tested in rabbits to date. By conducting the NOT and NST tests within the home cage the number of factors that may be eliciting the behavioural response in the rabbit at that time are reduced and the test can be considered to measure the rabbit's response to the novel object (Réale *et al.* 2007).

Human interaction tests are considered to be measures of boldness in rabbits (Rödel *et al.* 2015; Rödel *et al.*, 2017). Human-interaction tests that measure responses during handling restrict the rabbit's ability to react to the human with adaptive strategies such as approach or avoidance. In the current study, an initial pilot test was conducted that incorporated three stages of human interaction that were assessed in a previous study of adult rabbits (Mullan and Main, 2007). The pilot test rating tool was assessed for inter-rater and test-retest reliability to enable the development of the final test used in the following trials. Five subtests were then developed to measure the rabbit's reactivity to escalating advances from an unfamiliar human while in the home cage.

Stable rabbit populations at land-based college teaching units were selected to identify rabbit personality traits using a suite of behavioural tests including the OFT, LEOF, NOT, NST and HIT. These rabbits were selected for sampling based on the number of adult rabbits held at each site for a number of years, and ease of contact with college staff (i.e. all were known to the author or through the PhD supervisory team). The results from the behavioural tests measured across two trials, three to four months apart, were analysed to examine the possible underlying mechanisms between these tests and the variables measured in each test, thought to be measures of boldness and fearfulness in rabbits.

5.3 STUDY 1: HUMAN INTERACTION TEST TOOL DEVELOPMENT PILOT TEST

5.3 Methods

5.3.1 Tool development

A rating scale tool was developed to be used for the human interaction tests that included three steps based in part on those used in a previous study of adult rabbits, including 1) approach of human, 2) rabbit picked up and 3) rabbit held (Mullan and Main, 2007). A five-point Likert scale score was allocated to each rabbit per sub-test, with descriptions for the polar extremes of the scores being provided (Table 5.1). Raters were not trained on scoring (no practice on other animals beforehand) but the scoring method was described and discussed prior to use.

5.3.2 Procedure

Nineteen rabbits (eight females, all neutered and all over 1 year old) housed at the same site (site 1 in study two below (Appendix 2)) were used for testing the prototype human interaction rating tool. Each rabbit was handled by the same handler within the home cage and all rabbits were handled at the same time of day (within one hour, late morning). Testing took place in February 2016 and two trials took place one week apart.

As the rabbits were socially housed, the handler selected a rabbit to start with (usually the most accessible) and then moved on to the next rabbit closest to them following picking up the first rabbit. The handler approached each rabbit and crouched next to it, placing a hand in front of its face to prevent it moving forward if needed, then placing a hand over the rabbit's shoulders before scooping the rabbit up with the other hand underneath the abdomen. With the handler still crouched low to the ground, the rabbit

was then held against the handlers chest horizontally, supporting all four legs with the arm, for ten seconds before allowing it to jump down. All subtests were observed by two raters from outside of the enclosure and scores were recorded at the end of each subtest using a check sheet by both raters. The handler spoke into a Dictaphone in their pocket and transcribed the scores to a check sheet after exiting the enclosure. Ethical approval was granted by the Moulton College Research Committee on 2nd March 2015.

Table 5.1 Human-interaction pilot test scoring protocol for each of the three stages of testing. The descriptors were provided to the raters for review before use.

	Scores	Scoring descriptors
Approach		
	1	Allows approach, does not attempt to evade handler.
	5	Thumping ground, attempts to evade handler, continuously looking for places to escape / hide or aggressively charges at handler.
Pick-up		
	1	Does not struggle when picking up, no vocalisations or biting, calm being picked up.
	5	Struggles, kicks, may attempt to bite, may growl.
Being held		
	1	Remains still while being handled, can be moved between hands without struggling.
	5	Struggles throughout handling, attempts to nibble/ bite, vocalisations, kicking.

5.3.4 Data analysis

Inter-rater reliability analysis was conducted on the data from week one and week two separately, using intra-class correlation coefficients (two-way random effects model for consistency, single measures form). Test-retest reliability was conducted on the mean average scores across all raters for each week and Spearman's rank correlations were used, as the data were nonparametric. As the three subtests were thought to be assessing the same underlying construct (boldness, Rödel *et al.* 2015; Rödel *et al.*,

2017), the subtest scores (average across three raters from week 1 scores) were subject to a Spearman rank correlation to confirm convergent validity. The range of scores and distribution of scores around the mean are examined to understand if the items measured demonstrate variation within the population, as required to be considered a personality trait.

5.3.5 Results

5.3.5.1 Inter-rater reliability

Raters reliably scored the 'approach' and 'pick-up' subtests at both trials, however the 'being held' test was not consistently scored across the three raters at either trial (Table 5.2). When scores for just two raters (excluding the handler, as they would be feeling how the rabbit was reacting in addition to observing it) were considered, scores for subtest 'being held' still did not meet the threshold for inter-rater reliability (W1 ICC 0.360, W2 ICC -0.058)..

5.3.5.2 Test-retest reliability

Only the 'approach' subtest scores were reliable over time (*Rho*= 0.594, p<0.01).

Table 5.2 Human-interaction pilot test inter-rater reliability during three subtests (3 raters of 19 rabbits) for two trials (week 1 W1 and week 2 W2). The approach and pick-up scores were acceptable at each trial.

	ICC	95% Confidence Interval		F	df
		Lower Bound	Upper Bound		
W1 approach	.662***	.423	.838	6.87	18
W1 pick-up	.604***	.347	.805	5.57	18
W1 being held	-.132	-.303	.158	.65	18
W2 approach	.567***	.301	.783	4.93	18
W2 pick-up	.685***	.455	.851	7.53	18
W2 being held	.209	-.058	.525	1.79	18

***p<0.001

5.3.5.3 Analysis of distribution

'Approach' and 'pick-up' scores demonstrated good variation for all rabbits, although 'pick-up' scores had a lower mean in both weeks of testing (Table 5.3). Being held had a limited range of scores across both weeks of testing.

Table 5.3 Human-interaction pilot test scores varied for most subtests, as required, demonstrating that the tests can detect inter-individual variation. Scores for being held had the lowest distribution at both trials (week 1 W1 and week 2 W2) (*n*=19).

	Mean	SEM	Minimum	Maximum
W1 approach	2.26	0.27	1.0	4.3
W1 pick-up	1.77	0.21	1.0	4.0
W1 being held	1.16	0.05	1.0	1.7
W2 approach	2.25	0.24	1.0	4.3
W2 pick-up	1.40	0.13	1.0	2.7
W2 being held	1.19	0.07	1.0	2.0

NB: Scores from both raters were averaged (mean) for each rabbit and at each trial.

5.3.5.4 Construct validity

None of the three subtests were convergent (approach v pick-up *Rho*= 0.414, *p*= 0.078; approach v being held *Rho*= 0.358, *p*> 0.05; pick-up v being held *Rho*= 0.119, *p*> 0.005).

5.3.6 Development of the human interaction tool

The 'approach' subtest appeared to have a better range of scores within the sample population in comparison to the other two subtests, demonstrating inter-individual variation and sensitivity of the rating tool to detect differences in the rabbits. The 'approach' and 'pick-up' subtests had acceptable inter-rater reliability, however only the 'approach' scores were consistent over time when the rabbits were tested one week apart.

None of the subtests were convergent, suggesting that they may be measuring behaviours that are driven by differing underlying motivations. Alternatively, the limited range of scores for the ‘pick-up’ test and ‘being held’ test may have caused the strength of the relationship between variables to be underestimated. This warrants further exploration to understand what traits are being measured by the various types of human interaction tests in domestic rabbits. However, the ‘pick-up’ and ‘being held’ subtests did not meet the reliability measures in this study. Nor did the subtest ‘being held’ meet the criteria for a good range of scores and so is not incorporated into the final human interaction tool developed. The approach test was retained for further development to incorporate more measures of response to humans at varying distances and with different human interactions (described below as the HIT).

5.4 STUDY 2: SUITE OF BEHAVIOUR TESTS

5.4 Methods

All tests were designed to be relatively quick to conduct and using minimal specialist equipment, to ensure any tests deemed useful for measures of rabbit personality could be functional and realistic for use at rabbit shelters (Chapter 3.4.4).

5.4.1 Participants

Sites holding appropriate rabbits (teaching facilities) were contacted and volunteered to take part following receipt of a detailed participant information sheet and all provided consent via email for their rabbits to be used for the study. A total of 60 rabbits were sampled, however this varied by test (see Appendix 2 Table A2.1). Twenty-five males and 35 females were tested. Rabbits were required to be at least 1 year old at the time of testing, however exact ages of rabbits were rarely available. Rabbits were a range of breeds and most were housed socially in at least pairs (*n*=54). The home cage enclosures differed in size (min 1.42m^2 and max 5.25m^2) and three out of the four were situated inside a building with the fourth being an outdoor shed unit with no artificial heating but with lighting (Appendix 2, Table A2.2).

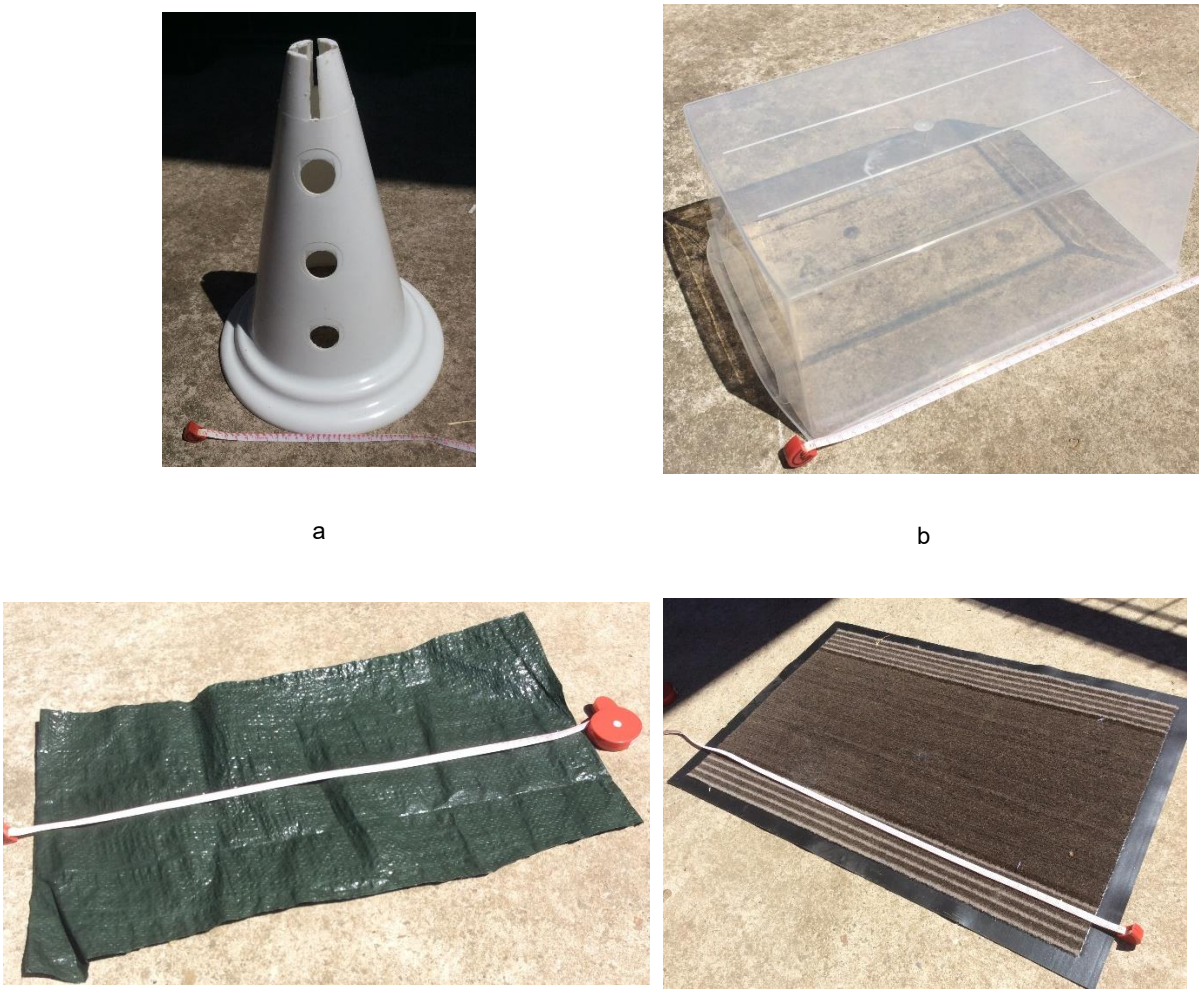
5.4.2 Materials

5.4.2.1 Test 1 Latency to enter open field and Test 2 Open Field test

A small, metal, square shaped animal pen, measuring 1.6m by 1.6m and 0.8m high, was used to create the novel arena (Figure 5.1). The pen had a small door opening that was used by the animal handler to enter and exit the enclosure and was secured during all testing. Black tarpaulin was used for the substrate in each test and was marked into nine evenly sized squares using masking tape, each measuring 53cmx

53cm. To create a slightly different set up during the open field test, a shade net was added to the top of the arena (Figure 5.1). All tests took place indoors in a room other than the rabbit's accommodation room, with the exception of rabbits from site 1 that were tested outside in a space adjacent to their normal accommodation for the open field tests only. This was justified to maintain environmental temperatures as much as possible as the rabbits at site 1 were housed in outdoor sheds with no artificial heating while all other rabbits were housed inside (in a building with heating) exclusively or inside with access to an outdoor area through a hatch. The arena was set up at a distance of at least 1m from any solid structure on any side, but distances varied depending on the site (walls were never more than 4m away from the arena). A battery operated FREDI 4K Ultra HD action camera was mounted on the top of the arena barrier to facilitate scoring from video at a later date. A Professional Go Cook timer was used to record time in the arena and this was calibrated against the action camera's internal clock prior to each testing day. For both tests, the rabbits were removed from the home cage and placed in a pet carrier and moved to the test room/area.

Figure 5.1 Open field set up during the latency to enter the open field test (L) and the open field test (R)



a

b

c

d

Figure 5.2 Novel object (a and b) and novel substrate (c and d) items used in the study. Items a and c were used at trial 1 and items b and d were used in trial 2.

5.4.2.2 Test 3 Novel substrates and Test 4 Novel large objects

Four novel objects were used in total, two substrates and two large objects (Figure 5.2). Each rabbit was exposed to one substrate and one large object at each testing time (one day apart), i.e. one substrate and one large object during trial 1 and a different large object and different substrate during trial two. Substrate 1 was a bottle green tarpaulin sheet measuring 52cm x 27cm. Substrate 2 was a door mat (rubber and carpet) measuring 60cm x 41cm. Large object 1 was a white cone measuring 33cm (h) x 25cm base diameter x 4cm top diameter and large object 2 was a clear plastic box measuring 32cm x 23cm x 14cm. Novel objects were added to the home cage during filming with CCTV cameras as part of the home cage observations described in Chapter 6, except at site 1 where the battery operated FREDI 4K Ultra HD action camera was mounted on the door of the enclosure.

5.4.2.3 Test 5 human interactions (5 subtests)

A single tester wore either a green (day one) or white (day two) laboratory coat and black or navy trousers and black boots for all HIT's. The HIT was conducted on both test days at each trial. A head mounted, battery operated FREDI 4K Ultra HD action camera was worn by the tester.

5.4.3 Procedures

Testing took place in spring or early summer (April – end of May) 2017 (site 1) or 2018 (all other sites) for trial one and July to the end of August 2017 (site 1) or 2018 (all other sites) for trial two. A minimum of three months interval was required (a maximum of four months was allowed). These dates were selected to fall outside of term time for the colleges used so that there would be minimal disturbance of the rabbits by other staff or students. Temperature in the home cage and the novel arena (for LEOF and OFT) was recorded (Appendix 2 Table A2.3).

Tests took place over two days at each trial (Figure 5.3). On day one the LEOF, one novel object (NOT or NST) and one human interaction test (green or white laboratory coat worn) were conducted. The OFT, the second novel object and a second HIT was conducted on day two. All tests were conducted between 10am and 3pm at each site due to ease of access to the rabbits at this time of day.

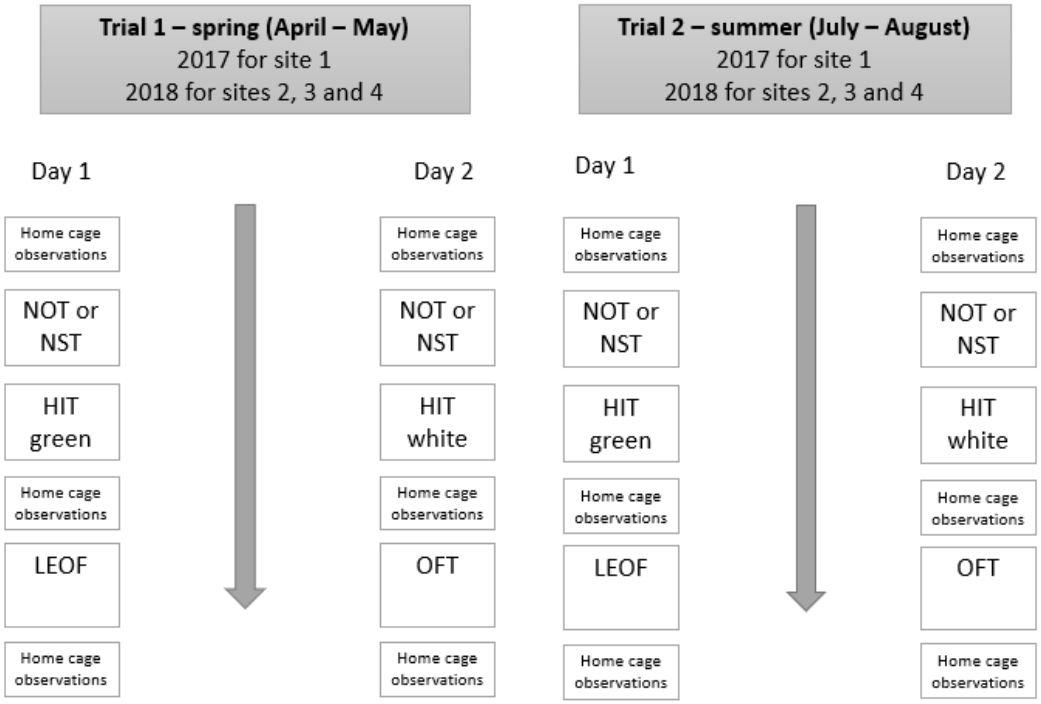


Figure 5.3 Test procedures and approximate order of completion during visits to each test site.

5.4.3.1 Test 1 Latency to enter the open field

This test was conducted in groups, i.e. rabbits housed in groups were tested together in the novel arena, or if the rabbit was housed alone it was tested alone. Rabbits were placed in a pet carrier in the home cage and moved to the novel arena where each cage was placed in a corner position with the front of the carrier facing towards the middle of the arena. The two handlers alternated for every second rabbit and followed

the same handling procedure where they picked up, held and then placed the rabbit in the carrier. Any rabbits that evaded the handler three times, thumped the ground three times within a minute of the handling attempt, or hid in a location that meant they were unable to be reached, were not used in this test. Any rabbits weighing over 4kg were not used in this test to avoid any risks to the handler and rabbit during handling, and this also enabled the same sized pet carriers to be used for all rabbits. Once the carrier was placed within the open field arena they were left for a minute before the camera was set to record and the carrier doors were opened by the handler from outside of the arena. The handler sat at ground level a minimum of 1.5m away from the door opening of the arena for safety and avoided staring at the rabbits or the arena. At the end of the tests the handler returned to the arena to place the rabbits back in the carriers and returned them to their home cage.

5.4.3.2 Test 2 Open field test

Rabbits were collected from their home cage as test 1 above with the same ethical cut off points. Rabbits were left in the carrier for one minute in the test area, but outside of the test arena, prior to being removed by taking the top off the carrier to enable smooth pick-up. They were then placed in the arena in the starting position (centre square). The timer was started, and the handler sat a minimum of 1.5m away (and maximum of 3m away) from the arena at ground level. At the end of the five-minute test, the handler re-entered the arena and returned the rabbit to the pet carrier then returned the rabbit to its home cage.

5.4.3.3 Test 3 Novel substrates and Test 4 Novel objects

The novel substrate and object were added to the home cage in a location at least one rabbit body length away from the nearest rabbit. The object was left in place for five minutes before being removed. An observer watched the animals via the CCTV

monitor to ensure the animals did not have any negative responses to each object (e.g. chewing or climbing on as an escape route). One test was ended early due to the rabbit chewing substrate two. Objects were disinfected with animal safe disinfectant (as used at each site) before each test, including prior to the first test at each site, to ensure all animals were exposed to the object with the same scent and one they had previously been exposed to.

5.4.3.4 Test 5 Human interaction test (HIT)

The five HIT subtests were conducted in order (Table 5.4), starting as the tester approached the door to the enclosure and stood still at the doorway for ten seconds (ST1 – approach enclosure). The tester then opened the door to the enclosure and crouched down for ten seconds (ST2 - crouched in doorway), except at site 4 where the tester remained stood up but with the wire mesh cover over the enclosure removed. For subtest three the tester sat inside the enclosure closest to the door/entry point, for one minute (ST3 – sat inside enclosure). Subtests one to three enabled the rabbit the option of approaching, retreating or remaining still upon the advance of the tester. For subtests four and five the tester attempted to stroke (ST4 – stroke) and pick-up (ST5 – pick-up) the rabbit.

All rabbits within each enclosure were tested at the same time and the tester conducted subtests 4 and 5 with the rabbit closest to them first, followed by the next closest in proximity. Endpoints of the test based on ethical grounds were the same as those listed above for collecting the rabbit from the home cage for the LEOF test.

5.4.4 Data collection

The LEOF, OFT, NOT and NST utilised traditional scoring measures with the addition of measuring the direction of movements the rabbit took while in the OFT (Table 5.4).

Rabbits that did not exit the carrier in the LEOF test or approach the novel objects were scored as 301 seconds for latency scores. The variables measured during the HIT (Table 5.4) incorporated measures utilised in studies with domestic cats and cattle and included, approach and avoidance (Walblinger *et al.,* 2003; Moore and Bain, 2013), proximity measures (Walblinger *et al.*, 2003) and latency to interact with the tester (Moore and Bain, 2013). Proximity to conspecifics were also recorded during the HIT for any socially housed rabbits during ST1 to ST4.

5.4.5 Ethical approval

Care was taken to design the tests to work in line with operating procedures at the colleges and detailed participant information sheets and risk assessments were provided to each centre prior to them agreeing to take part. Participation was voluntary with no reward or compensation offered. Ethical approval was granted by the Moulton College Research Committee on 12th March 2017.

Table 5.4 Variables measured during the suite of behavioural tests used to assess rabbit personality (three test paradigms containing nine subtests).

Test situation	Subtest	Variables measured	Possible scores
Open field	**Test 1 Latency to enter open field**	If the rabbit exited the carrier (1 indicated no, 2 indicated yes)	Dichotomous 1 or 2
		Latency to exit carrier (0 – 300 seconds, 301 if did not exit)	Continuous
		Frequency of times the rabbit came out of a carrier	Continuous
		Zones visited	Ordinal 0 - 9
	Test 2 Open field test	Zones visited (start square not counted unless returned to)	Ordinal 0 – 9
		Lines crossed	Continuous
		Clockwise movements made	Continuous
		Anti-clockwise movements made	Continuous
		Direction changes	Continuous
		If the rabbit returned to the centre square during the test (1 indicated no, 2 indicated yes).	Dichotomous 1 or 2
Novel object	**Test 3 Novel substrate and Test 4 Novel object**	Latency to approach object/substrate (0 – 300 seconds, 301 if did not move)	Continuous
		Latency to make contact with object/substrate (0 – 300 seconds, 301 if did not move)	Continuous
		Contact achieved rating (1 indicated no, 2 indicated yes)	Dichotomous 1 or 2
		Proximity score. 0 indicates no advances towards the object/substrate. 1, 2 and 3 indicate increasingly closer proximity was observed.	Ordinal 0 – 3
		Number of independent contacts with the object/substrate	Continuous
		Behavioural description for each contact	Qualitative
Human interaction	**Test 5a Approach enclosure (ST1)**	Location in enclosure recorded twice, at the start and after 10 seconds in doorway (higher score is calm advance towards the front of the enclosure).	Scale -6 to +6
		Social proximity score recorded twice, at the start and after 10 seconds in the doorway (rabbits housed alone were scored as 0).	Scale 0 to +4
		Any occurrence of specified behaviours was recorded within the ten seconds of the test.	Scale -8 to +2
	Test 5b Crouched in doorway (ST2)	Location in enclosure recorded at the end of 10 second test (higher score is calm advance towards the front of the enclosure).	Scale -3 to +3
		Social proximity score recorded at the end of the 10 second test (rabbits housed alone were scored as 0).	Scale 0 to +2
		Any occurrence of specified behaviours was recorded within the ten seconds of the test.	Scale -3 to +2
	Test 5c Sat inside enclosure (ST3)	Location in enclosure recorded at the end of 1-minute test (higher score is calm advance towards the front of the enclosure).	Scale -3 to +3
		Social proximity score recorded at the end of the 1-minute test (rabbits housed alone were scored as 0).	Scale 0 to +2
		Any occurrence of specified behaviours was recorded within the duration of the test.	Scale -3 to +3
	Test 5d Stroke (ST4)	Test outcome score (0 reflects rabbit unable to be stroked)	Dichotomous 0 - 1
		Social proximity score recorded at the end of the test (rabbits housed alone were scored as 0).	Scale 0 to +2
		Any occurrence of specified behaviours was recorded within the ten seconds of the test.	Scale -3 to +3
	Test 5e Pick-up and hold (ST5)	Test outcome score (0 reflects rabbit unable to be picked up)	Dichotomous 0 - 1
		Any occurrence of specified behaviours was recorded within the duration of the test.	Scale -3 to +2

For the HIT, test measures were developed based on previous work in cattle, cats and rabbits (Walblinger *et al.,* 2003 and Moore and Bain, 2013)

5.4.6 Data analysis

Data were collated and analysed in Microsoft Office 16 Excel (descriptive statistics) and IBM SPSS Statistics Version 22. Data analysis followed the psychometric testing protocol detailed in Chapter 2. All items from all tests were then also analysed for interactions between the items and tests to further our understanding of the underlying mechanisms that may determine behaviour in the suite of behaviour tests used.

5.4.6.1 Inter-observer reliability

Two observers reviewed video footage of the rabbit during each test. Observer one (CFE) coded 100% of footage and observer two (postgraduate in animal sciences) coded 20% of footage per test for the first trial (Uher and Asendorpf, 2008; Mirko *et al.,* 2013; and Horback *et al.,* 2013) (LTEOF = 11, OFT = 11, NST = 12, NOT = 12, HIT = 8).. The second observer was trained through instruction on the test design and scoring processes per test.

5.4.6.2 External validation

To determine if the retained components were the same by sex of the rabbits or by site used in testing, independent t-tests or Mann-Whitney U tests were used to examine differences of component scores by sex. One-way ANOVA with Tukey post hoc analysis and Kruskal-Wallis were used to determine if component scores differed by site tested for the behaviour tests. The Kruskal-Wallis test for independent samples was also used to determine if ‘site’ played a role in scores for each of the variables recorded during the LEOF, OFT, NOT and NST tests. Where significant differences were observed, Dunn’s multiple comparison *post hoc* test was used with a Bonferroni correction.

5.4.6.3 Interactions between test scores

Correlations (Spearman rank for ordinal and continuous variables as several were non-parametric and Kendall's Tau b for dichotomous variables) with Bonferroni corrections, were used to examine associations between OFT and LEOF scores to support further understanding the interpretation of these two versions of the OFT. The NST and NOT were also examined using correlations to explore the effect of the two types of objects used in the test.

5.4.6.4 Concurrent validity

Concurrent validity was completed for the HIT subtest variables, using handling categories allocated by site staff prior to the study. Staff at three sites used a traffic light system (green, amber, red) to identify which rabbits were good for student handling (green), could be handled but may be more difficult (amber) and were difficult to handle (red), which at one site also indicated that staff only should handle that rabbit. These scores were not known to the observers while reviewing the HIT videos. Due to just one rabbit being identified as a red, the rabbits were coded as 1 (amber or red) or 2 (green). To examine interactions between the handling categories and HIT scores across all subtests (14 variables including location, behaviour and outcome scores), Kendall's Tau b correlation was used. .

To examine cross tool validity, the three component scores generated from the RaBRT (Chapter 4) and the component scores generated from the behaviours tests were examined using Pearson correlations, following confirmation of normal distribution using a Shapiro-Wilk test.

5.5 Results

5.5.2 Rabbit demographic information

Due to challenges with visibility resulting from camera positioning during the HIT, NOT and NST, not all rabbits had a full record of scores for these tests. For the OFT and LEOF tests, some rabbits were unable to complete these tests as they met ethical end points during attempts to pick them up (3 during LEOF T1, 4 during OFT T1) or the decision was made to not pick them up due to their size (*n*=2). The population sampled contained male and female rabbits, all were over one year old and neutered at the time of the assessment (Appendix 2) and all had been at the sites where they were tested for at least three months prior to testing.

5.5.2 Analysis of distribution

A range of scores for each variable measured was observed in the sample population (Tables 5.5, 5.6, 5.7, 5.8, 5.9). This demonstrates that there was inter-individual variation within the sampled population for the items measured.

5.5.3 Inter-observer reliability

All items measured during trial one of the LEOF (*n*=55), OFT (*n*=53), NOT (*n*=60) and NST (*n*=60) demonstrated acceptable interobserver reliability (*Rho*= or Tb >0.5 (Koo and Li, 2016; Trevethan, 2017)). (Tables 5.5, 5.6, 5.7, 5.8). For the HIT, location measured during ST2 and ST3, behaviour measured in ST3, ST4 and ST5, and the outcomes measured for ST3 and ST5 were retained as they met the threshold for consistency between observers (Table 5.10). Outcome scores for ST4 were also retained as there were close to the 0.5 cut off. Social scores observed during subtests one to four were also retained.

Table 5.5 Analysis of distribution of scores and inter-observer reliability (Intraclass correlation coefficient, ICC) from the latency to enter the open field test (LEOF). Trial 2 always took place 3 – 4 months after trial 1. Only rabbits exiting the carrier are included in the latency to exit carrier scores, number of times out of carrier and zones visited.

Latency to enter open field	Number of rabbits to exit carrier (%)	Latency to enter OF (seconds)			Number of times out of carrier (frequency)			Zones visited (out of 9 zones)		
		Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC
Trial 1 (*n*=55)	39 (70.9)	2:176	44.9 (51.6)	0.956	1:8	1.6 (1.4)	1.000	2:9	7.3 (2.1)	0.991
Trial 2 (*n*=30)	29 (96.6)	3:120	16.1 (23.6)		1:8	1.4 (1.3)		2:9	7.79 (1.9)	

Table 5.6 Analysis of distribution of scores and inter-observer reliability (Intraclass correlation coefficient, ICC) from the open filed test (OFT). Trial 2 always took place 3 to 4 months after trial 1.

Open field test	Returned to centre after start	Zones visited			Lines crossed			Clockwise movements			Anti-clockwise movements			Total direction changes		
	Yes (%)	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC
Trial 1 (*n*=52)	30 (57.7%)	0:9	7.4 (2.4)	0.950	0:48	17.2 (11.6)	0.985	0:28	7.5 (6.2)	0.980	0:35	8.0 (7.4)	0.948	0:10	2.9 (2.6)	0.911
Trial 2 (*n*=29)	22 (85.9%)	0:9	7.9 (1.8)		0:45	18.8 (9.9)		0:19	8.1 (4.5)		0:30	9.4 (7.2)		0:7	3.0 (2.2)	

Table 5.7 Analysis of distribution of scores and inter-observer reliability (Intraclass correlation coefficient, ICC,) from the open novel substrate test (NST). Trial 2 always took place 3 to 4 months after trial 1.

Novel substrate	Proximity score (possible 0 – 3 where a higher score = closer proximity, a score of 0 = no approach to substrate)						Contact achieved (%)		Latency to approach substrate (seconds)			Latency to make contact with substrate (seconds)			Frequency of independent contacts with substrate		
	Median	Frequency of score 0	Frequency of score 1	Frequency of score 2	Frequency of score 3	ICC	Yes	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC
Trial 1 tarpaulin (*n*=60)	2	10	9	25	14	0.783	47 (78.3)	1.0	0:231	40.3 (52.4)	0.976	1:222	45.6 (47.7)	0.922	1:8	2 (1.8)	0.736
Trial 2 door mat (*n*=42)	1.5	8	13	15	6		35 (83.3)		1:287	35.8 (54.8)		1:292	48.6 (62.4)		0:9	2.6 (7.3)	

Table 5.8 Analysis of distribution of scores and inter-observer reliability (Intraclass correlation coefficient, ICC) from the open novel object test (NOT). Trial 2 always took place 3 to 4 months after trial 1.

Large novel object	Proximity score (possible 0 – 3 where a higher score = closer proximity, a score of 0 = no approach to object)						Contact achieved (%)		Latency to approach object (seconds)			Latency to make contact with object (seconds)			Frequency of independent contacts with object		
	Median	Frequency of score 0	Frequency of score 1	Frequency of score 2	Frequency of score 3	ICC	Yes	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC	Min:Max	Mean (SD)	ICC
Trial 1 White cone (*n*=60)	2	20	8	26	6	0.955	42 (70)	1.0	1:299	39.5 (67.4)	1.0	2:300	54.5 (70.5)	1.0	0:5	1.7 (1.6)	1.0
Trial 2 Clear plastic box (*n*=42)	2	12	6	22	2		27 (64.3)		1:222	44.1 (61.3)		1:293	47.3 (74.0)		0:7	1.8 (2.0)	

Table 5.9 The distribution of scores for each behaviour measured during the human-interaction test (HIT) for 38 adult rabbits. Sample sizes vary for some behaviours measured where rabbits were not housed socially or were out of sight during review of footage.

		n=	Minimum	Maximum	Median	Mean	Std. Deviation
Trial 1 Day 1 Green clothing	ST1 Approach Location	38	-3	1	-1	-0.4	1.1
	ST1 Approach Behaviour	34	-3	1	-1	-0.4	1.3
	ST1 Approach Social	33	0	2	1	1.0	0.9
	ST2 Doorway Location	38	-3	3	-1	-0.3	1.3
	ST2 Doorway Behaviour	33	-3	2	-1	-0.6	0.9
	ST2 Doorway Social	28	0	2	1	1.1	0.8
	ST3 Inside Outcome	38	0	1	0	0.1	0.3
	ST3 Inside Location	38	-3	3	-1	-0.5	1.3
	ST3 Inside Behaviour	36	-4	5	-1	-0.7	1.6
	ST3 Inside Social	32	0	2	0	0.9	1.0
	ST4 Stroke Outcome	38	0	1	1	0.6	0.5
	ST4 Stroke Behaviour	38	-4	5	0	1.0	2.7
	ST4Stroke Social	33	0	2	1	0.9	0.9
	ST5 Pick-up Behaviour	38	-5	0	-1	-1.3	1.5
	ST5 Pick up Outcome	38	0	1	0	0.4	0.5
Trial 1 Day 2 White clothing	ST1 Approach Location	38	-1	3	1	0.3	1.2
	ST1 Approach Behaviour	37	-3	2	-1	-0.3	1.4
	ST1 Approach Social	31	0	2	2	1.1	1.0
	ST2 Doorway Location	38	-3	3	-1	-0.3	1.5
	ST2 Doorway Behaviour	35	-2	2	0	-0.3	0.9
	ST2 Doorway Social	30	0	2	1	1.0	0.9
	ST3 Inside Outcome	38	0	1	0	0.2	0.4
	ST3 Inside Location	38	-3	3	-1	-0.2	1.7
	ST3 Inside Behaviour	36	-3	5	0	0.4	2.0
	ST3 Inside Social	31	0	2	1	1.0	0.9
	ST4 Stroke Outcome	38	0	1	1	0.6	0.5
	ST4 Stroke Behaviour	36	-6	5	0	0.2	2.3
	ST4Stroke Social	29	0	2	0	0.8	0.9
	ST5 Pick-up Behaviour	38	-5	0	-1	-1.1	1.4
	ST5 Pick up Outcome	38	0	1	0	0.2	0.4

ST1 Approach enclosure; ST2 Crouched in doorway; ST3 Sat inside enclosure; ST4 Attempt to stroke; and ST5 attempt to pick-up and hold rabbit.

Table 5.10 Human-interaction test (HIT) inter-observer reliability from Trial 1 tests with the handler wearing a green laboratory coat. Intraclass correlation coefficients (ICC) and p value are provided (*n*=38).

HIT trial 1	**ST1**	**ST2**	**ST3**	**ST4**	**ST5**
Location	0.263	1	1	n/a	n/a
Behaviour	-0.012	0.056	0.962***	0.984***	0.917**
Outcome	n/a	n/a	1	0.467	1
Social	1	0.906**	1	0.615^	n/a

p<0.01**
p<0.001***
^ p=0.071

5.5.4 Test retest reliability

Retained variables from each test, following inter-observer reliability testing, were tested for consistency over time (Table 5.11), with the addition of subtest four (stroke outcome) being added as it was just below the threshold for inter-observer reliability testing. For the HIT, the two trials were conducted one day apart. For all other tests the two trials were three to four months apart. .

HIT location scores were only stable over time during ST3 (p<0.01, *Rho*= 0.457). Behaviour scores were not stable over time during ST3, ST4 or ST5. Outcome scores were stable for all three subtests (ST3, p<0.01, T_b 0.516; ST4 p<0.001, T_b 0.733; ST5 p<0.01, T_b 0.542). Social scores recorded during the HIT were only stable during ST2 (p<0.01, *Rho*= 0.551).

Table 5.11 Test-retest correlations from the two trials for each behaviour subtest, including only variables that met minimum correlation and probability criteria (*Rho*=/T_b >0.43, p<0.05).

Subtest (sample size)	Variable measured	Test	*Rho*= / T_b
Human-interaction test (HIT) (*n*=38)	Location ST3	Spearman's	0.457**
	Social ST2	Spearman's	0.551**
	Outcome ST3 (approach)	Kendall's Tau b	0.516**
	Outcome ST4 (stroke)	Kendall's Tau b	0.733***
	Outcome ST5 (picked up)	Kendall's Tau b	0.542**
Latency to enter OF test (*n*=21)	Count of times out of carrier	Spearman's	0.442*
	Number of zones visited	Spearman's	0.479*
Open field test (*n*=27)	Count of zones visited (ordinal)	Spearman's	0.510**
	Count of lines crossed	Spearman's	0.588**
	Count of clockwise movements	Spearman's	0.515**
	Count of counter clockwise movements	Spearman's	0.497**
Novel substrate (*n*=39)	Proximity score	Spearman's	-0.615**
	Latency to approach substrate	Spearman's	0.480**
	Count of independent contacts with the substrate	Spearman's	0.452**
Novel object (*n*=42)	Count of independent contacts with the object	Spearman's	0.438**

p<0.05*
p<0.01**
p<0.001***

5.5.5 Dimension reduction

For inclusion in the initial exploratory PCA, each rabbit represented was required to have complete scores for each of the criteria retained (14 behaviour items) following inter-observer and test – retest reliability analysis (*n*=32). These 32 rabbits were mostly female (21) and pair housed (25), however four were singly housed and there was one group of three rabbits.

To improve the item to sample size ratio for conducting a PCA, two items, anti-clockwise and clockwise movements within the OFT, were removed as they were highly correlated with the number of lines crossed in the OFT (Rho= 0.804, $p<0.001$ and Rho= 0.821, $p<0.001$ respectively). Outcome scores for the three retained HIT sub-tests, if the rabbit approached the human, if it allowed the human to stroke it and if it was picked up, were combined to one overall HIT outcome score by adding the three scores together. The scores for 'social' behaviour during the HIT ST2 were also not included as no other measures that measured sociality were retained. This resulted in ten retained items for inclusion in the stage 1 PCA.

5.5.5.1 Stage 1 exploratory PCA

Sampling adequacy was acceptable (KMO 0.531, Bartlett's Test of Sphericity $p<0.001$). Four components had eigenvalues >1, accounting for 71.2% of the cumulative variance (Table 5.12). There were three complex items, all of which were retained at this stage and one trivial component (PC4) with only one item loading on to it above 0.55. This one item, 'NOT count of independent contacts with object', was removed from analysis prior to running the stage 2 PCA. Two items had negative relationships but only item 'NST latency to approach substrate' was reverse scored prior to running the stage 2 PCA, as the second item with a negative relationship 'NST

count of independent contacts with substrate' loaded positively on a second component (PC2) with better face validity.

Table 5.12 Stage 1 PCA (no rotation) using the ten retained behaviour test items for 32 rabbits. Items in bold were acceptable at this stage and retained for stage 2 PCA

Items	PC1	PC2	PC3	PC4
LEOF Zones visited	0.837			
OFT Zones visited	0.809			
OFT Lines crossed	0.680	0.549		
LEOF Number of times out of carrier	0.566			0.511
NST Count of independent contacts with substrate	-0.541	0.505		
NST latency to approach substrate		-0.809		
NST Proximity to item		0.639		
HIT Overall (combined) outcome score			0.758	
HIT T3 Location			0.744	
NOT Count of independent contacts with object				0.761
Cumulative variance explained	**26.3%**	**18.4%**	**14.8%**	**11.7%**

h^2 = communalties, defined as the proportion of each variable's variance that can be explained by the extracted components.

5.5.5.2 Stage 2 (rotated) PCA

Since it was not known if the components were independent, the PCA was rerun with the nine retained items using both orthogonal (Varimax) and oblique (Direct oblimin) rotations. The three newly generated component scores, generated using Bartlett's method, indicated that the three components were divergent (r= 0.0, p> 0.05 for all) and therefore the Varimax rotation solution was retained.

Sampling adequacy was acceptable (KMO 0.572, Bartlett's Test of Sphericity p<0.001). Three components were extracted accounting for 63.4% of the cumulative variance and there were no trivial components or complex items (Table 5.13). Communalities were good (closer to 1.0) for all but two items, 'LEOF number of times out of the carrier' and 'NST proximity to the item', which both had low communalities indicating these items may not be well explained by the components they are loaded

on to. Additionally, 'LEOF times out of carrier' also loaded below the 0.5 item loading cut-off required (Clark and Watson, 1995) and so was removed at this stage.

Table 5.13 Stage 2 PCA (Varimax rotation) using the nine retained behaviour test items for 32 rabbits.

	PC1	**PC2**	**PC3**	h^2
OFT zones visited	0.892			0.802
OFT Lines crossed	0.850			0.804
LEOF Zones visited	0.805			0.718
LEOF Times out of carrier	0.416			0.392
NST Latency to approach substrate		0.788		0.677
NST Count of independent contacts with substrate		0.707		0.607
NST Proximity to item		0.569		0.355
HIT Overall outcome score			0.833	0.704
HIT T3 Location			0.784	0.644
Cumulative variance explained	28.8%	18.9%	15.7%	

h^2 = communalties, defined as the proportion of each variable's variance that can be explained by the extracted components.

5.5.5.3 Internal consistency

Correlation matrices were examined for the three retained components using Spearman rank correlations with Bonferroni correction where adjusted p values were required to be <0.025 for PC1 and PC2 and <0.05 for PC3. The mean inter-item correlation for PC1 was 0.620 (Table 5.14), slightly higher than the ideal range of 0.15 to 0.05 (Clark and Watson, 1995), suggesting that there may be repetition in what the items are measuring. Only two items had a statistically significant correlation in PC2 (Table 5.15) and so item 'NST proximity to substrate' was removed prior to running the stage 3 PCA. The two items on PC3 had a satisfactory mean inter-item correlation (*Rho*=0.420, *p*=0.017).

Table 5.14 PC1 had acceptable internal consistency with a mean average inter-item correlation of *Rho*= 0.514 across the five items loading on this component (*n*=32).

	LEOF Zones visited	OFT Zones visited	OFT Lines crossed
LEOF Zones visited	1		
OFT Zones visited	0.562**	1	
OFT Lines crossed	0.499**	0.799***	1

** p<0.01

*** p<0.001

Table 5.15 PC2 had acceptable internal consistency with a mean average inter-item correlation of Rho= 0.307 across the five items loading on this component (n=32).

	NST Proximity to item	NST Count of independent contacts with substrate	NST latency to approach (reverse scored)
NST Proximity to item	1		
NST Count of independent contacts with substrate	0.348	1	
NST latency to approach (reverse scored)	0.151	0.424*	1

*p<0.025

5.5.5.4 Stage 3 PCA (Varimax rotation)

The seven retained items loaded across three components following the final PCA (Varimax rotation) (Table 5.16) and accounted for 75.2% of the cumulative variance. Sampling adequacy was acceptable (KMO 0.635, Bartlett's Test of Sphericity p<0.001). Communalities were good (>0.7) for all items. The three final component scores, generated using Bartlett's method, confirmed that the three components remained divergent (PC1 v PC2 Rho= 0.055, p> 0.05; PC1 v PC3 Rho= 0.033, p>0.05; PC2 v PC3 Rho= 0.001, P>0.05).

Table 5.16 Retained components following stage 3 PCA (Varimax rotation Varimax with Kaiser Normalization), for 32 rabbits.

Items	PC1 Exploratory	PC2 Boldness	PC3 Curiosity	h^2
OFT Zones visited	0.903			0.823
OFT Lines crossed	0.864			0.818
LEOF zones visited	0.792			0.683
HIT T3 Location		0.844		0.715
HIT Overall outcome score		0.840		0.715
NST Latency to approach substrate (reverse scored)			0.839	0.777
NST Count of independent contacts with substrate			0.781	0.733
Cumulative variance explained	**34.3%**	**21.9%**	**21.9%**	

h^2 = communalties, defined as the proportion of each variable's variance that can be explained by the extracted components.

5.5.6 External variation

No sex differences were observed for scores from any of the three components (PC1 U= 80.00, z= -1.408, p> 0.05; PC2 t(30)= 0.768, p> 0.05; PC3 t(30)= 0.279, p> 0.05). Additionally, there was no significant difference in PC1 or PC2 component scores across site (PC1 X^2= 0.133, p> 0.05; PC2 F(2,29)= 2.786, p> 0.05. However, for PC3, component scores were significantly different (F(2,29)= 3.384, p= 0.048). Tukey's post hoc analysis identified that site 4 had higher mean scores (0.736 SE 0.596) than site 3 (-0.288 SE 0.284), with a mean difference of 1.02 (95% CI 0.028 to 2.019), which was statistically significant (p= 0.042).

Examination of the individual variables measured during the NST identified that 'latency to approach the substrate' scores differed by site (n=49, F(df 3,3)= 13.3, p= 0.004). Pairwise comparisons indicated that sites 3 and 4 differed (f= 16.5, p= 0.024 adj.), where site 3 had a wider range of scores (mean 138.94 SE 28.5) and were slower to approach the item than site 4 (mean 15.0 SE 5.83).

None of the LEOF or OFT variable scores differed by site at trial T1 or trialT2. NST scores for latency to approach the substrate and latency to make contact with the substrate differed by site for both trials (Appendix 6, Figure A6.1). NOT scores differed

by site for proximity to object scores at both trials and latency to make contact with the object at T1 only (Figure A6.2).

5.5.7 Interactions between items and across tests

Correlations were conducted to test for interactions between each behavioural variable measured across all subtests, however, when correcting for experiment wise error using Bonferroni correction, none of the items from the NST, NOT, OFT, LEOF or HIT were correlated ($p > 0.05$ in all cases).

5.5.8 Concurrent validity of the HIT

The HIT subtest scores were examined for correlations with the handling category scores allocated by the centre staff (n=35 across three of the four sites). All three sites used a traffic light system (red, amber, green) to indicate rabbits that were fine to be handled by students (green rabbits) and those that were less favourable for handling (amber or red rabbits). Only one rabbit was identified as a red rabbit, 16 were identified as amber and 18 as green. Handling categories given to each rabbit by site staff were moderately and positively correlated with HIT ST5 (pick-up) outcome score (Tb 0.510, $p<0.01$) (Figure 5.4) and weakly correlated with overall outcome score (Tb 0.391, $p<0.05$).

Handling categories given to each rabbit by site staff were weakly and negatively correlated with ST2 doorway behaviour and ST5 pick-up behaviour scores (Tb -0.352, $p<0.05$ and Tb -0.344, $p<0.05$ respectively). Handling categories was also negatively and significantly correlated with the overall behaviour score from across the five subtests (Tb -0.484, $p<0.01$). None of the other outcome, behaviour or location scores correlated with the handling categories allocated to these 35 rabbits by site staff.

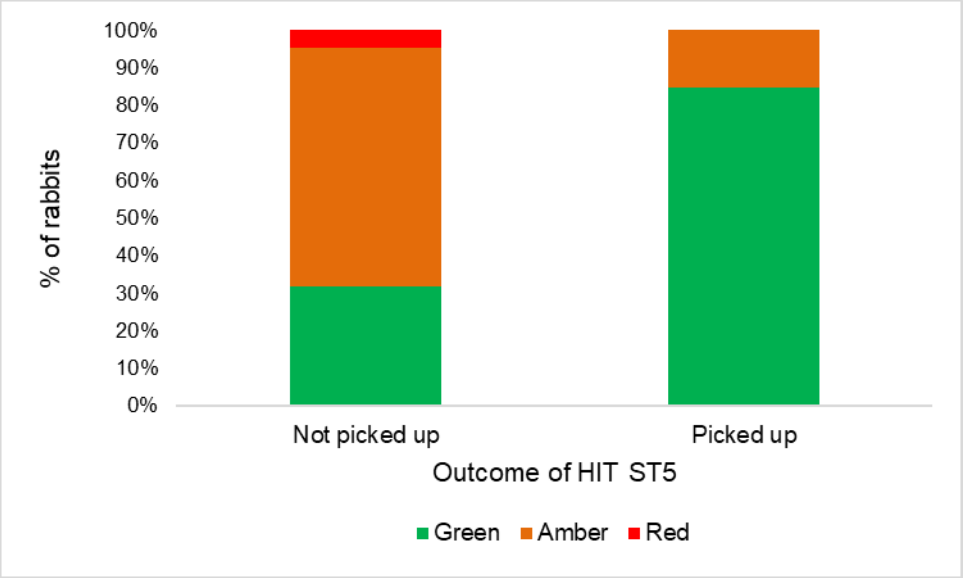


Figure 5.4 A larger proportion of rabbits identified as 'green', that is they were considered good for student handling, were picked up during the HIT ST5 (pick-up) (84.6% of rabbits that were picked up during the subtest were categorised as 'green' by site staff prior to the current study) (*n*=35, 24 were picked up).

5.5.9 Cross tool (concurrent) validity

Scores from RaBRT PC2 (labelled avoidance of humans) and PC1 from the behaviour tests, which contained items relating to activity with the open field tests, were negatively and statistically significantly correlated (r= -0.818, p= 0.021). However, only nine rabbits had scores for both tools available. None of the other components from either tool were correlated (p> 0.05 in all cases).

5.6 Discussion

A range of scores were observed for each variable measured during the five behaviour tests, demonstrating that the tests are able to detect differences within the population. Seven items across four tests met reliability criteria and were retained in the final three component solution.

5.6.1 Development of a novel test to measure rabbit-human interactions

The novel suite of subtests for measuring rabbit responses to human interactions demonstrated that some elements of rabbit responses can be reliably observed (although see note on inter-observer reliability below). Rabbit location during ST3 (approach when tester sat in the enclosure for one minute), proximity to a conspecific during ST2 (tester stood at door) and the three outcome scores from ST3 (sat in enclosure), ST4 (attempt to stroke) and ST5 (attempt to pick-up) were stable over time. However, none of the HIT scores of behaviour in the five subtests were stable over time and in the current study, HIT was only assessed for stability over time between two days. The initial pilot test that employed subjective scoring, demonstrated stability over one week of rabbits scored on their response to the approach of a human. Further exploration of responses over time would be beneficial to determine the utility of the HIT. A revised version of the HIT is therefore proposed for future studies, comprising the three outcome scores and ST3 location score. These items were also retained in the final component solution on PC2.

5.6.2 Reliability measures

Across the five behaviour tests employed for the sample population of neutered, adult rabbits, seven items measured were retained following reliability testing and loaded on to three divergent components. These three components reflect activity within the OFT and LEOF tests labelled ‘exploratory’ (PC1), responses to human interaction labelled ‘boldness’ (PC3) and the speed of approach and number of interactions with the novel substrate, labelled ‘curiosity’ (PC3). Internal consistency was acceptable for all three components. however there may be room for improvement in PC1 where the mean inter-item correlation was above 0.5, identifying that there is some repetition in what is being measured by the three items, which are all measures of activity with a novel

arena. High inter-item correlations may indicate that some items are measuring the same aspect of behaviour and therefore it may be beneficial to reduce the number of items to enhance test efficiency as some items may be measuring the same thing and therefore be redundant (Clark and Watson, 1995). PC1 included three measures of activity within a novel arena, two from the OFT (zones entered and lines crossed) and one from the LEOF (zones entered). It may be possible to only utilise the one of the tests, OFT or LEOF, in future studies and record just the number of lines crossed of the zones entered, if using the OFT, as measures of activity in rabbits, This would save time for future assessments. This would be particularly beneficial for any tool developed for use within a shelter setting where time constraints have been identified as a limiting factor for collecting behavioural information (Chapter 3, tables 3.4 and 3.5).

5.6.2.1 Inter-observer reliability

All measures observed during the commonly used open field (tests 1 and 2) and novel object (tests 3 and 4) test scenarios were reliably scored by the two observers. For the newly developed human-interaction test, some items measured were reliably recorded by the observers however many were not. Behaviour was not reliably recorded during ST1 and ST2 (approach enclosure and crouched in doorway) but observers were reliable at scoring behaviour across the other three subtests. Location was also not reliably observed during ST1 (approach enclosure). This may be due to the visibility of the rabbits on the videos, as they were easier to view once the tester was inside the enclosure in some cases, for example if the rabbit was under a shelter (personal observation). Social proximity scores were reliably scored across all subtests.

5.6.2.2 Test -retest reliability

From the 30 initial variables recorded during the five tests, 15 were found to be stable over time (between trial one and trial two), which took place three to four months apart (or over two days for the HIT). No previous rabbit personality research has examined the stability of personality in adult, domestic rabbits over this time frame. The only studies to date looking at the stability of responses over time in the OFT were conducted in young animals and at relatively short timeframes (Daniewski and Jezierski 2003; Buijs and Tuyttens, 2015). These studies showed habituation to the test, where rabbits had shorter latencies to enter the centre of the OF over time. Conversely, in the current study, latency to enter the OF was not stable over time, when tested 3-4 months apart in adult rabbits, suggesting the rabbits did not habituate to this test in the current study, although testing at additional intervals may be beneficial to conclude lack of habituation when tested 3-4 months apart.

5.6.3 Validity measures

The retained components have good face validity and were found to be discrete. While the open field and novel object tests were considered to be validated tests, differences in the shape and size of the OF and novel objects may result in poor standardisation (Marder, 2015) and so comparisons between studies should be made with caution. The novel HIT subtest-5 (attempt to pick-up) outcome score demonstrated moderate concurrent validity with the handling categories allocated to the rabbits by site staff. Four items from the HIT were retained to the final principal component solution, three of which were combined into an overall 'outcome' score and the location of the rabbits during subtest-3 (person sat inside entrance to enclosure). Additionally, the HIT measures had positive inter-observer and test – retest reliability, along with moderate concurrent validity for the ST5 outcome scores with the college's own rabbit handling

rating tool. Other variables that were not retained from all five behaviour tests in the three-component solution, may have practical value in describing individual behavioural profiles in rabbits. Therefore, the temporally stable variables were examined further in relation to concurrent validity with the RaBRT scores from Chapter 4 for nine rabbits and the home cage observations for 16 rabbits for which scores to all three tools were available (see Chapter 6).

5.6.3.1 Concurrent (cross tool) validity

There was concurrent validity with PC1 (exploration) from the behaviour tests and PC2 (labelled avoidance of humans) from the RaBRT, where the two components were negatively correlated. This may indicate that rabbits that were more likely to avoid humans, as recorded as a higher score on RaBRT PC2 were likely to do less exploring in the open filed. This may imply that measures of activity within the open filed may be more accurately described as a measure of boldness in domestic rabbits, rather than exploration. However, the sample size was small (n= 9) so the results should be considered cautiously and would benefit from further testing with a larger sample of rabbits.

Andersson *et al.* (2014) also utilised a suite of behaviour tests alongside a modified horse behaviour rating survey to assess personality in domestic rabbits, however they did not find consensus between the two methods in terms of the factors derived following data reduction.

5.6.4 Traits measured by tests used

Examination of the component solution can be used to identify possible traits underlying rabbit behavioural responses to the selected behaviour test situations (open field, novel objects and human interactions).

5.6.4.1 Open field test

From the retained three-component solution, PC1 items represent activity within the OFT and LEOF test. As all measures reflect levels of activity within this situation (latency to enter the OF was not included within the PCA as it did not meet test - retest reliability measures) the retained variables recorded within the OF may be considered measures of exploration (Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; Rödel *et al.,* 2017). However, as described above, the correlation of this behaviour test component to the avoidance of human component derived from the RaBRT, may imply that exploration within the open field is associated with boldness. Although PC2, labelled boldness and reflecting responses to humans, in the behaviour tests, was not correlated with PC1 scores. That said, there is evidence for cross-situational consistency in scores of 'avoidance of humans' (RaBRT PC2) and activity in the OFT (behaviour tests PC1) which was also reported in Rödel *et al.,* (2015 and 2017) where they identified the link between OFT exploration and response to handling in juvenile rabbits.

Studies using the LEOF version of the OFT test should consider that latency to enter the OF may not be synonymous with activity within the OF and so may support the theory that fearfulness is measured with this OF test variation. Andersson *et al.* (2014) identified three traits from a suite of behaviour tests used in adult domestic rabbits. The component 'exploration' in Andersson *et al.* (2014) may be thought as of similar to PC1 (exploration) in the current study, as both included measures of activity within the OFT, however in Andersson *et al*. the OF measures were responses to novel objects within the open field.

5.6.4.2 Novel object tests

Although not observed in the current study, recent research using novel objects in the home cage in a laboratory study, terminated this particular test part way through due to no discrimination being observed in the sample population responses to the novel objects (Krall *et al.,* 2019). The authors concluded that as the laboratory environment is relatively barren, the novel objects may be seen as an enriching stimulus and thus observed no neophobic responses as they had predicted. In the current study, between 64% and 83% of rabbits made contact with each novel object presented and so the novel object tests may be suited to use within the educational setting, however use on other settings, such as shelters, requires exploration as shelter settings may vary widely.

Novel objects added in the home cage have been reported to reflect reactivity and boldness in rabbits (Gacek *et al.,* 2012 and Andersson *et al.,* 2014). Novel substrates have not been used in previous studies with rabbits but were reported to be measures of fearfulness in equids (Lansade *et al*., 2016 and Gonzalez-De *et al.,* 2017). Only the novel substrate variables were retained in the final component solution (PC3) and the items retained included latency to approach the novel substrate. Similarly to Andersson *et al.'s* (2014) study with novel objects added within the home cage, in the current study, when latency to interact with the novel object increased, the number of contacts with the item was lower. This would be expected as with increased latency to explore the object the rabbit had less time to make repeated contacts with the object. While latency to approach the substrate may reflect shyness/boldness, the number of interactions with the object suggests exploration of novelty. Comments made to describe the nature of each rabbits' interactions with the novel substrate (Appendix 7) indicate that after sniffing, the most common interaction was to lift or move the novel substrates. Some rabbits also attempted to get underneath the substrates. The nature

of the interactions with the substrates was more complex than for the novel objects (cone and box) (Appendix 7). Investigation or examination of the substrates appears to have better face validity as an explanation for the rabbit's interactions with the novel substrates, rather than boldness. PC2 scores (containing latency to approach the novel substrate and the number of interactions with the novel substrate) could therefore reflect curiosity. Curiosity was previously identified as reflective of felid responses to novel objects (Gartner and Powell, 2012; Wielebnowski, 1999) and in hyenas from adjective ratings (Gosling, 1998). As PC1 (exploration) and PC3 (curiosity) were divergent, exploration in relation to novel environments and the examination of a novel object added in the home cage, may represent different underlying mechanisms driving behaviour in rabbits.

Andersson *et al.* (2014) tested two novel objects (wooden pyramid and rubber duck) in the home cage on separate occasions and both loaded on the same component following dimension reduction statistics. In the current study, no objects measured during the NOT were retained in the final component solution and no correlations were found between any individual test items measured during the NST or NOT. However, the specific interactions the rabbits had with the two types of items also appears to vary (Appendix 7). It can be concluded that the novel objects and novel substrates used in this study were not perceived in the same way by the rabbits sampled. The choice of object should therefore be considered in future designs of the novel object tests before drawing conclusions about the underlying mechanisms that drive responses in these tests.

In Andersson *et al.* (2014) the exploration of items in the open field were thought to be reflective of exploration and responses to novel objects in the home cage along with two measures of reactivity to a predator were identified as boldness. The authors also utilised an intraspecific social test and predator response test with scores from these

two tests loading on a component labelled anxiety. The inclusion of the predator test items along with the response to novel objects in the home cage in Andersson *et al's*. (2014) study, does imply an element of risk taking underlying this component, which differs to the component generated in the current study from interactions with a novel substrate presented in the home cage (PC2 labelled curiosity). Further work is needed to understand the traits measured using novel object tests within the home cage or novel environment for domestic rabbits.

5.6.4.3 Human interaction tests

Two HIT measures were retained in the final solution. One combined outcome score from the three outcome tests and a second identifying the location of the rabbit during subtest 2, when the handler sat inside the doorway to the enclosure. This component did not correlate with either other two components measured during the behaviour tests, reflecting exploration in the open field and curiosity. Nor, was this human response component correlated with the measure of human avoidance measured in the RaBRT. However, the sample size for both tests was small and therefore this may benefit from further testing with a larger sample.

A challenge with interpreting the outcome from human interaction tests in rabbits relates to the behavioural responses adopted by rabbits in response to a threat which may be active or passive (Verga *et al.,* 2007). Passive responses, where the rabbit may freeze rather than evade a threat, may appear to make a rabbit stay closer to the approach of a person and easier to pick up, despite them being fearful. Passive and active rabbit responses were incorporated into the behavioural measures recorded during the HIT (Appendix 5a), however none of these behaviours were not stable over time and were not retained to the final component solution. It may be that PC2 'boldness' measured using the behaviour tests in the current study, is a measure of ease of being able to pick up a rabbit, rather than reflecting the personality construct

of boldness. That said, the ease of being able to handle a rabbit is an important consideration where there are regular interactions with humans (Bradbury and Dickens, 2016) and so the HIT may be a beneficial test for interested in rating rabbits by ease of handling. As a measure of personality, the HIT requires further exploration to understand the underlying mechanisms driving responses to human interaction tests in rabbit and the inclusion of additional behavioural responses reflecting passive responses to humans would be beneficial.

5.6.5 Limitations

The use of video scoring of the home cage tests resulted in missing scores for some rabbits due to them being out of view, which limited the sample size. Live recording of test scores may be beneficial to ensure the observer can make efforts to view the focal animal as required throughout the tests, although implications for the effect this may have on the behaviour of the rabbits should be considered.

When observing the behaviour of group housed animals, the effect of the other animals and the shared housing conditions within sites and differing housing conditions between sites, may affect the behaviour expressed by the individual. Some rabbit behaviours were observed for different proportions of time in group versus singly housed laboratory rabbits (Podberscek *et al.,* 1991). As such, the sampled data for each individual is not independent, as assumed for some of the inferential tests utilised here. This limitation is not uncommon in studies of captive group-housed animals (Uher, 2008; Gartner *et al.* 2014; Williams *et al.,* 2019). Unfortunately, the nature of the ordinal data and limited sample size achieved in the current study made it difficult to account for the effects of grouping when looking at interactions between groups, i.e. sex differences. The results from inferential testing, specifically examining external validation examining sex and site differences, should be interpreted with caution.

By sampling rabbits housed at educational facilities, it was possible to sample stable rabbit populations, a mix of breeds and ensure that two individuals at two sites, with experience of each rabbit, were available to complete the behaviour rating survey. The limitations of sampling rabbits at different sites means that the rabbits are exposed to different environments and different schedules of human activity. To reduce the impact of human activities on the rabbits, testing took place outside of term time, meaning that there were less people about and limited activities that may impact the rabbits behaviour. The examination of behaviour test results by site indicated that only tests occurring in the home cage, not the novel arena tests, differed for some sites. The novel substrate tests and the novel object tests differed between sites. Typically, rabbits at sites 1 and 4 were quicker to approach the novel substrates at both trials, compared with rabbits at sites 4 and 3 (Appendix 6). However, at the component level, this was only relevant to the retained component 3, reflecting interactions with the novel substrate within the home cage, which were significantly different between sites 3 and 4 only. This difference between scores at the different sites may reflect the differences in the setup of enclosures at each site. While enclosure sizes were similar across sites (Appendix 2), site three was the only college to have elevated shelves in each enclosure and used a deep (approx. 40cm) straw substrate, both of which may have impacted the visibility of the substrate when added to the home cage and resulted in the slower approach times at this site. As the intended future use for these tests was within rabbit rehoming shelters, which are unlikely to have standardised enclosure setups, it may be challenging to ensure the novel substrate test in the home cage is standardised across sites. Therefore, it may be beneficial to generate guidance around the setup of the test area within the home cage, to provide some consistency between sites. For example, the home cage may have a surface that is always kept clear and is easily visible from all areas of the enclosure, including in hiding places. Testing novel items away from the home cage may also be beneficial, as done by Andersson *et al*.

(2014). Andersson *et al.* (2014) included novel item tests within the home cage and within a novel arena, with the two versions of the test loading onto two divergent components. It may be that the novel items within a novel environment elicit a different response to items added in the home cage, which may benefit from further investigation in future research.

5.7 Conclusions

- The use of behavioural tests generated three components following dimension reduction, which were reliable in terms of inter-observer and test re-test reliability.
- Activity in the OFT was found to be stable over time in the current rabbit population and measures of activity in the OFT were considered to reflect a trait of exploration (PC1).
- A second component was labelled boldness (PC2) and included two items reflecting a rabbits location within the home cage on approach of a human inside the enclosure doorway and an overall outcome score indicating if the rabbit approached the person, and if it was stroked or picked up by the person. However, it is suggested that this component may be measuring ease of picking up a rabbit, rather than being sensitive to rabbits that are more passive in their responses to humans, which may have been misinterpreted as easy to stroke and pick up.
- HIT scores for being picked up was correlated with categories given by college technicians at three sites to identify rabbits that were easier to handle or less easy to handle.
- The third component was labelled curiosity (PC3) based on the nature of the interactions the rabbits had with novel substrates presented within the home

cage. This trait requires further examination in future studies to exclude other possible explanations of motivating mechanisms in domestic rabbit responses to novel objects in various forms.

Chapter 6

Behavioural coding in the home cage

CHAPTER 6: BEHAVIOURAL CODING IN THE HOME CAGE

6.0 Objectives

Objective 2: Investigate personality traits in adult, domestic rabbits through the development of personality assessment tools that could be used within applied settings.

c) Development of behaviour coding tool for use within the home cage to measure personality traits in adult, domestic rabbits, examining reliability and validity criteria.

6.1 Summary

Observing the behaviour of individuals in a natural setting should be beneficial to identify personality traits that arise in animals, since behaviour is the output of the latent mechanisms determined by personality. Behaviour coding in natural settings has rarely been conducted in studies of rabbits to describe personality traits. The inclusion of observations in the natural setting for the domestic rabbits housed in educational facilities, as studied in chapters four and five, were incorporated into the current study to allow cross tool (concurrent) validation. An additional aim was to explore the use of a behavioural coding tool in the captive setting to further our understanding of personality traits that may exist in adult, domestic rabbits. Following a pilot study (*n*=12) exploring the range of behaviours that could be obtained with remote observation methods (CCTV) and exploring optimal times of day to observe the behaviour of the rabbits to gain the most insight, 16 rabbits were observed twice, three to four months apart. Just one behaviour was found to be consistent over to the two observations and it was not possible to utilise data reduction statistics with the sample due to not meeting the sampling adequacy standards required. Some, but not all, individual behaviour

items were correlated with items from the RaBRT and behavioural tests. It was not possible to confirm if the behaviours observed in the home cage reflected personality traits in the selected sample of rabbits. The correlations between activity observed in the home cage and both of the other tools suggests that all three tools do have the potential to identify this one aspect of rabbit behaviour within domestic rabbit populations.

6.2 Introduction

As personality manifests in the form of behaviour, observations of behaviour under non-experimental conditions (behavioural coding in a natural setting) are an important aspect of personality assessment that is often overlooked (Furr and Funder, 2007). However, there are challenges with measuring personality through natural setting observations, including the lack of opportunities to explore traits of interest and the time-consuming nature of such measurements (McDonald, 2008). Natural setting observations are rarely used in animal personality studies, with more studies of domestic species utilising behaviour tests or survey tools (Gartner, 2015). Research exploring rabbit personality in natural settings have predominantly included a semi-wild population of rabbits (Rödel *et al.,* 2006; Monclús and Rödel, 2009; Eccard and Rödel, 2011; Rödel *et al.,* 2015) and mostly explored social interactions through measures of approach and avoidance with intraspecifics. Only one study of domestic rabbits has utilised observations in the home cage (captive enclosure the individual resides in for the majority of its time) environment (Mullan and Main, 2007), however, the study did not use this information to explore personality traits, nor were observations conducted over time to demonstrate the stability of behaviour in this undisturbed pet accommodation situation.

In the current study, behavioural coding in the home cage was used to provide data for concurrent validation of the survey and behaviour test tools. The home cage setting

for the rabbits used was an animal care unit within four colleges in the UK. Observations were taken at two time points, three to four months apart using CCTV to avoid disruption to the animals during observations.

6.3 Ethogram generation

A literature search of terms (as described in Chapter 4.3.1) resulted in 26 behaviours being selected for the ethogram that represented the behaviours sampled with the RaBRT and behaviour tests (Chapters 4 and 5). The hypothesised traits reflected by these behaviours included boldness / shyness (vigilance), exploration, activity and intraspecific sociality (Table 6.1).

Table 6.1 Ethogram for rabbit behaviours utilised for focal, instantaneous sampling for state (s) behaviours and all occurrence (frequency) sampling for event (e) behaviours.

Categories	Behaviour name	Behaviour descriptions	References
Active Locomotion	Walk (s)	Locomoting where the forelimbs advance separately (asymmetrical). Considered to be slower movement than hopping.	
Active Locomotion	Hopping / running (s)	Locomotion where the forelimbs and hind limbs move symmetrically and the animal advances.	Mullan and Main, 2007; Buijs, 2011; Buijs *et al.,* 2015
Active Locomotion	Stand (s)	Body weight on all four feet, all limbs extended with abdomen off the floor.	Buijs *et al.,* 2015
Inactive Maintenance	Sit (s)	Four paws on ground and all supporting weight with hind limbs tucked under the rump and forelimbs extended. Ears against back or no higher than 45 degrees from back (or straight at sides for lop).	Kalagassy *et al.,* 1999; Hansen and Berthelsen, 2000; Dixon *et al.,* 2010
Inactive Maintenance Social	Huddling (s)	Within at least one body length of another rabbit, without any physical barriers. Laying on side or abdomen, head may be lifted or lowered, ears must be back against back (or no higher than 45 degrees from back) or straight at sides for lop. Eyes may be open or closed.	Mullan and Main, 2007; Reyes-Meza *et al.,* 2011
Inactive Maintenance	Rest (on own) (s)	Away from other rabbits. Laying on side or abdomen, head may be lifted or lowered, ears must be back against back (or no higher than 45 degrees from back) or straight at sides for lop. Eyes may be open or closed.	Kalagassy *et al.,* 1999; Hansen and Berthelsen, 2000; Mullan and Main, 2007; Dixon *et al.,* 2010
Inactive Vigilance	Alert or Scanning (s)	Alert – seated, standing on all four paws, or laying on side or abdomen with ears up (at least at 45 degrees up from back)(or drawn back/forwards for lop) and eyes open. Scanning – at least three feet on the ground. Stopped behaviour that it was doing, raised head, turned and looked at either side / behind self. Behaviours combined following pilot study	Hansen and Berthelsen, 2000; Monclus *et al.,* 2005; Rödel and Monclus 2011

Categories	Behaviour name	Behaviour descriptions	References
Inactive Avoidance	Hiding (s)	Rabbits head and /or entire body is underneath a box, substrate, shelf or inside a tunnel.	Rödel and Monclus, 2011
Active Maintenance	Digging (s)	Digging in substrate (straw or soil)	Mullan and Main, 2007; Schepers *et al.,* 2009
Active Maintenance	Foraging / eating / drinking (s)	Sniff food items or feeding equipment / substrate or eating or drinking from food bowl or hay ball / pile	Mullan and Main, 2007; Schepers *et al.,* 2009
Active Maintenance	Autogroom (s)	Grooms self, washes face, includes shaking - either from a seated or standing position	Hansen and Berthelsen, 2000; Mullan and Main, 2007; Dixon *et al.,* 2010
Active Social Affiliative	Allogrooming (s)	Grooming another rabbit	Mullan and Main, 2007; Schepers *et al.,* 2009; Rommers *et al.,* 2014;
Active Social Affiliative	Follow other (s)	Rabbit is hoping or walking behind another rabbit for at least 3 seconds, where the other rabbit may be locomoting but not evading.	
Inactive Exploration Vigilance	Rearing (e)	Standing or sitting on hind limbs with both forelimbs off the ground and head raised.	Monclus *et al.,* 2005; Schepers *et al.,* 2009; Reyes-Meza *et al.,* 2011; Andersson *et al.,* 2014; DiVincenti and Rehrig, 2017
Active Maintenance Comfort	Stretch (e)	Stretching front legs forwards and hind legs anchored on ground or up against barrier	Buijs *et al.,* 2011
Active Exploratory	Sniff non-food item (e)	Sniffing item / substrate / enclosure barrier / food source	Schepers *et al.,* 2009
Active	Manipulate non-food item (e)	Manipulating toy / enrichment in enclosure (chew / drag / lift / nudge)	Schepers *et al.,* 2009

Categories	Behaviour name	Behaviour descriptions	References
Exploratory			
Active Social Territorial	Chinning / scent marking (e)	Chinning / scent marking in enclosure with chin on item (not other rabbit)	Mullan and Main, 2007) (Andersson et al 2014)
Active Social Agonistic	Displace (e)	Pursue in a run / lunge at other rabbit (lasts less than 3 seconds), whereby the other rabbit moved to a new location	Kalagassy *et al.,* 1999; Rödel and Von Holst, 2009; Vervaecke *et al.,* 2010
Active Social Defensive	Displaced (e)	Rabbit moves away from other rabbit, lasts less than 3 seconds	Vervaecke *et al.,* 2010
Active Social Agonistic	Chase (e)	Rabbit runs behind another rabbit for at least 3 seconds, where the other rabbit is evading the focal rabbit. (considered to be an escalation of displace, by Rödel and Von Holst, 2009)	Rödel and Von Holst, 2009; Schepers *et al.,* 2009; Rommers *et al.,* 2014; DiVincenti and Rehrig, 2016
Active Social Defensive	Evade (e)	Rabbit moves away from other rabbit, lasts 3 seconds or more	Rommers *et al.,* 2014
Active Social Agonistic	Mounts rabbit (e)	Mounts another rabbit from rear, front or side (attempts to get on top of another rabbit)	Kalagassy *et al.,* 1999; Schepers *et al.,* 2009
Active Social Affiliative	Sniff other rabbit (e)	Sniffing (nose towards) other rabbit – may be in adjacent enclosure through barrier. May include anogential nuzzling, nose-to-nose or nose to body contact.	Kalagassy *et al.,* 1999; Rommers *et al.,* 2014; DiVincenti and Rehrig, 2016
Active Social Territorial	Urine sprays rabbit (e)	Scent marking another rabbit by urinating on them	Schepers *et al.,* 2009

Categories	Behaviour name	Behaviour descriptions	References
Active Social Defensive	Submit (e)	Crouches body low to ground when being approached or in contact with another rabbit	Rommers *et al.,* 2014
Other	Other	Any other behaviour observed during instantaneous sampling. Other events were not recorded.	

NB: Walk and follow other were included following an initial review of footage obtained as they were observed in the rabbits and considered to be distinct from other behaviours identified from the literature search.

6.4 STUDY 1: HOME CAGE BEHAVIOURAL CODING PILOT TEST

To test the feasibility of recording all behaviours identified from the literature search (ethogram) from the video footage, and to determine the most suitable time of day for filming, one site (site 1) was used for a pilot test during spring 2015.

6.4.1 Methods

6.4.1.1 Materials

Footage of rabbits from site 1 (Appendix 2) was obtained for three days at three time slots; morning 9am to 10am, midday 12pm to 1pm and afternoon 3pm to 4pm). The footage for twelve rabbits at the site was collected within 4 weeks (due to moving the cameras between enclosures). A CnM Secure H.264 CCTV system and cameras were mounted to the wall inside the enclosures at a height of approximately eight foot with all cables secured.

6.4.1.2 Procedure

Videos were coded by a single observer using instantaneous (30 second intervals for state behaviours) and all occurrence (for events) focal sampling. Additionally, the animal's location within the enclosure (Farnworth *et al.,* 2011) and proximity to a conspecific (if they were within one body length of the largest rabbit present), was recorded.

Sampling started from the time the rabbit was identifiable and visible on camera and on every minute or thirty second mark, as measured by the video playback software (Windows Media Player 10, Microsoft). As the cameras did not provide full coverage of the rabbit enclosures, the approximate percent of CCTV coverage was recorded for each enclosure (mean average 78%). Rabbits that were not able to be viewed for the

full hour of the first video were then sampled in the footage from the next day, up to the three days that footage was obtained. The number of videos needed to obtain the hour of footage per rabbit was also recorded (mean 1.98, SE 0.08).

Behavioural states (Table 6.1), location within the enclosure (front or rear) and proximity to a conspecific (< one body length of the largest rabbit (Rödel *et al.,* 2006) were recorded using instantaneous (30 second sampling), giving 120 sample points per rabbit, per time of day and 360 sampling points on total per rabbit. Behavioural events were again sampled using all occurrence sampling.

6.4.1.3 Data analysis

The data were reviewed for analysis of distribution for all rabbits by times of day and for the total of all time periods combined. This enabled further refinement of the ethogram to retain only behaviours that could be observed easily using the video footage and to exclude any that had never been observed in this setting. Any behavioural state that was never observed at any given time point or had a standard error less than two, was excluded from the next stage of data collection but retained to be recorded as behavioural events. Behaviours with very low variance around the mean were not considered to be beneficial to detect individual differences within the population. The time of day to use in future observations was determined based on the retained behaviours being sampled at each time of day and examination of the standard error for each behaviour at each time of day.

6.4.2 Results

6.4.2.1 Analysis of distribution

Eight behavioural states were excluded following examination of the distribution of data collected using the study methods (Table 6.2 items in bold were retained). The behaviour ‘hide’ was retained despite not meeting the required criteria, as the sample

population had limited hiding locations. 'Other' was also retained. Of the retained behaviours, the standard error of the frequency of observations at each time of day varied giving no clear time of the day with better scope for observing a maximum range of behaviours in the sample population.

Table 6.2 Behavioural data recorded for 12 rabbits over three hours in one day, showed that some behaviours were rarely observed, and some were absent for at least one time point. The behaviours in bold were retained for the next stage of observations.

Behaviour items	AM		Midday		PM		Day total			
	Mean	SEM	Mean	SEM	Mean	SEM	Min	Max	Mean	SEM
Walk	0.167	0.167	0.400	0.267	0.917	0.358	0	6	1.42	0.58
Hop/run	3.50	1.01	1.00	0.53	3.18	0.80	2	21	7.50	1.74
Stand	0.92	0.53			0.36	0.28	0	9	1.42	0.74
Sit	5.42	1.44	1.00	0.38	2.09	0.61	2	18	8.33	1.50
Huddle	20.50	8.50	35.71	9.04	33.36	7.36	0	138	73.50	13.38
Rest	27.75	7.89	41.57	10.70	32.91	11.32	12	282	111.17	23.60
Alert	14.75	2.61	15.29	6.13	17.46	5.34	13	114	44.75	8.51
Scan							0	0	0.00	0.00
Rearing	0.42	0.34			0.27	0.14	0	4	0.67	0.33
Hide	1.67	1.58					0	19	1.67	1.58
Dig	0.33	0.19					0	2	0.33	0.19
Foraging/eat/drink	24.58	7.59	17.57	5.86	17.46	4.64	7	165	52.42	13.11
Sniff item	1.58	0.48	0.14	0.14	1.82	0.81	1	12	3.42	0.96
Autogroom	12.75	2.61	4.86	1.92	8.36	1.91	7	52	24.67	3.67
Allogroom	4.75	1.51	2.14	0.88	1.27	0.59	0	18	7.25	1.51
Follow other	0.08	0.08					0	1	0.08	0.08
Evade					0.18	0.18	0	2	0.17	0.17
Other	0.83	0.34	0.14	0.14	0.36	0.15	0	4	1.25	0.35
Location front	47.83	9.03	81.86	16.56	60.46	11.45	32	293	179.17	21.38
Location rear	72.17	9.03	38.14	16.56	59.55	11.45	53	328	163.33	23.12
Proximity to rabbit/s	37.00	10.29	50.00	11.12	53.46	9.72	1	240	117.42	22.20

Excluded behaviours were retained as events for study 2.

6.4.3 Discussion

Nine behavioural states were retained to the next stage of data collection as they were readily observed using the video footage and demonstrated a good distribution of scores within the sample population. Behaviours were reflective of those observed during a ten-minute sampling period in domestic rabbits (Mullan and Main, 2007) and wild rabbits (Gibb, 1993). Location and proximity scores were readily obtained and demonstrated good distribution within the sample population. These measures were retained to the next stage of data collection.

While rabbits are naturally more active during dusk and dawn, the effects of external noise and daytime feeds during light periods can result in a predominantly diurnal activity pattern (Jilge, 1991). Laboratory studies have also demonstrated that feeding occurs throughout the day in domestic rabbits but peaks overnight (5pm – 5am) and autogrooming tends to peak in the morning (1am – 12pm). Of the retained behaviours, only ‘hide’ was limited in the time of day it was observed and other behaviours were more frequent at differing times of day. Therefore, future data collection incorporated observations at all times of day.

6.5 STUDY 2: HOME CAGE BEHAVIOURAL CODING

6.5 Methods

Home cage data is presented only for 16 rabbits (Appendix 2) that had full scores for the retained RaBRT components and retained components from the suite of behaviour tests. This enabled concurrent validation testing between the three tools; home cage behaviour observations, behaviour tests and the behaviour rating survey tool (RaBRT).

6.5.1 Materials

The refined ethogram was used to sample the video footage. Videos were obtained using either CCTV or one of two mountable cameras, a handheld Full HD 1080P 16MP handheld digital camera (spring observations only) or a FREDI 4k Ultra HD Sports Action Camera that were mounted using a universal 360-degree rotation flexible grip mount.

6.5.2 Procedure

To ensure the sampling period could be replicated in a working situation, such as a shelter or educational facility looking to assess the rabbit's personality, each rabbit was observed for five minutes of observations for three different time points (morning 9am to 11am, midday 11.30am to 1.30pm, afternoon 2.30pm to 4.30pm) in one day. Timing was determined based on accessibility to the rabbits at this time and as a range of behaviours were found to be able to be observed at these times during the pilot test. This gave 10 sampling points for behavioural states per time of day and 30 sampling points per trial. Each rabbit was sampled at two trials three to four months apart and on the same days as the behaviour tests described in Chapter 5 (Figure 5.3).

The internal clocks of the CCTV replay function and the video playback software (Windows Media Player 10, Microsoft), where handheld cameras and the FREDI 4k Ultra HD Sports Action Camera was used, were used for timing. Behaviour states were sampled using instantaneous (30 second) focal sampling. All occurrences of the retained events were recorded. Location and proximity (within one body length of another rabbit, based on the largest rabbit present) were recorded at the 30 second intervals. Scores from the human-animal interaction behaviour test (ST2) (Chapter 5) relating to intraspecific social behaviour measured using proximity to a conspecific during approach from a human in the home cage, was incorporated with the home cage behavioural observation data reduction analysis.

6.5.3 Data analysis

The frequency of occurrences of each behaviour for each day was divided by the total possible sampling points for the day (30), giving a proportion of time spent in each behaviour for each rabbit (Martin and Bateson, 2007). The resulting number (range between 0 and 1) was used for data analysis. Reliability, validity and dimension reduction statistics were conducted as described in the psychometric testing protocol detailed in Chapter 2.

Test -retest reliability analysis was conducted and an initial PCA, however sampling adequacy was not acceptable. Therefore, only concurrent validity was assessed between the home cage observation behaviour items, the three components from the rabbit behaviour rating tool components (RaBRT, Chapter 4, *n*= 9 rabbits) and the three components generated from the suite of behaviour tests (Chapter 5, *n*= 16 rabbits). The individual item scores from all three tools were also assessed using correlations (Spearman rank or Pearson correlations as determined following Shapiro-Wilk test of normality) with Bonferroni correction of p values and were completed in groups as follows: intra-specific social behaviours (10 items from the RaBRT and 5

social scores from the HIT behaviour test), responses to humans (24 items from the RaBRT and 10 from the HIT behaviour test), and exploration (15 items from the RaBRT and 9 from the OFT, LEOF, NST and NOT behaviour tests).

6.6 Results

Visibility on the cameras was better during trial 2 (80.1% of observation area was visible compared to 64.8% in trial 1). Data for 16 rabbits located at two sites (sites 2 and 3) that had full RaBRT and behaviour test scores are presented.

6.6.1 Analysis of distribution

Several of the state behaviours had a low variation of scores (Table 6.3), specifically hop/run and sniff item. Very few event behaviours were observed during the observations and so they were not analysed for distribution of scores due to the high frequency of nil observations.

Table 6.3: Behaviour data recorded for 16 rabbits at two sites, representing 15 minutes of observations with five minutes for each of three time points across the day.

	Trial 1				Trial 2			
	Mean	**SE**	**Min**	**Max**	**Mean**	**SE**	**Min**	**Max**
Hop/run	0.04	0.01	0.00	.13	0.03	0.01	0.00	0.13
Huddle	0.10	0.03	0.00	.30	0.10	0.03	0.00	0.33
Rest	0.12	0.04	0.00	.40	0.22	0.06	0.00	0.70
Alert	0.15	0.04	0.00	.60	0.09	0.03	0.00	0.47
Hide	0.18	0.06	0.00	.60	0.04	0.02	0.00	0.33
Foraging/eat/drink	0.27	0.06	0.00	.70	0.42	0.07	0.03	0.90
Sniff item	0.03	0.01	0.00	.17	0.02	0.01	0.00	0.10
Autogroom	0.08	0.03	0.00	.33	0.04	0.01	0.00	0.20
Allogroom	0.03	0.01	0.00	.13	0.04	0.02	0.00	0.27
Other	0.01	0.00	0.00	.03	0.01	0.01	0.00	0.03
Location front	0.50	0.07	.07	1.00	0.59	0.08	0.00	1.00
Location rear	0.50	0.07	0.00	.93	0.41	0.08	0.00	1.00
Proximity to rabbit/s	0.36	0.05	0.00	.70	0.47	0.05	0.17	0.80

6.6.2 Test -retest and dimension reduction

Only 'sniff item' (event) was reliably scored over time (*Rho*= 0.540, p<0.05). All thirteen items were therefore retained to enable initial exploration of the behavioural observations form the home cage with dimension reduction statistics, however, sampling adequacy was not acceptable (KMO 0.081, Bartlett's Test of Sphericity p<0.001) and so no further dimension reduction was completed. Individual item scores are considered for correspondence with component scores and item scores from the RaBRT and behaviour tests.

6.6.3 Concurrent validity

After adjusting the p value for multiple testing (Bonferroni method), only one component from the behaviour tests, PC2 which included items from the human interaction test, was statistically significantly and negatively correlated with one variable, ‘sniff item’, recorded during the home cage observations (*Rho*= -0.757, *p*= 0.012) for the 16 rabbits sampled with both tools.

When examining the interaction between all individual behaviours measured in the home cage and individual variables measured during the RaBRT (Chapter 4) (*n*= 9 rabbits) and behaviour tests (Chapter 5) (*n*= 16 rabbits), only three variables had significant correlations, after correcting for multiple tests (Bonferroni method), two from the rabbit behaviour rating tool (RaBRT) and one from the suite of behaviour tests. RaBRT Q 10 'East food while people are nearby' significantly correlated with amount of time the rabbits spent in different parts of the home cage (front *Rho*= 0.902, *p*= 0.049; rear *Rho*= -0.902, *p*= 0.049). RaBRT item Q6 'Is active' negatively correlated with the proportion of time the rabbits spent grooming themselves in the home cage (*Rho*= -0.901, *p*= 0.02). The number of contacts with the substrate measured during

the novel substrate behaviour test, negatively correlated with proportion of time spent at rest in the home cage (*Rho*= - 0.883, *p*= 0.038).

6.7 Discussion

Observations of rabbits in their home environment did not yield any clear identification of personality traits in the current study. As the test -retest threshold was only met for one item and the sampling adequacy was below the acceptable level to enable data reduction analysis to be conducted, the behavioural observation data were only used to support the understanding of the items measured in the RaBRT and behaviour test tool. The limitations of the data collection and data analysis are discussed below.

6.7.1 Reliability measures

6.7.1.1 Test – retest

Only one item measured 'sniff non-food item' was reliable over time when tested three to four months apart, however this behaviour was rarely observed and so this result may be a result of the limited range of scores observed. Previous studies assessing home cage observations in adult domestic rabbits did not conduct repeated tests over time (Mullan and Main, 2007) and so it is not possible to know if the lack of stability of behaviours over time is applicable to the wider domestic rabbit population. The limited time of observations (15 minutes over the day) and confounding effect of conducting the behaviour tests on the same days as home cage observations may have resulted in an interference effect and influenced the behaviour observed at each trial and as such, the home cage observations may not be reflective of undisturbed conditions for the rabbits. In future studies it would be beneficial to conduct the home cage observations on different days than the behavioural tests.

6.7.2 Exploration of personality traits in home cage observations

The behaviour of rabbits in the current study showed similar patterns to that of rabbits in other studies for comparable times of day. Observations were made between 9am and 4.30pm and at trial 1 feeding was the most frequently observed behaviour, followed by hiding, alert responses and resting. At trial 2, feeding was still the most commonly observed behaviour followed by resting including social resting (huddle) behaviour. This is similar to observational studies of laboratory and farm rabbits, that are also exposed to artificial daylight and human activities during the day, where rabbits are reported to spend the most time performing rest, maintenance and ingestive behaviours (Dixon *et al*., 2010; Szendrő and Dalle Zotte, 2011; Prebble *et al.,* 2015).

Four variables measured during the home cage were correlated with variables measured during the behaviour rating tool and suite of behaviour tests. This cross-tool examination may help to support the development of theories explaining the mechanisms underlying responses to the tests used. One variable, 'sniff non-food item', was correlated with PC2 from the behaviour tests 'boldness in response to humans' and was also temporally stable when tested on two occasions three months apart, but as discussed above, this may be due to this behaviour rarely being observed. Just six out of the sixteen rabbits were observed sniffing non-food items during trial 1 and eight of the same sixteen rabbits were observed performing this behaviour at trial 2.

6.7.3 Limitations

Behavioural coding while animals are undisturbed by experimental conditions, referred to as natural setting or home cage observations in the current study, have historically had limited sample size (Gosling, 2001). This is likely due to the time-consuming nature of such observations. In the current study, the limited visibility of the rabbits on the video cameras and reduced number of rabbits that had corresponding data from the RaBRT and suite of behaviour tests, also impacted the sample size for the home cage

observations. Boosting the sample size in future research, through improved visibility of the rabbits while in their home cage, would be very beneficial and help researchers to interpret the results from behaviour tests or rating tools through concurrent validity testing.

There were challenges with visibility of the rabbits from the home cage observations where hiding places obscured the view of the rabbits. Hiding places were often large enough that the rabbits may have been performing a range of behaviours within them and so it would be beneficial to observe behaviours that occur within the shelters also. The use of semi-translucent shelters, as used in laboratory rodent enclosures (Patterson-Kane, 2003), would enable observation without disturbing the rabbits, and may be useful for future research. Such shelters are not readily available and would require safety and welfare assessments initially to ensure they do not disturb the rabbit's behaviour or present safety issues if the material is chewable.

While it was not possible to conduct a PCA on the home cage behaviour observations, adhering to strict criteria to assess the suitability of the data for conducting a PCA, in this case the examination of the Kaiser-Meyer-Olkin with values required to be above 0.5 (Budaev, 2010), avoids drawing inaccurate conclusions. A solution in the current study may have been to reduce the number of variables being used in the PCA to boost the sample to variables ratio. This was not done however, as the purpose of this study was to explore traits that may occur in rabbits without assuming specific behaviours are representative of any traits. Additionally, as the home cage observations only identified one temporally stable behaviour, possibly due to the limited duration of observations, the data had not met test -retest reliability criteria.

No published research could be found to describe the activity budgets of rabbits housed in educational facilities and laboratory and farm studies predominantly look at the effect of changes in cage size and group housing on physiological (i.e. growth rates

and frequency of injuries) and behavioural (i.e. aggression) measures. From a welfare perspective, further research describing behaviour patterns in this setting is required. In terms of measuring personality through observations of home cage behaviour in rabbits housed in educational facilities, future research will ideally seek to obtain a larger sample size and control for rabbit group and enclosure confounding variables. In the current study, the home cage observations demonstrated limited concurrent validity to a few items measured using the RaBRT and behaviour tests.

6.8 Conclusions

- Behavioural patterns in the home cage were similar to that of rabbits housed in laboratory and farm settings.
- The results from the behavioural observations in the home cage were not suitable for dimension reduction statistics and so it was not possible to extract components of grouped behaviours that may be reflective of personality traits from this tool.
- One variable measured, 'sniff non-food item', was temporally stable when tested three months apart and associated with PC2 'boldness in response to humans' from the behaviour tests.
- Time in different parts of the enclosure, front or back, were correlated with the score for RaBRT item 'eats food near to people', provided by animal care technicians.
- Rabbits that spent more time resting in the home cage made less contacts with the novel substrate, added to the home cage at different times, but on the same day as home cage observations.
- Larger sample sizes and improved methodology to enable observations in hiding places would be beneficial for future research exploring personality in a natural setting in domestic rabbits.

Chapter 7

Final discussion

CHAPTER 7: FINAL DISCUSSION

The study of animal personality has progressed over the last few decades and several researchers have looked at the applied use of measuring animal personality to match animals to specific roles in human society (Richter and Hintze, 2019). The domestic rabbit has received little attention in this area despite being the third most frequently kept companion animal in the UK and there being extensive keeping of rabbits in a range of settings, including farms and laboratories. The aim of the current study was to use three different animal personality measurement tools to determine if these tools could have benefits for use in a shelter setting to support the rehoming processes for domestic rabbits. The second aim was to understand what traits could be measured using these tools following detailed reliability and validity analysis.

7.1 Reliability and validity of three personality assessment tools

Some animal personality research to date has received criticism for the lack of reporting of critical validation and reliability information (Foyer *et al.,* 2013). The current study has attempted to be thorough in regard to the retention of suitable measures to assess rabbit personality and as such, only limited variables measured have been retained through the process of psychometric analysis. The current study utilised strict criteria to support the use of the term personality trait to the components generated from the three tools used. To demonstrate that any specific personality traits existed in the rabbits, that could be measured using the three tools employed, the variables measured needed to demonstrate "*consistent[cy* across situations] *and repeatable behaviour at the level of the individual"* (Carter *et al*., 2012a, p.153) and demonstrate between-individual variation within the population (Stamps and Groothuis, 2010; Carter *et al.,* 2013). From the three tools used, six components were identified. However, only three traits (exploration, boldness and curiosity) identified using the behaviour tests

could be said to meet the criteria above in terms of repeatable behaviours that demonstrated a range of scores within the sampled population (Table 7.1). However, the RaBRT appeared to show potential benefit for measuring traits reflecting intraspecific sociality, boldness in relation to the environment and avoidance of humans, although inter-rater and test -retest reliability criteria were not yet achieved for this tool.

Both tools, the behaviour rating tool and behaviour tests, resulted in the identification of three components that were divergent. Examination of concurrent validity, testing for convergence between tools, identified that PC2 from the RaBRT labelled 'avoidance of humans' was negatively correlated with PC1 from the behaviour tests, labelled exploration and containing measures of activity with the open field test, which is considered to provide further evidence for the existence of personality in rabbits where it supports past research findings of cross-situational (Rödel *et al*., 2015; Rödel *et al*., 2017).

While the home cage behaviour observations were limited in duration and ultimately were not suitable for examination with the principal component analysis, three behaviours and rabbit location in the enclosure were found to be correlated with items measured during the other two tests (Table 7.1). It would appear that some behaviours performed in the home cage and the location of the rabbit in the home cage may be associated with how raters score the rabbit for some behaviours on the RaBRT, but ideally more correlations would have been identified to provide evidence that the RaBRT is a valid measure of rabbit personalities. A previous study of domestic rabbits by Andersson *et al. (*2014) did not find convergence between a suite of behaviour tests and an adjective rating survey. Designing future rating questionnaires to more accurately measure the traits under examination in any behaviour tests, or likely to be

observed in undisturbed observations in the home cage, may provide useful in future research.

7.2 Application of the behaviour rating tool and suite of behaviour tests to assess domestic rabbit personality

A number of behaviour and personality assessment tools exist for selecting working dogs (Serpell and Hsu, 2001; Harvey *et al.,* 2016) and matching pet dogs to new homes in shelters (Dowling-Guyer *et al.,* 2011; Valsecchi *et al.,* 2011; Duffy *et al.,* 2014). Currently, no such tool is in use for domestic rabbits, which are also used within working roles such as animal-assisted therapies (Nimer and Lundahl, 2007) and frequently relinquished to rehoming shelters each year (Ellis *et al.,* 2017). The traits identified using behaviour tests in the current study, exploration, boldness with humans and curiosity, may have value for generating a personality profile for each rabbit for potential adopters. However, currently the function of these tests to address the need for information concerning intraspecific interactions, may be limited.

Recent research has attempted to utilise cage-side assessments of rabbit responses to humans and location within the cage to differentiate anxious from non-anxious rabbits to help to refine laboratory operation procedures (Krall *et al.,* 2019). Such research demonstrates a function for the use of behavioural tests in this species that can differentiate rabbits and support management practices that provide improved welfare standards for the individual rabbit. The RaBRT and HIT tests developed in the current study may have applications for the selection of rabbits, where interactions with humans is relevant to the situation in which the rabbit lives. However, the benefits of the RaBRT tool in relation to determining social rabbits from those that are less sociable is not clear and would benefit from further testing in relation to predictive

validity (Protopopova and Gunter, 2017), where a rabbit may be measured using the RaBRT pre-grouping and post-grouping with other rabbits.

The outcome of behaviour tests used in the current study to measure human-rabbit interactions were found to be reliably rated and demonstrated consistency over time. Additionally, the HIT tool had moderate concurrent validity with independent scores provided by site staff that identified rabbits that were better or less good for being handled. Subtests three, four and five from the HIT, that take less than one minute to complete, could be beneficial for use in applied settings to differentiate rabbits that are more approachable and those that actively avoid human interaction. This would provide a quick and relatively non-invasive assessment of rabbits, since the rabbits do not need to be handled if they avoid the handler, where there is no knowledgeable person available to rate the rabbit.

There is very little research about the information gathering processes at shelters and how this information is used to support the relinquishment to rehoming process. Vinic *et al.* (2019) reported that behavioural history information was held by shelters in the United States of America, however behavioural evaluation information was only held for dogs with none being reported for cats and no other species were explored. Shelter staff surveyed in the current study, reported a range of functions for the use of behavioural and personality information to support their work with rabbits, much of which reflected the recommended practice for shelters to generate profiles for each individual animal to aid the rehoming process (CAWC, 2011; Protopopova and Gunter, 2017). Matching the rabbit to a new home and matching rabbit pairs were commonly reported uses for behavioural and personality data. Currently, shelters are predominantly using informal observations to collate this information. Resource issues, particularly staff time, were identified as challenges the shelters face to be able to spend more time collecting and maintaining information on rabbit personality. Practical

uses for the behaviour and personality information whilst the rabbits were at the shelter, included deciding on accommodation for the rabbit and creating a management plan. Reports from past owners were considered by some shelter staff to not be reliable, and so a suite of assessments incorporating previous owner surveys and on-site behaviour assessments should ideally be implemented, as recommended when measuring animal personality (Gosling, 2008; Carter *et al.*, 2012a; Carter *et al.*, 2013). The use of the OFT, HIT, NST evaluated herein could make a relatively quick resource available for shelter staff. Such tests would take less than 20 minutes to complete and require physical resources that are likely readily available at centres, i.e. an exercise run away from the home cage, novel substrate items. In its current state, the RaBRT would require further reliability testing before it is ready for application.

7.3 Rabbit personality traits

Three traits were identified from the behaviour tests (OFT, LEOF, HIT and NST), reflecting exploration (PC1) and boldness in response to humans (PC2) and curiosity (PC3). The items on these components demonstrated good inter-rater reliability and test – retest reliability. While the OFT has been reported to be a measure of fearfulness in a number of domesticated species (Forkman *et al.,* 2007), exploration has been highlighted in several recent studies as an explanation for rabbit activity in the open field test (Rödel and Monclús, 2011; Buijs and Tuyttens, 2015; Rödel *et al.,* 2017). This explanation was retained in the current study to describe component one derived from the behaviour tests (activity in the open field test and activity in the latency to enter open field test). This interpretation is further supported by the correlation identified between PC1 scores and scores from the behaviour rating tool PC2, reflecting avoidance of humans, which has also been observed in previous research exploring rabbit personality (Rödel *et al*., 2015; Rödel *et al*., 2017).

The second component retained from the behaviour tests, labelled boldness in response to humans, may be tricky to interpret due to the passive or active nature of rabbit responses to threats, as discussed in section 5.6.4.3. This component negatively correlated with the amount of time a rabbit spent sniffing non-food items in the home cage, which could indicate that a less active rabbit, as measured by its exploration of non-food items in the home cage, was more likely to be stroked and picked up. It may be that these observed correlations reflect that a more passive rabbit is less likely to explore the environment. Recent advances using cognitive bias testing in animals (e.g. Mendl *et al.,* 2009) may be a beneficial area for future research to enable the discrimination of passive and more active coping styles in rabbits in relation to engagement with the environment and responses to human interactions.

Just one previous study has attempted to identify personality traits in domestic rabbits using a bottom-up approach (Andersson *et al*., 2014) and generated three components from a suite of behaviour tests, two of which reflected interactions with novel objects during two separate situations, the home cage and an open field. Responses of rabbits to the addition of novel objects have been reported to reflect boldness (Andersson *et al*., 2014), reactivity (Gacek *et al.,* 2012) and fear and anxiety (Buijs and Tuyttens, 2015). However, a range of novel objects have been used across studies and novel objects are sometimes presented in a novel environment, rather than the home cage. Responses to the novel substrate in the current study reflect interest in this type of novel object (latency to approach substrate and count of independent contacts with substrate) and so the component was labelled as curiosity. While distinct from the existing literature defining responses to novel objects in rabbits, this interpretation was justified on the basis of the nature of the item being different (Forkman *et al.,* 2007) and the responses to the novel substrates in this study differing to responses to the novel objects used in this study, which were large items. The number of independent

contacts with the novel objects and the novel substrates in the current study were weakly, negatively, correlated, suggesting that responses to these different types of substrates may reflect different underlying motivations. A personality trait labelled curiosity has been observed in other animal species including cats using novel object tests (Gartner and Powell, 2012; Wielebnowski, 1999), dogs using adjective rating scales and behavioural tests (Svartberg *et al.,* 2005) and hyenas using adjective rating scales (Gosling, 1998), but this is the first time it has been described in rabbits. To date, the novel object test has not been used commonly in rabbit personality research. Therefore, responses to a variety of novel items warrants further exploration in rabbit behaviour research, accounting for the environment that the rabbit has been exposed to prior to the point of testing, as done by Buijs and Tuyttens (2015) to explore the underlying motivation affecting behaviour in the OFT. This would ensure that clearer guidance can be provided in the interpretation of novel item tests for rabbits.

The RaBRT tool generated three components following dimension reduction statistics but these lacked inter-rater reliability, possibly due to the nature of the rater's experiences with the rabbits not allowing for observations of the full range of behaviours measured. While staff in an educational facility have not scored individual animals in their care in any past research that could be identified, previous research with zoo housed animals identified weaker inter-rater reliabilities where staff had only limited types of interactions with the target animals (Highfill *et al.,* 2011). Ratings by knowledgeable people are considered to have greater consensus when the rater is more familiar with the target individual and when more overt traits are being assessed (McDonald, 2008). It would be beneficial to conduct further testing of the RaBRT with a larger population of individuals that are familiar with rabbits in work and pet settings.

As with past research using the OFT (Kersten *et al.,*1989; Rödel *et al.,* 2006), no sex differences were observed in the current study for any of the components generated

from the RaBRT or behaviour tests. No sex differences were observed in ratings of rabbit responses to humans using the RaBRT, contradictory to pet owner survey results in Mullan and Main (2007) and d'Ovidio *et al.* (2016). However, a large number of rabbits in the current study were neutered and this appeared to play a role in ratings for PC2 on the RaBRT (avoidance of humans). The ratio of neutered to non-neutered rabbits was heavily skewed towards neutered rabbits, and the p value was only just significant. Neutering has been reported to affect responses to humans and was particularly evident in castrated male rabbits in a previous survey study (d'Ovidio *et al.,* 2016).

Aspects of the social interactions during development have been demonstrated to play a role in early appearing traits in rabbits (Rödel *et al*., 2006; Hudson *et al.,* 2011). Social grouping has also been reported to affect results from the LEOF, with juvenile group housed rabbits being more likely to enter the open field (Trocino *et al.,* 2014). The majority of rabbits in the current study were socially housed and entered the open field at a similar rate (trial 1 70% and trial 2 90%) to the group housed rabbits in Trocino *et al.* (2014) (81.2%). Other factors correlated with activity in the OFT have included handling condition during the first 10-20 days after birth (Kersten *et al.,* 1989). While the rearing history of the study rabbits was not known, the mean number of lines crossed by rabbits in the current study, exceeded that identified for handled rabbits during the first trial in Kersten *et al.'s* study (1989). An average of 17.2 line crossings in current study compared to 14.3 during the first trial in Kersten *et al.* (1989), however the later trials had much higher mean line crossings over 35.

Table 7.1 Reliability and validity measures met for each of the three rabbit personality tools developed.

	Inter-rater reliability	Test - retest	Components retained	Internal consistency	External variation (sex, site)	Construct validity	Concurrent validity
RaBRT	Not yet satisfactory	Not attempted	1 – Intraspecific sociability 2 – Shy / bold (humans) 3 – Boldness (environment)	>0.5 for PC1 and PC2 Ideal for PC3 (0.471)	Scores did not differ by sex, age or housing type. Neutered status differed for PC2	Components were discrete	• RaBRT PC2 'avoidance of humans' -ve correlation with behaviour test PC1 'exploration. • RaBRT item 'eats food near to people' +ve correlation with proportion of time in front of enclosure in home cage. • RaBRT item 'eats food near to people' -ve correlation with proportion of time in rear of enclosure in home cage. • RaBRT item 'active' -ve correlation with proportion of time auto grooming in home cage. • Behaviour test PC2 'boldness in response to approach from human' -ve correlation with home cage proportion of time sniffing non-food item. • Behaviour test number of contacts with novel substrate -ve correlation with time proportion of time resting in home cage.
Behaviour tests	Good	15 items acceptable over 3 to 4 months^	1 – Exploration 2 – Boldness (humans) 3 - Curiosity	>0.5 for PC1 Ideal for PC2 (0.424) and PC3 (0.426)	Scores did not differ by sex of the rabbits or site housed at except for PC3 and sites 3 and 4.	Components were discrete	
Home cage observations	Not attempted	One item (sniff non-food item) was acceptable over 3 to 4 months	Unable to extract components	Unable to analyse	Unable to analyse	Unable to analyse	

^ five HIT behaviour test items were retested one day apart, not over 3-4 months.
-ve indicates a negative correlation was identified
+ve indicates a positive correlation was identified
RaBRT – rabbit behaviour rating tool

7.4 Limitations

The exploratory nature of the study and requirements to work around the working practices of the sites holding the rabbits, impacted experimental design. This resulted in possible order effects and lack of independence of samples where rabbits were housed and tested together for the novel item tests and home cage observations. No research could be found to describe the behaviour of rabbits living within educational facilities and so the most comparable captive environments may be with zoos, where group housed animals are often treated as independent samples (Uher, 2008; Gartner *et al.* 2014; Hopper *et al.,* 2018; Williams *et al.,* 2019). For ethical reasons, it was not desirable to separate the rabbits in the current study or to remove them from their natural grouping for significant lengths of time. An alternative approach to ensuring the data was independent for inferential statistics analysis would have been to sample just one rabbit per enclosure (Martin and Bateson, 2007), but due to time limitations and the exploratory nature of the study, it was decided to aim for a larger sample of rabbits, which included mostly pair housed rabbits. As such, this limits what can be interpreted from tests conducted within the home cage, including the novel substrate and human interaction tests, which were retained to the final component solution in chapter 5. It would be beneficial for future research to explore the impact that a co-housed rabbit may have on the focal rabbit, as has been explored with captive elephants (Williams *et al.* 2019). For example, exploring if individuals housed with less sociable and more aggressive rabbits, or those housed with more or less compatible rabbits, had different measures of activity and interaction with items added to the environment, such as the novel substrate in the current study. Taking account of the nature of the relationship between the individuals, for example, are encounters more affiliative than agonistic, may enable consideration of the impact of group housing on rabbit behaviour at the individual level.

The samples size available for the behaviour tests and home cage observations was limited. While some domestic dog and cat personality research has achieved larger sample sizes over ten thousand (Gartner, 2015), Jones and Gosling (2005) reported much smaller samples sizes for studies that review reliability and validity criteria, generally less than 100 animals. Similarly, small samples sizes have been reported in studies of farm animals (O'Mally *et al.,* 2019; Costa *et al.,* 2020) and zoo animals (Freeman and Gosling, 2010; Hopper *et al.,* 2018; Williams *et al.,* 2019) and are an issue broadly across the behavioural sciences (Wilson *et al*., 2019). Obtaining larger samples sizes should be a target for future exploratory studies of rabbit personality traits to ensure the results obtained meet statistical requirements and are representative of the wider population.

There were challenges with utilising camera footage for the home cage behaviour observations, particularly due to the rabbits being hidden from view in shelters. The provision of hiding places is important for rabbit welfare (Ottesen *et al.,* 2004; Rommers *et al.,* 2014) and so the use of a semi-translucent shelter may be beneficial for future research (Patterson-Kane, 2003). This would enable the rabbits to be visible when in the shelter, however such shelters would need to be assessed for safety to ensure the rabbits would not chew and digest the material and that semi-translucent shelters provide the same perceived safety to the rabbit as a solid shelter.

The background of the rabbits that were used in the current study for the behaviour tests and home cage observations was not able to be accounted for. Past research has demonstrated that rabbit behaviour is affected by early experiences, with specific emphasis in the literature on early handling experiences on responses to humans in later life (Kersten *et al.,* 1989; Rödel *et al.,* 2006; Buijs and Tuyttens, 2015). In addition to the effect of early life experiences, the experiences of the rabbits in their current environment, for example how they are handled by students at the colleges, may also

contribute to their response to the behavioural tests. It may be possible to account for such experiences by using laboratory rabbits that are housed in identical housing, experience the same daily routines and have known breeding histories. There are so few studies on domestic rabbit personality to date, with just 16 published articles identified from the literature review reported in chapter 1. To enable the exploration of factors that contribute to the development of specific personality traits in this understudied species, further work is needed that explores rabbit personality in a range of settings.

7.5 Future research

While the RaBRT tool was developed in the current study from rabbit behaviour literature, to further develop the rating tool it would be beneficial to incorporate additional behaviours. Based on the adjectives provided by respondents to the RaBRT, it may be beneficial to include behaviours that are considered signs of friendliness or affection towards people, inquisitiveness and play behaviour which were all used by over 10% of respondents to describe the personality of their rabbits and were also terms used by rabbit owners in Mullan and Main (2007). Additionally, seeking input from rabbit behaviour experts and those working with rabbits in specific settings, i.e. animal assisted therapists and those rehoming rabbits, would ensure that the behaviours measured reflect traits of interest to the humans selecting rabbits for work roles or as pets.

Lack of socialisation and handling in early life has been highlighted as a concern for rabbit welfare in relation to the impact this has on the rabbits' experience with humans in later life (Rioja-Lang *et al.,* 2019). A recent review has highlighted that rabbit handling may be a significant welfare concern (Bradbury and Dickens, 2016) and past studies have highlighted that owners are not always confident handling their rabbits

(Mullan and Main, 2007; Oxley *et al.,* 2018). Behavioural assessments that provide a quick estimate of a rabbit's willingness to be approached by people, or be handled, may have benefits in terms of rabbit welfare and support those that work with rabbits to identify individuals that would benefit from a training and desensitisation plan. The HIT developed in chapter 5 could be utilised along with the RaBRT items relating to avoidance of humans, to assess rabbit reactions to humans over the duration of positive-reinforcement training or the use of alternative handling methods (see Bradbury and Dickens, 2016 for review of handling methods). The non-invasive nature of both tests, the RaBRT and HIT where the rabbit is not required to be picked up and has opportunities to approach the person if it wishes, could support those working in close proximity to rabbits in laboratories, educational facilities or shelters as a measure of success of any training initiative.

While the novel substrate test has been utilised with equids and goats in previous research, further exploration of what this test measures in rabbits is required. It would be beneficial to test rabbit responses to a range of novel items, incorporating substrates but also other types of novelty, for example, novel foods, novel structures such as hiding places, novel scents and objects that may produce a startle response, such as opening an umbrella. This would enable more detailed measurements of how rabbits respond to the different types of novelty. This would be in line with guidance to ensure multiple measures of the same trait and dissimilar traits are measured to understand the convergent or divergent validity of any given test (Carter *et al*., 2013) and could help to confirm the presence of a curiosity trait in rabbits. Additionally, it would be beneficial to test substrates of varying sizes, where it may be possible to force the rabbit to walk over the new substrates to reach a preferred resource, as the substrate used in the current study could be avoided as it was relatively small.

7.5 Conclusions

In summary, this thesis has addressed a gap in rabbit behaviour research, by utilising three different tools and a bottom-up approach to attempt to describe personality in adult, domestic rabbits, while also adhering to strict criteria to assess each tool against reliability and validity criteria. Two novel tools (survey rating tool and human-interaction behaviour test) have been created and tested for their reliability and validity in assessing rabbit personality traits. The use of the retained items from the rabbit behaviour rating tool (RaBRT) could be beneficial to describe the behaviour of rabbits by knowledgeable raters in relation to avoidance of humans, intraspecific sociality and boldness in relation to the environment. However, the tool needs further testing to ensure it is reliable between raters and over time. The OFT was again confirmed as a measure of exploration, this time in adult, neutered, domesticated rabbits, and alongside a novel substrate test, was useful for identifying rabbits that were more explorative and curious. A human interaction test appeared to offer a quick test to measure if rabbits allowed the approach of a person and interaction, including being picked up, which correlated moderately with independent assessments of the ease of handling the rabbits. With further scrutiny of tools designed to measure personality in animals, we can ensure tools being used in applied settings, where consequences of such tests may have consequences for the welfare of the animals, are fit for purpose and meet the needs of those looking to incorporate such tests into their operations. While there are further steps needed to test the tools retained in the current study in a shelter setting, it is my hope that this work can go some way to indicate useful future directions for the measurement of personality in domestic rabbits.

Appendices

Appendix 1: Survey questions regarding Information gathering process in rabbit rehoming shelters

Opening statement and ethical note

Please read this information carefully before deciding to take part in this research. If you are happy to participate please then go on to complete the questionnaire. This will be taken as your consent to take part in the study.

What is this research about?

I am a PhD student at the University of Northampton and Moulton College and I am currently trying to find out information about the information collection process that takes place at UK rabbit rehoming centres, from intake of a new rabbit to adoption to a new home.

This information will be used to inform the development of a tool to assess behaviour in rabbits during the relinquishment /adoption process.

Why have I been chosen?

This study has been advertised to rabbit rehoming centres and should be completed with the consent of the centre manager and be completed by a person with knowledge of the rabbit rehoming procedure.

You must be at least 18 years old to complete this questionnaire.

What will happen to me if I take part?

You will be asked to complete a series of questions as honestly as possible and there are no right or wrong answers. The questionnaire is split into six parts and should take no more than 30 minutes to complete.

A progress bar is at the top so you know how much you have completed.

Participation is entirely voluntary. You may quit at any time during the survey (up until you complete the survey).

Are there any benefits in my taking part?

Free prize draw for one of two rabbit enrichment parcels! (Only UK participants eligible)

By taking part (completing all compulsory questions) and providing a contact email address and / or telephone number (one of which can be validated as linked to a rabbit rehoming centre), your centre can be entered into a free prize draw for one of two ‘rabbit enrichment’ parcels. You may opt in / out of this free prize draw at the start of the survey.

There is no alternative prize to the ‘rabbit enrichment’ parcel.

The contents of the parcel are store bought rabbit enrichment items (see photo for contents of one pack) and no responsibility is assumed by the researchers or associated institutions for any injury, illness or damage caused by any items. They are received and used entirely at the centre's / participant's own risk.

There is no affiliation with the companies that produce these products and the researcher conducting this study.

The information gathered will hopefully be published in a journal. As a result, this research will help inform other researchers and organisations about practices to collect information about rabbits during the relinquishment / adoption process and any challenges faced by centre staff in collecting such data.

Are there any risks involved?

There are no risks in taking part, however, due to the length of time it may take to complete the survey, it is advised that you take breaks from looking at the screen during completion.

Will my participation be confidential?

All participation will be confidential. The data be anonymised and will have no information that could lead to the identity of individuals. No centres will be individually identified in any data published but participation may be acknowledged.

Data will be kept on a password protected computer / data storage device.

What happens if I change my mind?

If you feel you do not wish to continue with the questionnaire, you have the right to withdraw at any time during the completion of the survey (up until you submit the survey) without your legal rights being affected.

Where can I get more information?

If you have questions about this research please contact

Clare Ellis - Moulton College / University of Northampton (BBBS@Northampton.ac.uk).

Consent

I have read and understood the information given above. In consenting, I agree to take part in this research project and agree for my data to be used for the purpose of this study. I understand that my participation is voluntary and I may withdraw at any time.

(*indicates a mandatory question)

Question page 1

* 1. I would like to be entered in to the free prize draw for one of two rabbit enrichment parcels for our rehoming centre. (UK participants only)

- Yes please (provide organisation / centre email below).
- No thank you.

* 2. Rehoming centre name (include organisation name and local centre name if part of a chain of centres).

3. Please enter a contact email address for your centre.

* 4. What is your role in the organisation / centre? e.g. owner, centre manager, rabbit section leader, volunteer.

* 5. Which of the following best describes the organisation / centre that you are completing the survey for?

- Home based (single centre, may include use of foster carers)
- One centre, NOT based in a residence (may include use of foster carers)

- Multiple centres / sites, all part of the same organisation
- Other

* 6. Which of the following best describes the centre you are completing the survey for? (Please only complete for the centre that you are completing the survey for, not all centres that might be part of a larger organisation)

- Employs more than 20 members of paid staff
- Employs between 10 and 20 members of paid staff
- Employs less than 10 members of paid staff
- No members of paid staff (volunteers only)
- Home / family run, no staff or volunteers

* 7. Where is the centre located? (Please only complete for the centre that you are completing the survey for, not all centres that might be part of a larger organisation).

- England
- Northern Ireland
- Scotland
- Wales
- USA
- Europe (Please use 'other' box below to state country)
- Australia
- Other

Question page 2

* 8. Please select ALL of the species that are rehomed at this centre (the centre that you are completing survey for, not all centres that might be part of a larger organisation).

- Amphibians
- Cats
- Dogs
- Equines species
- Exotic mammals (e.g. sugar gliders)
- Farm bird species
- Farm mammal species
- Invertebrate species
- Pet / exotic bird species
- Rabbits

- Reptiles
- Small mammals (e.g. guinea pigs, rodents, ferrets, chinchillas)

* 9. What, if any, onsite behavioural / temperament assessments do you complete with the rabbits whilst they are at the centre?

- Informal, ad hoc, observations (e.g. whilst completing other care tasks, e.g. health checking)
- Formal observation tool that has been validated in a scientific study. Please describe below.
- No behavioural / temperament assessments used for rabbits.
- Other, please describe below

* 10. If informal or formal behaviour / temperament observations are completed, please comment on these THREE points in the box below:

1. When are these tests conducted? (Arrival / after a few days / immediately prior to rehoming?

2. Name of any software or tool used to write results or store observation findings?

3. Who has access to the findings of these observations?

If you do not collect this data, please write N/A in the box below.

* 11. How is behavioural / temperament information used? Including information collected from the person relinquishing the rabbit and / or data collected whilst on site. Please select all that apply.

- To match a rabbit to another rabbit
- To match a rabbit to an appropriate new owner
- To enable staff to manage the rabbit whilst on site
- To determine which accommodation to keep the rabbit in whilst on site
- To generate a training / socialisation plan
- To identify any potential underlying medical conditions
- The data is not used in this way
- This data is not collected
- Other, please state below.

Question page 3

* 12. Please describe any factors that affect whether you collect data about a rabbit's behaviour / temperament whilst at your centre, or if you do collect this data, any factors that affect how you collect this data. e.g. time, resources, knowledge, usefulness of information gained, owners interest etc.

* 13. Describe factors that affect your ability to collect information at the following stages of the relinquishment to rehoming process:

- As the rabbit arrives / is handed over.
- Whilst the rabbit is on site.
- At the time of adoption (about the potential new home / owners).

Question page 4

* 14. If a rabbit is to go to a home with other rabbits, how is a suitable rabbit selected? i.e. what factors are used to determine if a rabbit is suited to the pets currently owned?

* 15. What support is available to a new owner to support the mixing process of the adopted rabbit and the current rabbits kept?

Question page 5

* 16. Thinking about information you collect during the intake process (when a rabbit enters your centre) please indicate if you collect the following types of information and how you store this information.

Do you currently collect and store information about...

	Information is NOT collected.	Collected but not written or typed (verbal communication only).	Paper based (e.g. paper records, door Information sheets, notice boards).	Computer based or online (e.g. spreadsheet, database or specialist software).	Other
> The reason the rabbit is entering the centre (e.g. intake route, stray / relinquished pet, reason for relinquishment of pet)					

> The rabbit's previous daily care (e.g. handling, grooming, bedding / substrate used).					
The rabbit's previous accommodation (e.g. type of accommodation, where the accommodation is kept).					
> The rabbit's previous social opportunities (e.g. frequency / type of interactions with other rabbits, other species, human caregivers).					
> The potential new rabbit owner's current pet rabbit's behaviour and temperament (e.g. how it responds to people, other animals or its surroundings, it's general character)					
> The rabbit's health history.					
> The rabbits neutered status.					

Question page 6

* 17. Thinking about information you collect whilst a rabbit is at your centre, please indicate if you collect the following types of information and how you store this information.

Do you currently collect and store information about...

	Information is NOT collected.	Collected but not written or typed (verbal communication only).	Paper based (e.g. paper records, door Information sheets, notice boards).	Computer based or online (e.g. spreadsheet, database or specialist software).	Other
> The rabbit's diet during its stay on site.					
> The rabbit's health during its stay on site.					

> The potential new rabbit owner's current pet rabbit's behaviour and temperament (e.g. how it responds to people, other animals or its surroundings, it's general character)					

18. If you collect any of the above information in a different way, please describe below.

Question page 7

* 19. Thinking about information you collect during the rehoming process, please indicate if you collect the following types of information and how you store this information. This may include information collected on-site, over the telephone or during a home check.

Do you currently collect and store information about....

	Information is NOT collected.	Collected but not written or typed (verbal communication only).	Paper based (e.g. paper records, door Information sheets, notice boards).	Computer based or online (e.g. spreadsheet, database or specialist software).	Other
> The potential new rabbit owner's knowledge / experience of pet rabbits.					
> The potential new rabbit owner's home environment (e.g. how many people live there, what type of home?).					
> The age of children living with the potential new rabbit owner.					
> The potential new rabbit owner's current pets (NOT RABBITS) kept.					

> The potential new rabbit owner's current pet rabbit's behaviour and temperament (e.g. how it responds to people, other animals or its surroundings, it's general character)					
> The potential new rabbit owner's current rabbits kept if any (e.g. sex, neutered status, past experience with other rabbits).					
> The potential new rabbit owner's plan to accommodate the rabbit (e.g. type of accommodation).					
> The potential new rabbit owner's current pet rabbit's behaviour and temperament (e.g. how it responds to people, other animals or its surroundings, it's general character).					
> Information about a potential new owners' expectations of a new pet rabbit?					

20. If you collect any of the above information in a different way, please describe below.

Question page 8

* 21. Of the information you store, what information is passed on to a potential / new owner?

	Yes, this information is provided to a new / potential owner.	No, this information is not provided to a new / potential owner.	We do not collect or store this information for rabbits.
The reason the rabbit came to the centre.			
The rabbit's daily care in previous home.			
The rabbit's accommodation in previous home.			
The rabbit's social opportunities in previous home.			
The rabbit's behaviour, as described by previous owner.			

The rabbit's temperament, as described by previous owner.			
The rabbit’s health history.			
The rabbit’s health during its stay on site (including any treatments / procedures whilst at the centre).			
The rabbit’s behaviour during its stay on site.			
The rabbit’s temperament, as assessed during its stay on site.			

22. Please use the space below to add any additional comments about information collection or the contents of this questionnaire.

Appendix 2: Life history information, tests each individual is represented in for the studies completed in Chapters 4, 5 and 6 (Table A2.1) and enclosure information (Tables A2.2 and A2.3) .

Table A2.1 Life history information for 64 rabbits studied at four land-based college sites and utilised in the behaviour rating tool, behaviour tests and home cage observations.

Site	Rabbit study ID No.	Weight	DOB	Breed	Sex	RaBRT	LEOF T1	LEOF T2	OFT T1	OFT T2	NOT T1	NOT T2	NST T1	NST T2	Human interaction pilot	HIT T1 (GREEN)	HIT T2 (WHITE)	Home cage pilot	Home cage
1	1	2.3	May-11	Lop	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	2	3.05	May-11	-	Female	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
1	3	2.4	Jun-11	-	Female	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	4	3.4	Apr-09	NZW	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	N	N
1	5	2	Jan-09	Dutch	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	6	4.2	Jan-09	NZW	Female	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
1	7	2.39	Sep-08	-	Female	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
1	8	-	Feb-10	-	Female	Y	N	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	9	-	Nov-11	Rex	Female	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N	N	Y	N
1	10	2.36	Oct-11	Rex	Male	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
1	11	2.3	Oct-11	Rex	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	12	-	Oct-11	Rex	Male	N	Y	N	Y	N	Y	Y	Y	Y	Y	N	N	N	N
1	13	-	Oct-11	Rex	Male	N	Y	N	Y	N	Y	Y	Y	Y	Y	N	N	N	N
1	14	2.2	Oct-11	Rex	Female	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	15	1.9	Mar-12	Dutch	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N

Site	Rabbit study ID No.	Weight	DOB	Breed	Sex	RaBRT	LEOF T1	LEOF T2	OFT T1	OFT T2	NOT T1	NOT T2	NST T1	NST T2	Human interaction pilot	HIT T1 (GREEN)	HIT T2 (WHITE)	Home cage pilot	Home cage
1	16	1.8	Mar-12	Netherland Dwarf	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	17	1.6	Mar-12	Dwarf	Male	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	18	1.8	Jan-13	-	Female	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	19	1.6	Jan-13	-	Female	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N
1	20	1.2	Apr-14	Lionhead	Female	**Y**	N	N	Y	Y	Y	Y	Y	Y	N	N	N	N	N
1	21	-	Apr-14	Lionhead	Female	Y	N	N	Y	Y	Y	Y	Y	Y	N	N	N	N	
2	22	1.88	Mar-10	-	Female	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
2	23	2.14	Feb-14	-	Male	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
2	24		Jun-17	Lionhead cross	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
2	25	-	Jun-04	-	Female	Y	Y	N	N	N	Y	N	Y	N	N	Y	Y	N	N
2	26	3.48	Nov-12	Rex	Male	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
2	27	2.25	Jan-13	-	Male	N	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	N
2	28	2.15	Sep-13	-	Male	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
2	29	2.31	Oct-14	-	Female	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	Y
2	30	2.32	May-10	-	Female	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	Y
3	31	2.06	-	English spot	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
3	32	3.21	-	Havana	Male	**Y**	Y	N	Y	N	Y	Y	Y	Y	N	N	N	N	N

Site	Rabbit study ID No.	Weight	DOB	Breed	Sex	RaBRT	LEOF T1	LEOF T2	OFT T1	OFT T2	NOT T1	NOT T2	NST T1	NST T2	Human interaction pilot	HIT T1 (GREEN)	HIT T2 (WHITE)	Home cage pilot	Home cage
3	33	2.28	Mar-17	Lionhead	Female	N	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	N
3	34	4.05	-	Giant continental cross	Male	N	N	N	N	N	Y	Y	Y	Y	N	Y	Y	N	N
3	35	-	-	Dwarf lop	Male	N	Y	N	N	N	Y	N	Y	N	N	N	N	N	N
3	36	2.13	Mar-17	Lionhead	Male	N	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	N
3	37	2.09	Nov-15	English cross	Male	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
3	38^	-	Apr-16	-	Male	N	Y	N	Y	N	Y	N	Y	N	N	N	N	N	N
3	39	2.73	-	English spot	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
3	40	2.96	Sep-10	lop eared	Male	**Y**	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	Y
3	41	2.48	-	French lop	Female	**Y**	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	Y
3	42	2.16	-	Cottontail	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
3	43	2.1	-	Rex	Male	**Y**	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	N	Y
3	44	3.85	-	Belgian hare	Female	N	Y	N	Y	N	Y	N	Y	N	N	Y	Y	N	N
3	45	2.8	-	British giant	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
3	46	5.28	-	Giant continental	Female	N	N	N	N	N	Y	Y	Y	Y	N	Y	Y	N	N
3	47	-	-	Dwarf lop	Male	N	Y	N	N	N	Y	N	Y	N	N	N	N	N	N
3	48	3.11	-	-	Female	N	Y	N	Y	N	Y	N	Y	N	N	Y	Y	N	N
3	49	2.79	-	Harlequin cross lop	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y

Site	Rabbit study ID No.	Weight	DOB	Breed	Sex	RaBRT	LEOF T1	LEOF T2	OFT T1	OFT T2	NOT T1	NOT T2	NST T1	NST T2	Human interaction pilot	HIT T1 (GREEN)	HIT T2 (WHITE)	Home cage pilot	Home cage
3	50	2.9	-	Havana	Female	**Y**	Y	N	Y	N	Y	Y	Y	Y	N	N	N	N	N
3	51	2.74	-	Havana	Female	**Y**	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y
3	52	3.02	-	Havana	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
3	53	3.28	-	-	Female	N	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	54^	-	Feb-16	-	Male	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	55	-	Aug-16	N dwarf cross	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	56^	-	Mar-12	Dutch	Female	N	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	N	N
4	57	-	Feb-12	Mini-lop	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	58^	-	Jan-12	Mixed	Male	N	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	59	-	2015	-	Male	N	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	N	N
4	60	-	2015	-	Female	N	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	N	N
4	61	-	Dec-11	Harlequin	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	62^	-	Sep-09	Lion head	Female	N	Y	N	Y	N	Y	N	Y	N	N	Y	Y	N	N
4	63^	-	Feb-15	Lop	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N
4	64	-	Aug-16	N dwarf cross	Female	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N

RaBRT in bold indicate the rabbit was included in the reliability and validity testing (12 rabbits)
^ Rabbits housed alone
- Missing data

Table A2.2 Home cage enclosure dimensions for a single enclosure and locations of enclosures for each site. Exercise runs were not included in home cage observations or the below dimensions.

Site number	Enclosure location	Length (cm)	Width (cm)	Floor space	Height (cm)
1	Outdoor sheds with exercise runs	300	175	5.25m^2	250 (approx.)
2	Indoor building – rabbits transported to outdoor runs, not attached	200	115	2.3m^2	120
3	Indoor building with outdoor exercise runs	230	180	4.14m^2	91
4 Single housed rabbits	Indoor building with outdoor exercise runs	105	135	1.42m^2	65
4 Group housed rabbits		200	135	2.7m^2	65

Table A2.3 Average temperatures taken within the home cage at each site prior to each test taking place and in the novel area prior to each test (OFT and LEOF) at each trial. The difference of the novel arena temperature from the home cage temperature is also provided.

Site number	Time and location of testing	Mean temperature in degrees Celsius	Standard deviation
1	Trial 1 home cage	25.6	2.3
	Trial 1 novel arena	24.0	2.4
	Difference between home cage and novel arena	-1.6	
	Trial 2 home cage	20.1	1.4
	Trial 2 novel arena	22.2	2.9
	Difference between home cage and novel arena	2.1	
2	Trial 1 home cage	16.5	1.9
	Trial 1 novel arena	22.0	0.0
	Difference between home cage and novel arena	5.5	
	Trial 2 home cage	24.0	0.9
	Trial 2 novel arena	23.3	0.5
	Difference between home cage and novel arena	-0.7	
3	Trial 1 home cage	17.2	1.8
	Trial 1 novel arena	18.0	1.4
	Difference between home cage and novel arena	0.8	
	Trial 2 home cage	26.9	1.7
	Trial 2 novel arena	23.3	2.1
	Difference between home cage and novel arena	-3.6	
4	Trial 1 home cage	17.5	1.7
	Trial 1 novel arena	18.8	1.3
	Difference between home cage and novel arena	1.2	
	Trial 2 home cage	17.7	1.9
	Trial 2 novel arena	19.0	2.4
	Difference between home cage and novel arena	1.3	

Appendix 3: Online survey – rabbit behaviour rating questionnaire (RaBRT)

Rabbit personality survey: Introduction and data protection

What is this survey about?
For my PhD research I am attempting to develop a questionnaire that can be used to identify individual differences in the behaviour of rabbits. Part one of this survey requires you to indicate how much each statement or word describes a rabbit that is currently known to you and asks questions about this rabbit. Part two of the survey asks questions relating to your perceptions of rabbits and your experience with rabbits and has been adapted from Arhant & Troxler, 2017.

The primary researcher is Clare Ellis, PhD Candidate at the University of Northampton and Moulton College. The survey has been approved by the Postgraduate Ethics Committee at the University of Northampton on 15th January 2018.

Why take part?
Animal personality research has become very popular in recent years, but unfortunately not all animal personality questionnaires have been tested for reliability (ability to be used by different people in different settings) and validity (actually test what they are supposed to), which are important to ensure these tests stand up to criticisms and are useful to people in the long term.

- By taking part in this survey you will be contributing to research that will be tested for reliability and validity, and will hopefully be further developed for practical use by animal shelter staff and others interested in rabbit personality.
- This study is the first phase of the development of a subjective behaviour rating questionnaire on rabbit personality. At this stage it is not possible to provide information about any individual rabbit's personality; however your contributions will help us to understand important aspects of rabbit personality and to develop valid tools for assessing this.

Please be assured that there are no right or wrong answers.
No part of this survey is designed to make judgements about the care you provide for your rabbit/s.

Who should take part in this survey?

- This survey is for **rabbit owners**, **or** those that **care for / work with rabbits,** that are aged over 18 years of age, and live in any country, although it is only available in the English Language.

- You need to have known the individual rabbit that you describe for at least 3 months of regular contact (at least 10 minutes with the rabbit for at least 3 days each week, or equivalent), and the **rabbit should be at least 1 year old** at the time of completing the survey.

What will happen if you take part?
Once you have entered your responses about ONE rabbit in part one, you will be asked if you would like to complete part two about your perceptions of rabbits in general.

We really appreciate your time in completing this important research about rabbits and so as a thank you for your time, there is a chance to opt in to a free prize draw (Sorry, UK participants only, but everyone is welcome to take part). If you choose to **complete BOTH parts of the survey,** you will be eligible to be entered into a **free prize draw for a £30 (GBP) Amazon gift voucher**. A valid email address and completion of all compulsory questions is required to be eligible for the prize draw. One winner will be selected at random on 30th April 2018.

How long will the survey take?
The survey is broken down into two parts. Part one contains 66 questions about your rabbit and your rabbit's behaviour and should take about 10 minutes to complete. Part two contains 58 questions about your experience with rabbits and your perceptions of rabbits and should take about 10 minutes to complete.

No specialist knowledge or background of rabbits is required as I am interested in your own experiences and perceptions of rabbits and the behaviour of one rabbit in particular.

How will your data be stored and used?
All information provided for this survey will be stored on password protected accounts and storage devices, in accordance with the Data Protection Act 1998. No personal identifying information will be shared with any third parties. No personal identifying information will be used for data analysis. All data will be securely deleted once deemed out of date. This will be reviewed five years post publication of the PhD thesis.

Email addresses supplied for participation in the prize draw will be stored securely and separately to the questionnaire responses and will be destroyed once the prize winner has been drawn and has confirmed they have received the prize.

The results will form part of my PhD thesis and will hopefully be published within a peer-reviewed journal and presented at academic and industry conferences.

If you are interested to know the outcome of this study, please follow my Twitter @rabbitphd or ResearchGate accounts
https://www.researchgate.net/profile/Clare_Ellis

What if you no longer want to take part?
You may withdraw from the survey at any time prior to completing and submitting the survey. Any partial responses to each part of the survey will not be included in data analysis and will be deleted. Due to the data being stored anonymously, it will not be possible to extract individual responses after this.

If you wish to ask further questions about this study, prior to taking part, please email Clare Ellis at BBBS@northampton.ac.uk

Please indicate that you have read the above and agree to take part in the study as described above.
* I confirm that I have read and understood the participant information sheet provided and the purpose of the research is clear to me.

- Yes
- No

* I understand that my involvement is completely voluntary. I am free to withdraw from the study at any point throughout the completion of the survey and for any reason, without having to give an explanation (data cannot be withdrawn once the survey has been submitted).

- Yes
- No

* I voluntarily consent to taking part and my data being used as described above.

- Yes
- No

* I am over 18 years of age.

- Yes
- No

ABOUT YOUR RABBIT

Please select **ONE rabbit** that you currently own or work with to complete this survey about.

If you own or work with more than one rabbit, please select a healthy rabbit that is closest to two years old. If you know / have two rabbits of the same age, please select one of your choice.

You **need to have known this rabbit for at least 3 months** and the **rabbit must be at least 1 year old** at present.

* This rabbit is (sex)

- Male
- Female
- Unknown

* Rabbit age (must be at least 1 year old)

- Unknown
- 1 year
- 2 years
- 3 years
- 4 years
- 5 years
- 6 years
- 7 years
- 8 years
- 9 + years

* Does this rabbit live with other rabbits?

- Yes, currently
- Not currently

* Is this rabbit neutered?

- Yes
- No
- Unknown

If yes, at what age was this rabbit neutered?

- Unknown
- up to 1 year
- 1 year
- 2 years
- 3 years
- 4 years
- 5 years
- 6 years
- 7 years
- 8 years
- 9 + years

* What breed is this rabbit? If a mixed breed, please simply state ‘mixed’, if breed in unknown, please indicate ‘unknown’.

* What is the main colour of your rabbit?

* Is this rabbit currently suffering any health issues that may affect its behaviour?

- Not to my knowledge
- Yes (Please briefly describe the health condition)

How long have you known this rabbit? (numbers only, no text)

* Years

Months

* Which of the following, best describe this rabbits living arrangements? (select all that apply)

- Hutch/cage (single level)
- Hutch/cage (multi-level)
- Exercise run attached to hutch/cage
- Shed/outbuilding
- House rabbit
- Outdoor only rabbit
- Other

* Is this a rabbit that you own or work with?

- I own this rabbit (e.g. pet, companion, show rabbit, breed at home)
- I work with this rabbit (e.g. in a rescue centre, zoo, breeder as business or laboratory)

* Approximately how many hours in a normal work week do you spend with rabbits? (numbers only, no text)

How long have you owned / kept this rabbit? (numbers only, no text)

* Years

Months

How many people live in the home where this rabbit lives? (numbers only, no text) If none, please indicate with 0 (zero).

* Adults (16 years and over)

* Children (under 16 years)

YOUR RABBIT'S CHARACTER

Please answer the question on this page thinking about the same rabbit as the previous page.
*

In your own words, please describe the character of this rabbit in two or three sentences.

ABOUT YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

* How often does your rabbit display the following GENERAL behaviours?

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Rests in a shelter (e.g. box, house), if available	O	O	O	O	O	O
Ignores new toys / items	O	O	O	O	O	O
Runs where space allows	O	O	O	O	O	O
Rests with legs outstretched and belly to the floor or on its side	O	O	O	O	O	O
Explores new items / toys confidently	O	O	O	O	O	O
Is active	O	O	O	O	O	O
Explores new items / toys cautiously (slow approach, and body may be lowered to the ground)	O	O	O	O	O	O

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ABOUT YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate (tick the line) how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

* How often does your rabbit display the following behaviours AROUND PEOPLE IN GENERAL?

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Evades handling by moving away when approached	O	O	O	O	O	O
Struggles when being held or restrained	O	O	O	O	O	O
Eats food while people are nearby	O	O	O	O	O	O
Rests close to people	O	O	O	O	O	O
Sprays urine on a particular person	O	O	O	O	O	O
Sprays urine on any person	O	O	O	O	O	O

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ABOUT YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate (tick the line) how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

*** How often does your rabbit display the following GENERAL behaviours?**

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Rests in the open	O	O	O	O	O	O
Explores new places confidently	O	O	O	O	O	O
Stands on back legs looking around in response to a loud noise	O	O	O	O	O	O
Runs and jumps in the air, turns mid-air and kicks legs out before landing (binkies)	O	O	O	O	O	O
Freezes, crouching in response to a loud noise	O	O	O	O	O	O
Explores new items by picking them up and tossing them	O	O	O	O	O	O
Explores new places cautiously (slow approach, and body may be lowered to the ground)	O	O	O	O	O	O
Rubs underside of chin on items in the environment	O	O	O	O	O	O

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ABOUT YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate (tick the line) how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

*** How often does your rabbit display the following behaviours around UNFAMILIAR people?**

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Stands still and alert when an unfamiliar person approaches	O	O	O	O	O	O
Readily approaches unfamiliar people	O	O	O	O	O	O
Takes food by hand from an unfamiliar person	O	O	O	O	O	O
Attempts to hide when being approached by an unfamiliar person	O	O	O	O	O	O
Rubs underside of chin on unfamiliar people	O	O	O	O	O	O
Thumps the ground when approached by an unfamiliar person	O	O	O	O	O	O
Attempts to bite unfamiliar people	O	O	O	O	O	O
Runs away to avoid being touched by an unfamiliar person	O	O	O	O	O	O

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ABOUT YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate (tick the line) how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

*** How often does your rabbit display the following behaviours around FAMILIAR people?**

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Stands still with ears alert when a familiar person approaches	O	O	O	O	O	O
Takes food by hand from a familiar person	O	O	O	O	O	O
Runs away to avoid being touched by a familiar person	O	O	O	O	O	O
Attempts to bite familiar people	O	O	O	O	O	O
Readily approaches familiar people	O	O	O	O	O	O
Thumps the ground when approached by a familiar person	O	O	O	O	O	O
Rubs underside of chin on familiar people	O	O	O	O	O	O
Attempts to hide (in a shelter of some sort) when being approached by a familiar person	O	O	O	O	O	O

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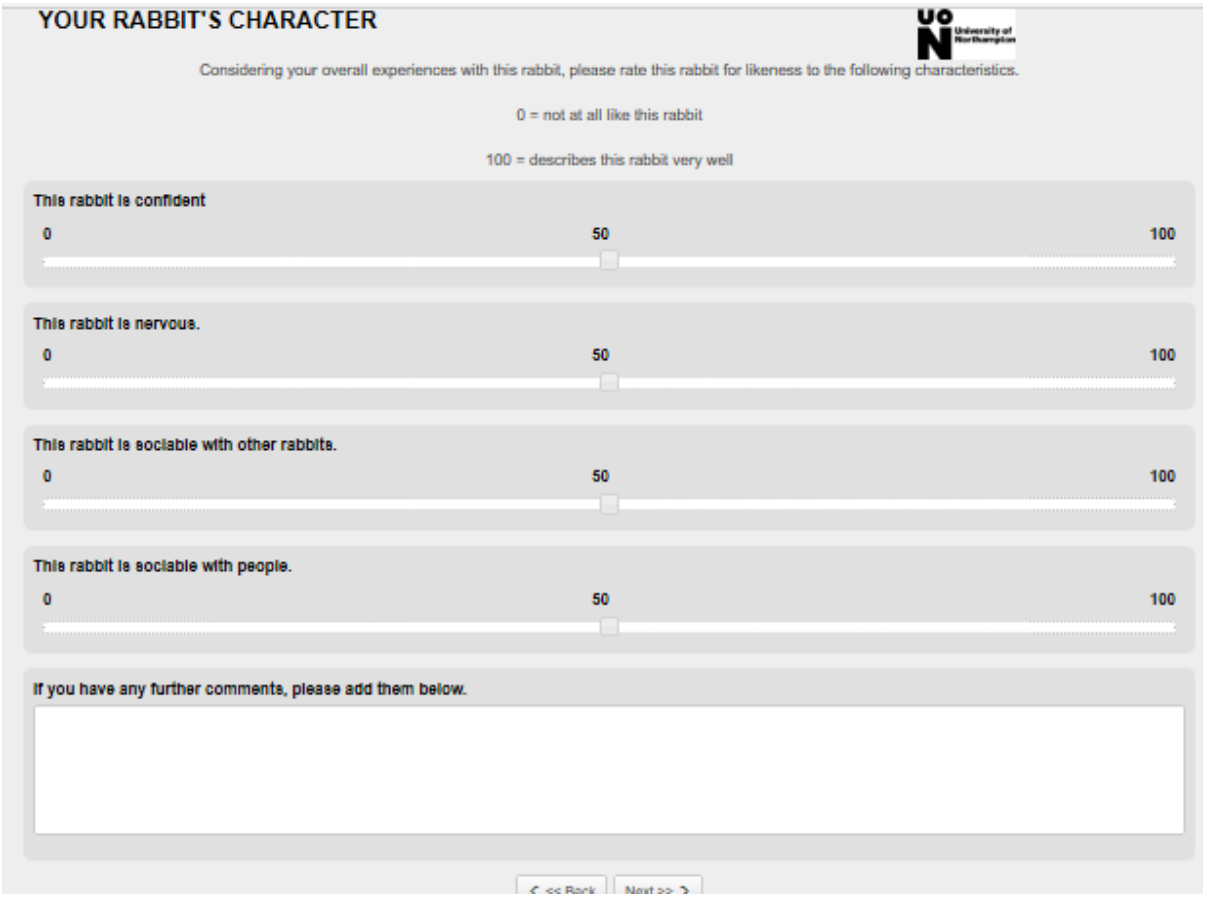
YOUR RABBIT'S BEHAVIOUR

Considering your experience with this rabbit during the last few months, please indicate (tick the line) how frequently you have observed each of the following behaviours.

If you have not had the opportunity to observe this rabbit in this way, please indicate by selecting 'no opportunity to observe'.

*** How often does your rabbit display the following behaviours around other rabbits?**

	Never	Rarely	Occasionally	Quite often	Very frequently	No opportunity to observe this behaviour
Chases any other rabbits to make them move (displaces)	O	O	O	O	O	O
Explores the environment and new toys while close (within one body length) to other rabbits.	O	O	O	O	O	O
Grooms / washes other rabbits	O	O	O	O	O	O
Attacks any other rabbit (includes kicking, biting and persistent chasing)	O	O	O	O	O	O
Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits	O	O	O	O	O	O
Rests close (within one body length) of another rabbit / other rabbits	O	O	O	O	O	O
Urine sprays other rabbits of the same gender	O	O	O	O	O	O
Urine sprays other rabbits of the opposite gender	O	O	O	O	O	O
Feeds close (within one body length) of another rabbit / other rabbits	O	O	O	O	O	O
Keeps body crouched and head low when being approached by another rabbit	O	O	O	O	O	O


YOUR RABBIT'S CHARACTER
Considering your overall experiences with this rabbit, please rate this rabbit for likeness to the following characteristics.
0 = not at all like this rabbit
100 = describes this rabbit very well
This rabbit is confident
0
50
100
This rabbit is nervous.
0
50
100
This rabbit is sociable with other rabbits.
0
50
100
This rabbit is sociable with people.
0
50
100
If you have any further comments, please add them below.

Appendix 4: Stage two PCA output following rotation (Oblique) of retained 18 items from the RaBRT.

Table A4 Oblique (Direct oblimin) rotated solution to 18 items of RaBRT. The four components reflected similar item loadings to the orthogonal rotated solution however discriminate validity testing demonstrated that the components were not correlated and so the orthogonal rotated solution was retained (*n*=1,234 rabbits).

	PC1	PC2	PC3	PC4	
Q31Runs away to avoid being touched by a familiar person	-.881				
Q8Evades handling by moving away when approached	-.790				Reflects PC2 of orthogonal rotated solution
Q36Attempts to hide (in a shelter of some sort) when being approached by a familiar person	-.744				
Q33Readily approaches familiar people	.490				
Q42Rests close (within one body length) of another rabbit / other rabbits		.943			
Q44Feeds close (within one body length) of another rabbit / other rabbits		.926			
Q41Grooms / washes itself whilst close (within one body length) to another rabbit / other rabbits		.923			Reflects PC1 of orthogonal rotated solution
Q39Grooms / washes other rabbits		.845			
Q38Explores the environment and new toys while close (within one body length) to other rabbits.		.681			
Q14Explores new places confidently			.768		
Q5Explores new items / toys confidently			.663		Reflects PC4 of orthogonal rotated solution
Q13Rests in the open			.584		
Q11Rests close to people	.445		.453		
Q23Takes food by hand from an unfamiliar person				.792	
Q26Thumps the ground when approached by an unfamiliar person				-.713	Reflects PC3 of orthogonal rotated solution
Q24Attempts to hide when being approached by an unfamiliar person				-.686	
Q22Readily approaches unfamiliar people				.622	
Q28Runs away to avoid being touched by an unfamiliar person	-.450			-.568	

Appendix 5A: Human-interaction behaviour data collection sheets.

The test should be conducted in the following order and filmed, ideally using a head mounted camera. Scoring should be completed retrospectively using the footage and the below scoring system.

Rabbit ID……..

Date……

Colour lab coat of handler……

Starting scores

Location

Score the rabbits location prior to starting test 1 and then at the end of sub-tests 1, 2 and 3.
The front is considered approximately 50% of the enclosure closest to the entry point being used to access the enclosure.
The back is approximately 50% of the enclosure furthest away from the entry point being used to access the enclosure.

Rabbit moves from the back to the front of the enclosure in a relaxed, non-threatening manner	+3 points	
Rabbit is at the front of the enclosure and stays at the front of the enclosure	+1 point	
Rabbit is at the back of the enclosure and stays at the back of the enclosure	-1 point	
Rabbit moves from the back to the front of the enclosure in an aggressive charge	-2 points	
Rabbit moves from the front to the back of the enclosure	-3 points	
	Total	

Social

Rabbit not housed socially Rabbit is not near to the other rabbit in the enclosure	0 points	
Rabbit is within one body length of another rabbit	+ 1 point	
Rabbit is in physical contact with another rabbit	+2 points	
	Total	

Sub-test 1 – Approach enclosure (stood at closed door for 10 seconds)

Location

Score the rabbits location prior to starting test 1 and then at the end of sub-tests 1, 2 and 3.

The front is considered approximately 50% of the enclosure closest to the entry point being used to access the enclosure.

The back is approximately 50% of the enclosure furthest away from the entry point being used to access the enclosure.

Rabbit moves from the back to the front of the enclosure in a relaxed, non-threatening manner	+3 points	
Rabbit is at the front of the enclosure and stays at the front of the enclosure	+1 point	
Rabbit is at the back of the enclosure and stays at the back of the enclosure	-1 point	
Rabbit moves from the front to the back of the enclosure in an aggressive charge	-2 points	
Rabbit moves from the front to the back of the enclosure	-3 points	
	Total	

Social

Rabbit not housed socially Rabbit is not near to the other rabbit in the enclosure	0 points	
Rabbit is within one body length of another rabbit	+ 1 point	
Rabbit is in physical contact with another rabbit	+2 points	
	Total	

Behaviour (select all that occur in 10 seconds)

Rabbit approaches person in a non-threatening way	+2 points	
Rabbit moves away from person	-1 point	
Rabbit is alert, ears up at least 45 degrees from back (or drawn forward/back for lops) and rabbit may be looking around or rearing (stood on back legs)	-1 point	
Rabbit thumps the ground	-2 points	
Rabbit attempts to hide	-2 points	
Rabbit charges at person and/or bites person	-3 points	
	Total	

Sub-test 2 – Crouch at enclosure entry point (door open for 10 seconds)

Location

Score the rabbits location prior to starting test 1 and then at the end of sub-tests 1, 2 and 3.
The front is considered approximately 50% of the enclosure closest to the entry point being used to access the enclosure.
The back is approximately 50% of the enclosure furthest away from the entry point being used to access the enclosure.

Rabbit moves from the back to the front of the enclosure in a relaxed, non-threatening manner	+3 points	
Rabbit is at the front of the enclosure and stays at the front of the enclosure	+1 point	
Rabbit is at the back of the enclosure and stays at the back of the enclosure	-1 point	
Rabbit moves from the front to the back of the enclosure in an aggressive charge	-2 points	
Rabbit moves from the front to the back of the enclosure	-3 points	
	Total	

Social

Rabbit not housed socially Rabbit is not near to the other rabbit in the enclosure	0 points	
Rabbit is within one body length of another rabbit	+ 1 point	
Rabbit is in physical contact with another rabbit	+2 points	
	Total	

Behaviour

Rabbit approaches person in a non-threatening way	+2 points	
Rabbit moves away from person	-1 point	
Rabbit is alert, ears up at least 45 degrees from back (or drawn forward/back for lops) and rabbit may be looking around or rearing (stood on back legs)	-1 point	
Rabbit thumps the ground	-2 points	
Rabbit attempts to hide	-2 points	
Rabbit charges at person and/or bites person	-3 points	
	Total	

Sub-test 3 – Sit inside enclosure, nearest to doorway (1 minute)

OUTCOME: Rabbit approached the person

Yes	1 point	
No	0 points	

Location

Score the rabbits location prior to starting test 1 and then at the end of sub-tests 1, 2 and 3.
The front is considered approximately 50% of the enclosure closest to the entry point being used to access the enclosure.
The back is approximately 50% of the enclosure furthest away from the entry point being used to access the enclosure.

Rabbit moves from the back to the front of the enclosure in a relaxed, non-threatening manner	+3 points	
Rabbit is at the front of the enclosure and stays at the front of the enclosure	+1 point	
Rabbit is at the back of the enclosure and stays at the back of the enclosure	-1 point	
Rabbit moves from the front to the back of the enclosure in an aggressive charge	-2 points	
Rabbit moves from the front to the back of the enclosure	-3 points	
	Total	

Social

Rabbit not housed socially Rabbit is not near to the other rabbit in the enclosure	0 points	
Rabbit is within one body length of another rabbit	+ 1 point	
Rabbit is in physical contact with another rabbit	+2 points	
	Total	

Behaviour

Rabbit makes physical contact with person (non-threatening/ non-aggressive)	+3 points	
Rabbit approaches person in a non-threatening way	+2 points	
Rabbit moves away from person	-1 point	
Rabbit is alert, ears up at least 45 degrees from back (or drawn forward/back for lops) and rabbit may be looking around or rearing (stood on back legs)	-1 point	
Rabbit thumps the ground	-2 points	
Rabbit attempts to hide	-2 points	
Rabbit charges at person and/or bites person	-3 points	
	Total	

Sub-test 4 – Attempt to stroke rabbit whilst in enclosure*

OUTCOME: Was it possible to stroke the rabbit?

Yes	1 point	
No	0 points	

Social

Rabbit not housed socially Rabbit is not near to the other rabbit in the enclosure	0 points	
Rabbit is within one body length of another rabbit	+ 1 point	
Rabbit is in physical contact with another rabbit	+2 points	
	Total	

Behaviour

Rabbit makes physical contact with person (non-threatening/ non-aggressive)	+3 points	
Rabbit approaches person in a non-threatening way	+2 points	
Rabbit moves away from person	-1 point	
Rabbit is alert, ears up at least 45 degrees from back (or drawn forward/back for lops) and rabbit may be looking around or rearing (stood on back legs)	-1 point	
Rabbit thumps the ground	-2 points	
Rabbit attempts to hide	-2 points	
Rabbit charges at person and/or bites person	-3 points	
	Total	

Sub-test 5 – Attempt to pick up rabbit (and 10 second hold) whilst in enclosure*

OUTCOME: It was possible to pick up the rabbit

Yes	1 point	
No	0 points	

Behaviour

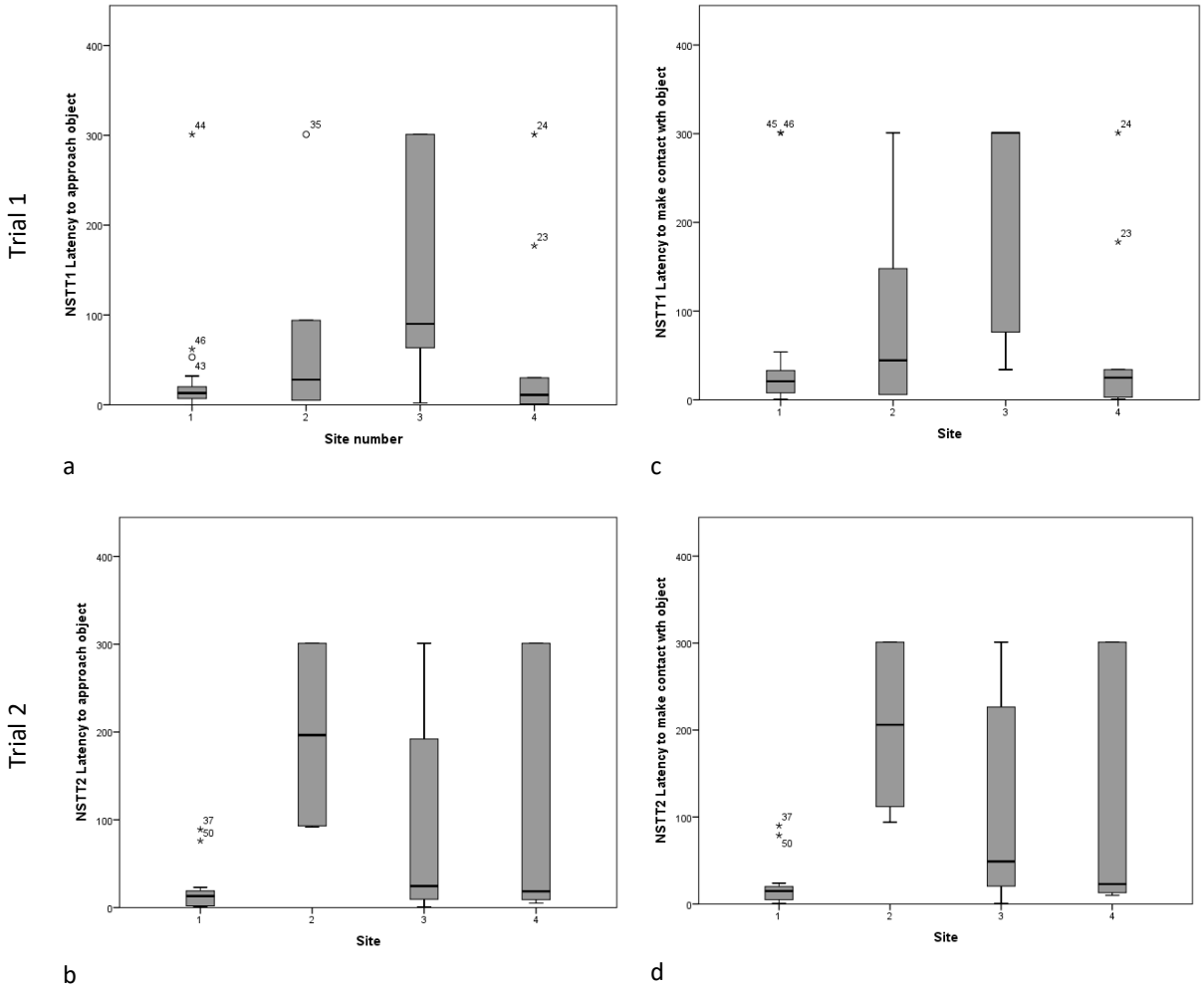
Rabbit approaches person in a non-threatening way	+2 points	
Rabbit moves away from person	-1 point	
Rabbit is tense, possible freezing in one position pushing its body towards the ground.	-1 point	
Rabbit thumps the ground	-2 points	
Rabbit attempts to hide	-2 points	
Rabbit charges at person and/or bites person	-3 points	
	Total	

Appendix 5B: External validity of retained items from the RaBRT, examining female and male loadings.

Table A5 PCA (Varimax rotation) item loadings for RaBRT when conducted separately for female (*n*=564) and male (*n*=670) rabbits.

Females				Males			
Items	**PC1**	**PC2**	**PC3**	**Items**	**PC1**	**PC2**	**PC3**
Q42	.938			**Q42**	.931		
Q44	.935			**Q41**	.921		
Q41	.914			**Q44**	.907		
Q39	.857			**Q39**	.815		
Q38	.696			**Q38**	.699		
Q28		.814		**Q28**		.821	
Q31		.784		**Q31**		.778	
Q8		.776		**Q8**		.742	
Q24		.706		**Q36**		.730	
Q36		.653		**Q24**		.710	
Q26		.550		**Q26**		.562	
Q14			.765	**Q14**			.788
Q5			.705	**Q5**			.694
Q13			.696	**Q11**			.545
Q11		-.415	.517	**Q13**			.471

Appendix 6: Interactions between measured variables across the novel substrate and novel object behaviour tests.



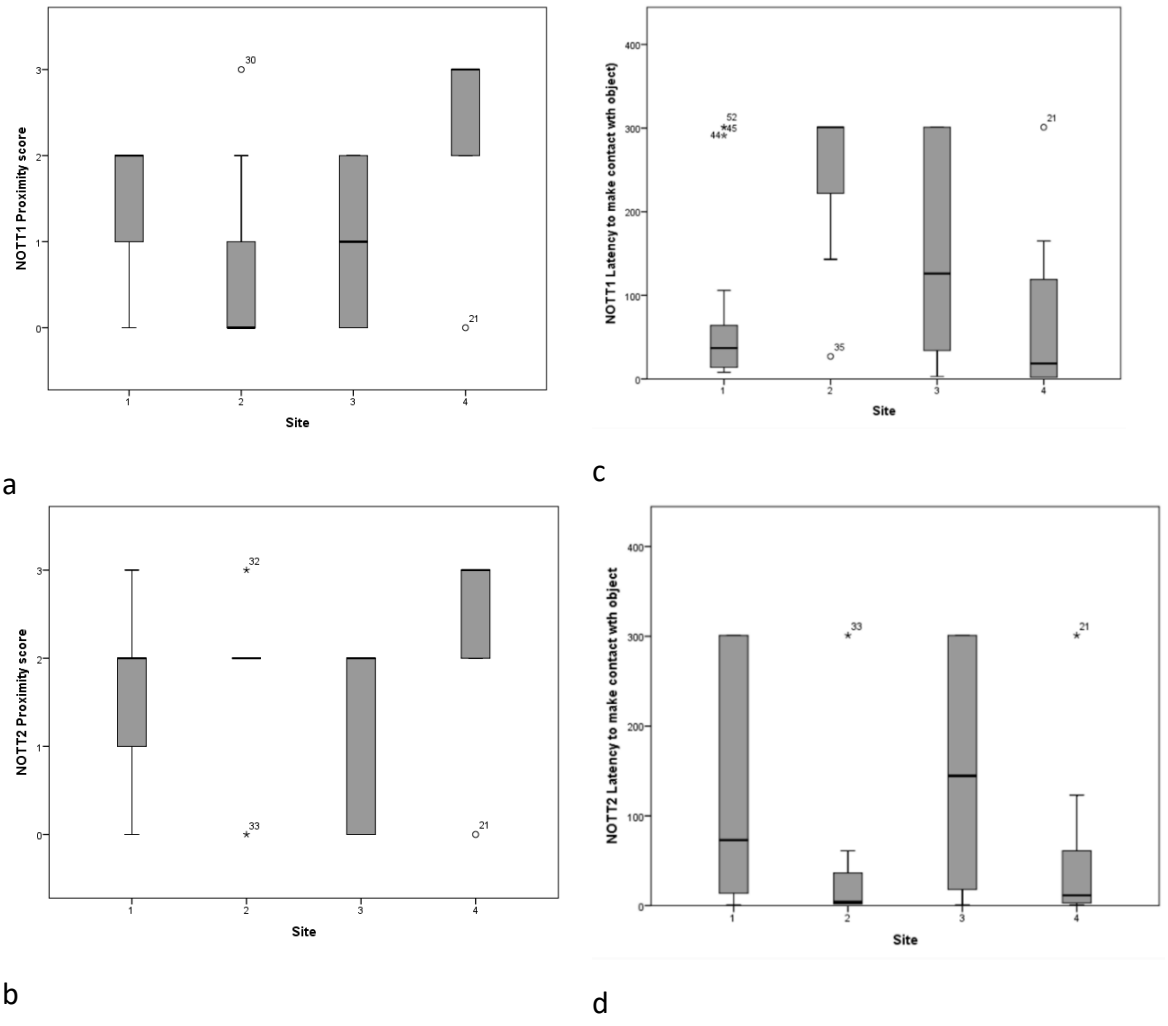
a) NST T1 score differed by site (*n*=49, 13.3, df 3=3, p=0.004). Pairwise comparisons indicated that sites 4 and 3 differed (16.5, p=0.024 adj.) and sites 1 and 3 (-16.2, p=0.006 adj.)

b) NST T2 score differed by site for latency to approach object differed by site, (*n*=49, 12.06, df=3, p=0.007). Sites 1 and 2 differed (-22.89, p=0.004).

c) NOT tT1 by site latency to make contact scores differed by site (17.66, *n*=49, df=3, p=0.001). Pairwise sites 4 and 3 (18.78, p=0.006 adj) and sites 1 and 3 (-18.64, p=0.001) differed.

d) NST T2 by site Latency to make contact with item differed with site during trial 2 (*n*=49, 15.56, df=3, p=0.001). Sites 1 and 3 (-13.43, p=0.041 adj) and sites 1 and 2 (-24.56, p=0.002 adj) differed.

Figure A6.1 a-d Site was identified to be a factor affecting scores of rabbits during NST latency to approach and latency to make contact with items during trial 1 and trial 2.



a) Proximity to item score during NOT T1 differed by site (*n*=57, 15.57, df=3, p=0.002). Sites 2 and 4 (-21.28, p=0.015) and sites 3 and 4 (-20.02, p=0.002) differed.

b) Proxmity to item also differed by site during NOT T2 (*n*=52, 11.74, df=3, p=0.008). Site 3 and 4 differed (-17.92, p=0.007).

c) NOTT1 latency to make contact with item scores differed by site (*n*=52, 11.7, df=3, p=0.009). Sites 4 and 2 differed (21.63, p=0.020).

d) Latency to make contact during NOT T2 difffered by site (*n*=52, 9.060, df=3, p=0.028), however closer examination fo the pairwise comparison showed that there were no significant interactions between the sites at this level.

Figure A6.2 a-d Site was identified to be a factor affecting scores of rabbits during the NOT for proximity to the object scores at both trails. Latency to make contact with the object scores also differed by site but post hoc analysis demonstrated that this was only true at T1.

Appendix 7: Descriptions of behaviours observed during the novel object and novel substrate tests.

Table A7 Frequency of different behaviours observed in response to the different types of novel objects used at trial 1 (T1) and trial 2 (T2) of the behaviour tests at three land-based colleges (T1 *n*=60, T2 *n*=42).

Test	Responses	T1 green tarpaulin	T2 carpet doormat (grey and black)
NST	Attempt to get underneath	2	3
	Chew	0	5
	Chin	1	2
	Lift	8	4
	Move	12	3
	Sniff	44	46
		T1 white cone	**T2 clear plastic box (upside down)**
NOT	Attempt to get underneath	0	2
	Chew	0	1
	Chin	1	4
	Lift	0	2
	Move	1	4
	Sniff	35	37

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