Laboratory rabbit welfare: An investigation of the social and physical environment

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Contents

Declaration

I declare that this thesis is my own composition. All assistance and references have been acknowledged.

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Abstract

The aims of this thesis were: 1) to identify resources that may be important in the housing of laboratory rabbits (from a survey of the pharmaceutical industry, visits to laboratories and consultation with the industry) and 2) to test the motivation of rabbits for the identified resources. From the survey and behavioural observations of rabbits in different housing systems, it was decided that further investigations would focus on female New Zealand White rabbits and the importance of social contact and platforms within cages. The importance of these resources for rabbits was assessed using both short and long-term motivational tests and observations in laboratory cages. An initial experiment to develop motivational tests identified that pushing through a weighted push-doors was perceived by rabbits as costly, in terms of the effort taken to overcome it, but moving through a water bath and approaching an air-stream were not. Short-term motivational tests were set up to give singly and pair caged rabbits the opportunity to push through a weighted push-door to gain a short period of visual and minimal tactile contact with another rabbit. The rabbits pushed through heavier weights to gain social contact than for no reward. Olfactory cues were found to be important, as several rabbits did not push through even the unweighted push-door when the other rabbit was removed. Also, socially housed rabbits pushed through heavier weights for social contact when they were housed out of olfactory contact with their cage-mate. A closed economy consumer demand experiment using weighted push-doors was set-up to test longer term motivation for resources. Two different economic measures (maximum price paid and total expenditure) were used to rank the importance of food, visual and minimal tactile contact, a platform and an empty cage. Both measures showed food and social contact to be of equal and most importance, whilst the importance of the platform varied with the economic measure used. When in the social contact cage the rabbits spent just over half their time in direct visual contact with the other rabbit. In the platform cage the majority of time was spent lying in front of the platform, suggesting that being near to a bolt-hole was important. Platform use was found to be affected by the presence of visual and olfactory cues from conspecifics. The different approaches used found that rabbits were motivated to work to gain visual

and minimal tactile contact with conspecifics and to gain access to a platform. It is recommended that visual and minimal tactile contact should be allowed between rabbits in adjacent cages (as well as providing a means of avoiding contact) and that caged rabbits should be provided with a platform.

Chapter 1 – Introduction

The animals used in research and teaching represent less than 0.25% of the total deaths of animals through human activities in the food industry, hunting and keeping of companion animals and this figure is even lower when deaths of animals from land clearing, pest eradication, road and building construction and deforestation etc. are included (Brennan, 1995 – as cited in Brennan, 1997). Despite this, and due to the nature of the procedures carried out, the use of animals in research and how they are housed are tightly regulated in the UK, by the Animals (Scientific Procedures) Act, 1986 and the associated Code of Practice (Home Office, 1989).

Within the last decade, there has been an increase in concern regarding the welfare of laboratory animals and a corresponding increase in the volume of research carried out in this area has been seen. Although research has previously been carried out on the housing and welfare of laboratory rabbits, the research reported in this thesis will investigate rabbit housing using a different approach to previous studies. The motivation of rabbits for resources will be measured to assess the importance of aspects of the environment to rabbits. The findings will be used as a basis for making recommendations for laboratory rabbit housing. In order to explore the welfare of laboratory rabbits, extensive background research was carried out and this chapter will discuss types of rabbit housing found within the laboratory industry and the advantages and disadvantages of each for the rabbits and laboratory technicians. In addition, the natural behaviour of the rabbit will be discussed and comparisons made between the behaviour of wild and domestic rabbits. Research carried out to date on the housing and welfare of laboratory rabbits and the use of behavioural and physiological measures to assess the welfare of animals will also be discussed, although this thesis will focus on different types of behavioural measures.

1.1 The laboratory industry

1.1.1 Rabbits within the laboratory industry

The rabbit is the fourth most commonly used mammal for scientific procedures within the UK, with 27,389 being used in 2000 (Home Office, 2000). Mice are the most commonly used (1,605,722 in 2000), followed by rats (524,168 in 2000) and guinea-pigs (56,358 in 2000). Rabbits are suitable for several experimental purposes, particularly for antibody production and orthopaedics research as well as cardiac surgery, studies on hypertension, infectious diseases, virology, embryology, toxicology, experimental teratology, arteriosclerosis and serological genetics (Batchelor, 1999). The rabbit is an induced ovulator, only releasing an egg in response to mating (some ten hours later; Sandford, 1996), which allows an accurate assessment of conception to be made. The rabbit also has no seasonal anoestrus and a short gestation period. These features make the rabbit useful in reproduction studies (Batchelor, 1999).

Breeds of rabbit used in the laboratory include the commonly used New Zealand White as well as the Dutch and Half-lop (Batchelor, 1999), with other cross breeds occasionally being bred within the laboratories themselves. As a result of breed differences some breeds of rabbit are more suitable for some types of study than others. The large New Zealand White, for example, is docile and has large ear veins making blood collection easier than in small breeds, whereas the pigmentation of the Dutch rabbit is a useful feature for certain types of study. A further advantage of the New Zealand White rabbit is that it has been found to be better adapted to living on wire mesh flooring than other breeds of rabbit, due to the thick layer of hair on the soles of their feet providing extra padding (Lebas *et al.*, 1997). Both breed and strain differences have been found in laboratory animals, for example, Dutch rabbits are found to be less bold than New Zealand White rabbits (Gerson, 1996). Certain strains can be very aggressive and their housing has to be carefully considered (Barnett, 2001). Selective breeding for certain characteristics has been intense in laboratory rats and mice and strain differences are most notable in these species (e.g. Broida and Svare, 1982; Nevison *et al.*, 1999).

Differences are, however, also seen in rabbits and can influence the type of studies they are used in as well as how they are housed (Harlan UK, pers comm). Differences in behaviour may also be found between animals of different ages and gender and between animals from different suppliers (Batchelor, 1999).

1.1.2 Laboratory rabbit housing

There are several ways of housing rabbits within the laboratory environment. Female rabbits can be housed singly or socially, in cages or in pens on the floor. It has been found that male rabbits cannot be housed socially due to severe aggressive behaviour after the age of around three months (e.g. Bigler and Oester, 1996). Attempts have been made to group house castrated male laboratory rabbits, although this has been found to be both successful (Gunn, 1994) and unsuccessful (Raje and Stewart, 1997). Male fattening rabbits have also been successfully group housed following castration (Lebas *et al.*, 1997). Cages may be made of metal or more recently mainly of plastic, although it was found in a preference test carried out by Stock (1997) that when given a choice, rabbits spent more of their time in metal cages than in plastic cages. Cages may be fitted with a platform, which provides additional floor space and a darkened, enclosed area underneath. Floor pens range both in dimensions and in group number. The use of social cages rather than floor pens can be more economical, as the cages can be used as single cages or linked to form social cages depending on experimental requirements, thus preventing the need for both cages and floor pens. There are advantages and disadvantages of each type of housing, for both the rabbits and the laboratory technicians. The type of housing used can affect several aspects of rabbit behaviour and health.

1.1.2.1 Activity levels

The behavioural repertoire of group housed rabbits has been found to be greater than that of singly caged rabbits. The larger area and increased height of floor pens allows more locomotory behaviour to be performed, such as hopping and running, and allows the rabbits to rear up onto their hind legs and to dig in the substrate (Metz, 1987). The restricted environment within single cages prevents the rabbits from performing a normal hopping locomotion (Lehmann, 1987) and caged rabbits show a lower frequency of rearing than floor penned rabbits (Podberscek *et al.*, 1991). Pair caged rabbits have been found to be more restless than rabbits housed in groups in floor pens, showing significantly more frequent changes between behaviours (Lehmann, 1987). Rabbits housed in cages without a platform have similarly been found to be more restless than rabbits housed with a platform and were more easily affected by their environment (Hansen and Berthelsen, 2000). Lehmann (1987) described such restlessness as "not a characteristic of well-being".

Rabbits reared in cages have been found to show skeletal damage in the form of inactivity atrophies in the proximal femur and distortions of the backbone (Lehmann, 1984 and Wieser, 1984 respectively – as cited in Lehmann, 1987). Martrenchar *et al.* (2001) found that rabbits housed in pens had significantly greater bone diameter and lower deformation before bone breakage compared to rabbits housed in cages. Rabbits in pens also tended to have higher bone weight and breaking strength than rabbits housed in cages, although this was not significant. In social cages, although the space is not as restricted as the single cages, there is still a restriction on locomotion and therefore the potential for the associated bone weakness found in some singly caged rabbits.

Whary *et al.* (1993) found that the food intake of rabbits housed in groups in floor pens was significantly greater than that of singly caged rabbits, although the growth rates of the two groups did not differ. It was suggested that this might be due to the calorific requirements of the group housed rabbits as they are able to exercise more. Contrary to this, Metz (1987) found that rabbits in groups in cages spent more time eating pellets than rabbits in groups in floor pens, and found that the rabbits in floor pens spent just over ten percent of their time eating and investigating their straw bedding.

1.1.2.2 Social behaviour

The rabbit is naturally a social species, living in small mixed sex groups (e.g. Bell, 1983; see section *1.2.2.1*). Single cages prevent rabbits from having full physical contact with other rabbits and whilst some types of single cages may provide the rabbits with the opportunity for visual and perhaps tactile contact with rabbits in adjacent cages others, particularly older designs, may not. Housing rabbits singly decreases their behavioural repertoire (e.g. Gunn, 1994), preventing behaviours such as allogrooming. Stereotypic behaviours may be shown by rabbits in single cages (e.g. Morton *et al.*, 1993; Gunn and Morton, 1995) and include behaviours such as bar-biting and fur pulling. It has been recommended that rabbits should be housed in groups where possible (Home Office, 1989; Morton *et al.*, 1993). However, being induced ovulators, mounting of rabbits by other females can cause them to have pseudopregnancies (Huls *et al.*, 1991), which may interfere with some experiments.

The prevention of social contact in single housing does however avoid the potential for aggressive interactions between individuals. When rabbits are housed socially, no matter what the group size, the social hierarchy has to be established and this will inevitably result in aggressive interactions. Depending on the extent of the competition between individuals, the consequences of the establishment of the hierarchy can vary from fur pulling to severe injuries, which may result in individuals being removed from the group (e.g. Batchelor, 1991). Once the group is stable, aggressive behaviour has often been found to be rare (Vastrade, 1986), although it can occur and may appear to be for no reason. Group housing therefore cannot be guaranteed to be successful. Aggressive behaviour is perhaps of even more concern in social cages than in floor pens. Dominant individuals may be able to defend the smaller area more easily and it being such a restricted space may mean it is difficult for rabbits to avoid each other and therefore to avoid aggression, and perhaps injury.

One of the concerns of group housing of rabbits is that it may be stressful for the subordinate individuals of the group (e.g. Batchelor, 1991). Wild rabbit populations consist of territorial breeding groups, with other individuals living on the edges of the territory of a breeding group (Bell, 1983; see section *1.2.2.1*). In the laboratory situation rabbits are unable to drive out the subordinate rabbits and regulate the group size. The subordinates may consequently be the focus of aggressive behaviour, as they are unable to escape. Morisse and Maurice (1997) investigated the behaviour of ten-week old rabbits in groups of six, seven, eight and nine rabbits per cage, in cages of a constant size. It was found that the rabbits in the group of six showed more social behaviour than the rabbits in the larger groups, and the rabbits in the larger groups showed more comfort and investigatory behaviours and less locomotory behaviour than the groups of six rabbits. This was interpreted as being due to the fact that with the increased group size the social and locomotory activities were impaired and redirected towards body care and the environment.

Wiepkema and Schouten (1988) included an inappropriate group size in a list of examples of social stressors, as in large groups the individuals of the group are less familiar with each other and this increases the occurrence of encounters between unfamiliar individuals. This could increase aggression in groups of rabbits as the dominance of individuals has to be established frequently. Held *et al.* (1995) allowed subordinate female rabbits the choice of being social in a group pen or living in a solitary pen, and found that the rabbits preferred to be in the group pen. The choice of the social or solitary pen was, however, also linked in with the choice of environmental complexity and therefore which of the two aspects of the pens the rabbits were choosing could not be accurately determined. It has been found in some species that the dominant individuals show more signs of social stress (in the form of increased levels of glucocorticoids) compared to the subordinates as they frequently have to re-affirm their position, whereas the subordinate individuals can avoid aggressive interactions (Creel *et al.*, 1996). If this were also true of rabbits, social stress would have an effect on more than just the subordinate rabbits of the group. It was however found by Whary *et al.* (1993) that group housing did not significantly affect immune or physiological responses compared to singly caged rabbits, and therefore did not influence the research they could be used for. This was also found by Turner *et al.* (1997), who found that the social rank of the rabbits in a group did not affect their immunocompetence.

Group housing of female rabbits is most successful if the rabbits are siblings or are introduced when young, before reaching maturity. Once they reach approximately three months of age and become mature, introductions are more difficult due to aggressive behaviour and should be avoided as much as possible. It is therefore preferable for groups to remain stable, with no new introductions made. Wiepkema and Schouten (1988) listed the introduction of strange individuals to a well established group, or indeed the removal of core members of such a group as a social stressor, as this disrupts already existing relationships. This may result in aggressive behaviour as the rabbits re-establish the hierarchy. Gunn (1994), for example, introduced two unfamiliar rabbits into an already established group and found an increase in aggressive behaviour and mobility throughout the whole group. The new rabbits performed limited behaviour, showing no grooming, foraging or lying stretched out and were suggested to be suffering as a result of being introduced into the group. The dominant rabbit showed play type behaviour, picking up and throwing straw, which was interpreted as being frustration in the form of displacement activity, as a result of not being able to chase away the unfamiliar rabbits.

1.1.2.3 Monitoring

Singly caging allows rabbits to be closely monitored in terms of their food and water intake, urine and faeces output and also their behaviour, and any changes can be quickly detected. These are particularly important following experimental procedures. Housing rabbits singly also means that they can be easily identified and can be easily caught for procedures or health checking. Housing rabbits in a group in a floor pen means that identification and monitoring of individuals is more difficult. The method used to identify individuals of a group should be harmless to the rabbits, simple to apply and maintain, easy to read, be sufficiently permanent and not interfere with experiments (Barnett, 2001). Methods used include keeping rabbits in groups of mixed breeds (although experiments may require the use of one breed), the use of dyes to mark individuals and fur clipping, all of which allow recognition of individuals at a distance. Other approaches include ear tattooing, ear and toe punching, ear studs/tags (Barnett, 2001), as well as micro-chipping and ear marking with indelible ink, however, these forms of identification are only visible once the rabbits have been caught and are not very useful if trying to find one particular rabbit to be caught. Group housing makes it almost impossible to monitor food and water intake and urine and faeces output and small changes in behaviour may go undetected, or take longer to detect. Changes in how an animal interacts within the group can, however, be a useful indicator of reduced health. Socially housed rabbits in cages can be more easily monitored, identified and caught than those in floor pens due to the restricted space, although not as easily as when rabbits are housed singly.

1.1.2.4 Injury/disease

Several types of injury and disease are associated with cages, for example, intestinal disorders have been found in rabbits housed in cages and these are thought to be as a result of stress (Jackson, 1991). In the past, cage floors were typically wire grid which caused sores on the feet due to the weight of the rabbit being distributed unevenly over the foot on the high point of the grid (Barnett, 2001). Wire grid has now widely been replaced by plastic 'dimple' flooring and the problem is now much less common. Morisse *et al.* (1999) found meat production rabbits to prefer wire mesh to straw deep litter. The rabbits used in this study were however only 32 days old and as fattening rabbits are generally slaughtered at 10-12 weeks of age their preference of wire flooring does not have any real consequence for the condition of their feet as they do not live long enough for real problems to occur. Problems with feet are more likely to develop in nervous rabbits that frequently stamp their feet when startled and there is evidence that a tendency for sore hocks is inherited (Sandford, 1996). Using wire or dimple flooring rather than solid flooring allows for urine and faeces to be collected in trays below the cage which can be removed and emptied. If cage trays are not cleaned out frequently enough there can be a build up of ammonia, which can cause respiratory diseases (Wolfensohn and Lloyd, 1994). This may be even more of a problem when rabbits are housed socially in cages, as in addition to the increase in waste and ammonia due to increasing the group size there will be a higher temperature and relative humidity within the cage which increases the risk of disease as well as creating an unpleasant environment (Barnett, 2001). Caging can therefore be a labour intensive method of housing (although the cages themselves are cleaned in automatic cage washers) and group housing rabbits in cages may therefore be more labour intensive than group housing in floor pens. With groups of rabbits it is possible that any infections or diseases may spread throughout the whole group (e.g. Akintunde *et al.*, 1994) although some studies have shown this not to be the case (Love and Hammond, 1991; Whary *et al.*, 1993). If any infections or diseases do get into the group, treatment may be difficult to administer, for example, if antibiotics are put into the water it is impossible to know how much treatment each rabbit has received.

It should be noted that of the listed advantages and disadvantages of single and group housing that the advantages of single housing are mainly for the technicians and for certain types of study rather than for the rabbits, and that the disadvantages of group housing are mainly for the rabbits. Many people believe that animals have moral rights which are violated by their use in laboratories and that rather than trying to improve their housing they should not be in laboratories at all (e.g. Regan, 1997). Of those who agree with the use of animals in laboratories, it is widely accepted that if animals are to be used for human benefit that we have an ethical and moral obligation to do the best we can for them (e.g. Beauchamp, 1997; Baumans, 1999; Stafleu *et al.*, 1999), with respect to both the procedures carried out and the way in which they are housed. It may therefore be expected that the type of housing used should be that which is most suitable for the rabbits. In determining the method of housing to be used however, experimental constraints, money, ease of use and space may also be major considerations to be taken into account, rather than just the consequences the housing may have for the rabbits (Barnett, 2001). Perhaps this is due to the fact that there is little scientific evidence to convince the laboratory industry that one method of housing is significantly more suitable for rabbits than another.

1.1.3 Research on rabbits used within the laboratory

In 1959, as requested by the Universities Federation for Animal Welfare, 'The Principles of Humane Experimental Technique' was written by Russell and Burch (as cited in Balls, 1997). They introduced the concept of the Three Rs in laboratory animal science - replacement, reduction and refinement. Replacement refers to replacing animals in research with alternatives and was defined as "any scientific method employing non-sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates". Reduction involves lowering "the number of animals used to obtain information of a given amount and precision". The concept behind refinement was described as any development leading to a "decrease in the incidence or severity of inhumane procedures applied to those animals which have to be used". Replacement and reduction are variables that are easily quantifiable, whereas refinement is a gradual, ongoing process which is never completed (Stauffacher, 1997). It has been suggested by Morton (1997) that there should be a subset of Rs within refinement which should be taken into account to avoid or alleviate the adverse effects imposed on animals within laboratories, and that these should include recognition, recording, relieving, retraining, respecting and responsibility. As well as refinement with regard to the procedures themselves (e.g. Flecknell, 1994), refinement is now often discussed in terms of the refinement of housing and husbandry (e.g. Morton, 1999).

Research into the refinement of laboratory animal housing and husbandry has increased over the last decade, with much of this research focusing on rats and mice. Research has been carried out into types of enrichment for laboratory rabbits, although much less than for the rodent species. Rodents are often housed in cages with solid floors therefore allowing the use of bedding and nesting material and thus increasing the potential for environmental enrichment. Some of the studies carried out on environmental enrichment for rabbits have investigated the use and effects of devices such as wood sticks and aluminium cans (Brooks *et al.*, 1993), parrot toys and brass wire balls (Huls *et al.*, 1991) and the effect that hay has on the behaviour of caged rabbits (Berthelsen and Hansen, 1999). Studies have also been carried out into social enrichment, mainly for female rabbits, although these tend to investigate whether or not group housing can be successful (e.g. Heath and Stott, 1990; Batchelor, 1991; Stauffacher, 1992), rather than whether or not the rabbits actually want to be housed socially. One such study where rabbits were given a choice was carried out by Huls *et al.* (1991). Pair housed rabbits were allowed to choose between being in a cage alone or in a cage together and it was found that the rabbits showed a preference for being in a cage together. Fewer studies have been carried out on social contact for male rabbits as they are notoriously difficult to socially house due to aggressive behaviour (e.g. Bigler and Oester, 1996). Some problems with aggression have also been found with castrated males (Raje and Stewart, 1997). It is important that research is carried out to assess the effectiveness and potential consequences of enrichment rather than assuming that the animals will benefit from it. It has been found that providing a particular strain of male mice with enrichment increased aggression (McGregor and Ayling, 1990) and certain enrichment devices for rabbits have actually been found to be unsafe (Shomer *et al.*, 2001).

1.2 The wild European rabbit

Before an assessment can be made of the welfare of a species in captivity it is important to investigate the natural behaviour of that species and, for domestic species, how their behaviour has been affected by domestication.

1.2.1 History and domestication

The wild European rabbit, *Oryctolagus cuniculus*, was discovered by the Phoenicians around 1000 BC (Lebas *et al.*, 1997). The word *Oryctolagus* translates from Greek into 'hare-like digger' (McBride, 1988, p13), whilst the word *cuniculus* translates from Latin into 'underground passage' (Thompson and Worden, 1956).

In the first century BC the Romans kept wild rabbits in walled enclosures known as 'leporaria'. Rabbit foetuses or newborns were known as 'laurices' and were eaten as a delicacy. By the sixth century rabbits were being kept almost as pets by monks, who experimented with breeding for size and tameness (McBride, 1988, p18). Rabbits were, however, not really accepted as pets until the sixteenth century, when several breeds of rabbit were known to exist. During the 1800s rabbit breeding in hutches began all over Europe in both rural areas and city suburbs as the Industrial Revolution moved people from the countryside to towns and cities. Even at this stage the importance of hygiene and breeding standards were recognised, as was the importance of housing. The breeding rabbits were housed in separate hutches due to aggressive behaviour shown if kept in groups in confined spaces. Young fattening rabbits were housed in groups, with the males being castrated (Lebas *et al.*, 1997).

Small-scale production of rabbits encouraged the use of large breeds of rabbits. Just after the middle of the 20th century the numbers of New Zealand White (NZW) rabbits and their offshoot, the Californian rabbit, exploded and those of the traditional European breeds declined. The NZW and Californian were found to be more suitable for living on wire mesh floors in cages due to the pads of their paws having a better covering of hair (Lebas *et al.*, 1997).

1.2.2 Wild rabbit behaviour

1.2.2.1 Social structure

The European rabbit is the only one of over 40 species within the family Leporidae known to form structured and stable social groups (Cowan and Bell, 1986), living within warrens in small territorial breeding groups. Territories are found within a larger 'home range' and the ranges of adjacent breeding groups may overlap, with members of more than one group sharing these 'communal' grazing areas during the peak feeding times of dawn and dusk (Bell, 1983). It is possible to find single pairs in areas where the population density is low but in larger groups the females usually outnumber the males (e.g. Mykytowycz, 1958; Stodart and Myers, 1964; Vastrade, 1986). As well as the breeding groups, there may be additional rabbits living in relatively close proximity to a particular group. These rabbits are known as 'satellites' and may be of either sex. As a result of failing to gain acceptance into the breeding group, male satellites will be chased away from the females in the breeding groups by the breeding males and female satellites will be chased away from breeding sites within the warren by females of the breeding group (Bell, 1983). The dominant breeding females give birth frequently and regularly in a breeding chamber which is an extension to the main burrow. The low ranking females dig out short breeding stops (i.e. small burrows) away from the warren, and may be forced to site these in areas which may be less safe, for example, prone to flooding. This may be one of the reasons why the young of subordinate rabbits have a much lower survival rate and growth rate than those of dominant females (Mykytowycz, 1968). The young may eventually extend these breeding stops to develop a new warren (Thompson and Worden, 1956).

1.2.2.2 Habitat use

The size of groups of rabbits is dependent on the habitat, for example, the available area suitable for burrowing (Bell, 1983) as well as the population density in the area. The warren structure varies between areas depending on factors such as the soil composition as this has an impact on the speed at which rabbits can excavate the soil and on the amount of soil that can be moved by the rabbits in each digging bout. Where soil is hard and the digging of burrows is difficult, the social system tends to be as described in section *1.2.2.1*, with the breeding group controlling the warren and the dominant females chasing the lower ranking females away from the warren. However if the soil is sandy and therefore easier to burrow in, all females will be able to dig their own long, deep and safe burrow for raising their young and it has therefore been suggested that rabbits living in areas of sandy soil may experience a higher rate of survival of the young (Kolb, 1985).

In addition to providing a place for the females to have and raise their young, the warren provides protection from the weather and from predators. As well as the main warren and breeding stops, rabbits will construct a further type of burrow, known as a bolt-hole. The main entrance to a warren is noticeable from the mound of earth on the surface, which the rabbits have kicked backwards during excavation. Bolt-holes, however, do not have this obvious feature, as they are dug from within the warren. They are small, often descend vertically and their entrance is usually concealed in the undergrowth (Thompson and Worden, 1956). The use of burrows by rabbits seems to be dependent on the amount of cover available from vegetation. If an area has no vegetation the burrow will provide the only refuge from predators, whereas if the vegetation cover is dense it perhaps provides more protection from predation (Kolb, 1991). In a study where both burrows and ground cover were available there was found to be variation in the use of burrows by males (Kolb, 1994). This was found to be related to weight and was assumed to therefore be due to age. The older males were found to use burrows more often than the younger males, and when disturbed the older males were more likely to bolt into burrows than they were to stay on the surface. Younger males however spent more of their time on the surface and would often avoid burrows when disturbed. This was also found by Mykytowycz (1960) who suggested this was due to social exclusion with the older, dominant rabbits preventing the younger subordinate ones from using the bolt-holes. These lower ranking males bred poorly and it was suggested that they would have migrated to another area if they had been able to, however the population was within the confines of an enclosure to enable easier tracking and observations of the rabbits. Kolb (1994) found female rabbits tended to spend less time on the surface than males, although this difference was not significant.

1.2.2.3 Territorial behaviour

The boundaries of the rabbits' territory are clearly defined and are patrolled regularly. The territory is marked using glands situated in the anal region and under the chin. The anal glands of male rabbits are larger than those of females and the size of the anal gland is related to the social status of the animals, with the more dominant rabbits having larger glands than the subordinates (Mykytowycz, 1968). Mounds of faecal pellets, known as 'latrines' (Thompson and Worden, 1956) or 'dunghills' (Mykytowycz, 1968) are formed around the warren area and the age, sex and social status of rabbits have been found to affect their behaviour at latrines (Sneddon, 1991). Dominant males tend to make regular deposits on a large number of latrines whereas adult females have been found not to make regular visits. When females do visit latrines they spend a greater proportion of time resting and grooming and therefore spreading the scent across their body, probably as a means of acquiring the scent of the dominant male. Young males and females appear to visit latrines mainly to investigate the odours. Rabbits also use urine to mark their territory and may mark each other through urine spraying during aggressive disputes (Mykytowycz, 1968). As well as using scent from urine and faeces to establish their territory, rabbits also scent mark using a gland under the chin, particularly male rabbits. Similarly to the anal glands, the chin glands are larger in male rabbits than in female rabbits and those of dominant males are larger than those of subordinate males (Mykytowycz, 1962). As well as marking areas already marked with faeces and urine, this gland is used to chin features of the territory such as logs and branches as well as burrow entrances, faecal pellets of other rabbits and its own weathered pellets (Mykytowycz, 1968).

1.2.2.4 Senses

Rabbits spend most of their time in semi- or complete darkness. They spend their days in their underground burrows, emerging at dusk to feed, to court and to patrol their territories. In such a world, vision is not the most important sense and for the most part communication relies on scent, with sound playing a minor role (McBride, 1988, p60). Rabbits are vigilant animals with highly developed senses of hearing and vision as well as smell. Their feeding behaviour epitomises the alert state of the rabbit. A rabbit will frequently interrupt its grazing, lifting its head to survey the area, often rearing up onto its hind legs to get a better view. The frequency with which an individual interrupts its feeding depends on how many rabbits are close by. The more rabbits, the less time an individual needs to spend being vigilant and the more time it can spend feeding. Rabbits may have to be more or less alert depending on where they are feeding. For example, rabbits are very vigilant if close to a hedgerow or if in the middle of the field, as predators may be camouflaged in the hedgerow and if in the middle of the field the rabbits may not be able to reach cover quickly enough. Between these two areas the rabbits can afford to be less vigilant (McBride, 1988, p44).

1.2.3 Comparison of the behaviour of wild and domestic rabbits

Studies have been carried out to compare the behaviour of wild rabbits to that of domestic rabbits, often in semi-natural conditions, i.e. within the confines of a large natural habitat enclosure. Stodart and Myers (1964) compared the behaviour of wild and domestic rabbits in separate groups in enclosures, and found that they showed very similar behavioural repertoires and similar daily time budgets, with both dividing their day into two distinct periods of resting during the day and activity during the night. Differences found were that domestic rabbits rested above ground during the day whereas the wild rabbits were found to rest underground. The decrease in activity from night into day and the increase from day into night was more gradual in the domestic rabbits and these rabbits always showed some form of activity during the day whereas this was relatively uncommon with the wild rabbits. One further difference was that more of the domestic rabbits were found to rest between dawn and dusk compared to the wild rabbits. Reproductive behaviour was found to be almost identical, with female domestic rabbits showing the same nesting behaviour as wild rabbits, i.e. building and lining nests, plugging the breeding burrow entrances with soil and defending the burrows aggressively. Domestic rabbits drank water more often than wild rabbits, rather than relying on moisture from their food. Both wild and domestic rabbits showed the same response of bolting for cover if a bird of prey flew over the enclosure.

Stodart and Myers (1964) also compared the behaviour of three pairs of wild and domestic rabbits released into a large enclosure together. Two of the domestic males were reared in small cages and the third male and the three females were born and reared in enclosures. The two cage reared males did not survive, with one breaking its back and the other starving to death. The wild rabbits had been caught from properties near to the experimental site. The wild and domestic rabbits formed almost completely separate groups, with one wild male joining the one remaining domestic male and three domestic females. One wild male behaved as a 'satellite', wandering over the whole enclosure rather than becoming part of either of the two groups. A dominance hierarchy was found among the female domestic rabbits, however the wild females showed no aggression towards each other so that any hierarchy present could not be determined. The adult wild rabbits had a higher rate of survival, however, the domestic does were found to have higher reproductive efficiency, having more litters and a higher mean litter size than the wild rabbits.

A further two studies by Stodart and Myers (1964) involved releasing cage-bred and enclosure-bred rabbits into groups of wild rabbits, either in enclosures or in a paddock. These studies found that if domestic rabbits were not cage bred, but were raised in freedom they were able to form breeding groups and reproduce in competition with wild rabbits, and in fact were found to have higher reproductive rates than wild rabbits. The rabbits raised in cages suffered a high mortality rate due to back injuries, disease, predation or exposure. Overall, they found that domestic rabbits showed behaviours very similar to those of wild rabbits in terms of feeding and resting patterns, social, reproductive and maintenance behaviour and responses to predators although they were not adapted to the environmental challenges they met in the wild. Vastrade (1986) concentrated on the social behaviour of free-ranging domestic rabbits and similarly found their behaviour to be akin to that of wild rabbits.

The fact that rabbits have retained species-typical behaviours throughout their domestication is perhaps to be expected, as they were selectively bred for features such as appearance (for showing), fur quality, size (for meat production) and reproductive success rather than for behavioural traits other than that of docility. Rabbits used in the laboratory have undergone further selective breeding for characteristics appropriate to the laboratory environment and experimental procedures, however, wild rabbit behavioural characteristics are found in laboratory rabbits years after laboratory domestication. Whilst this suggests that these traits are inherited, it is likely that the restriction of social interaction, lengthy isolation and increased longevity will have had an effect on behaviour (Heath, 1972).

1.3 Pet rabbits

The rabbit is now the third most commonly kept mammalian pet, or companion animal, in the UK (McBride, 1998, p14), having rapidly increased in popularity over the last few decades and many books have been written on their behaviour (e.g. McBride, 1998) and how to care for them (e.g. Crush, 1990; Brown, 2001). Some of these books discuss the natural behaviour of rabbits and use this to advise owners on how best to care for them in relation to this (e.g. James, 1997). Rabbits are traditionally kept outdoors in hutches and pet rabbit books usually give recommendations for hutch dimensions and almost always state that rabbits require regular exercise. James (1997), for example, suggests that rabbits should have at least three hours of exercise a day. 'The Official RSPCA Pet Guide' to caring for pet rabbits by Crush (1990) recommends that female rabbits housed in pairs should have a hutch measuring 9,000cm^2 and 60cm high for two small to medium sized rabbits and 16,200cm^2 and 90cm high for two large rabbits. Brown (2001) recommends a hutch size of 9000cm^2 and 70cm high for one rabbit, large enough to allow the rabbit to exercise. James (1997) recommends 5,400cm^2 (45cm high) for a small rabbit, 7,200cm^2 (60cm high) for a medium sized rabbit, 9,000cm^2 (60cm high) for a large rabbit and 13,500cm^2 (75cm high) for a giant breed. Several web sites have been developed to advise people on how to care for their rabbits and they give similar hutch dimensions to those listed. Most books recommend that female rabbits should not be housed alone and usually suggest that females housed together are neutered and that if rabbits of the opposite sex are housed together, that they are both neutered. Non-castrated male rabbits are always said to be very aggressive towards each other after three months of age and should therefore be housed singly. These books also tend to recommend providing rabbits with enrichment in their hutch and exercise area, including tubes, pipes, boxes, branches, ramps and a variety of plants.

It is becoming increasingly common for rabbits to be kept indoors as house rabbits. This type of housing may mean that rabbits are given more exercise than outdoor rabbits, particularly during the winter months and in spells of cold or wet weather when outdoor pet rabbits may receive little time and attention from their owners. Rabbits kept singly in a house are likely to receive more human contact than outdoor rabbits although this very reason may mean that house rabbits may be more likely to be kept singly as their owners think of human contact as being a substitute for contact with another rabbit.

Recommendations for pet rabbits tend to suggest larger cage sizes than those that are required for laboratory rabbits and even those recommended in the code of practice (Home Office, 1989). The code of practice gives recommendations of floor area and cage height in relation to the body weight of the rabbits. A singly housed rabbit less than 2kg in weight is recommended to have a cage area of 2000cm^2. For a rabbit weighing 2-4kg the recommendation is 4000cm^2, for a rabbit of 4-6kg the recommendation is 5400cm^2 and rabbits over 6kg in weight are recommended to have 6000cm^2. The height recommendations are 40cm for rabbits less than 2kg and 45cm for rabbits of 2kg and above. Rabbits housed in groups are recommended to have approximately 35% less than the above floor area measurements per rabbit. To allow comparisons with the measurements given from the pet rabbit books, a rabbit of 2kg is likely to be what the books described as a small rabbit, 4kg as medium, 6kg as large and over 6kg as giant. It is also widely recommended in pet rabbits books that rabbits should be housed with social contact with a conspecific and that rabbits should be allowed to exercise daily. The code of practice for laboratory rabbits also recommends that rabbits be housed socially where possible, however experimental 'needs' overcome this and unless rabbits are housed in groups in pens they are unlikely to be able to exercise. Although laboratory rabbits tend to be provided with some form of enrichment and several studies have been carried out to investigate suitable forms of enrichment (see section *1.1.3*) laboratory rabbits can not be provided with the range of items recommended for pet rabbits. All enrichment objects to be placed in a laboratory cage must be sterilised first and not all objects can be successfully treated. These points perhaps emphasise the extent to which compromises are made between the needs of the rabbits and the constraints of the experiments, space and money. It also raises the issue of whether the recommendations for rabbit housing should differ depending on whether they are kept for human use or human pleasure despite there being no differences in the animals themselves.

1.4 Methods of welfare assessment

The assessment of the suitability of an environment for an animal involves determining whether the needs of the animals are met. The term 'need' has been defined by Broom and Johnson (1993) as "A requirement, fundamental in the biology of the animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus. To have a 'need' is to have a deficiency which can be remedied by obtaining a specific resource or stimulus." If an animal is unable to satisfy its needs it will remain motivated to do so and will show behavioural and physiological responses to remedy the need and allow the animal to cope with its environment. If a need cannot be satisfied the welfare of an animal can be poor (Broom and Johnson, 1993). If the performance of a behaviour is not possible the animal's thwarted attempts to carry out the behaviour may become apparent in the form of stereotypic behaviour or displacement activity or may be directed towards the environment or themselves, in the form of wall licking or self-mutilation. Stereotypic behaviour is a repeated, relatively invariate sequence of movements which has no obvious purpose (Fraser and Broom, 1990), for example, weaving, pacing and bar-biting. Displacement activity has been defined as an activity which is performed in a situation where it appears not to be in the context in which it would normally occur (Fraser and Broom, 1990), such as preening shown by hungry domestic fowl when presented with food covered with perspex (Duncan and Wood-Gush, 1972). Needs are generally identified through deprivation (Poole, 1992), by identifying the cause of such behaviour if and when it occurs. As well as implying that an animal is prevented from performing a behaviour that it would perform if not restricted, deprivation also implies that adverse affects will arise as a result (Dawkins, 1988).

Animals in captivity are exposed to a number of potential problems in terms of adapting to their environment, such as the restriction of space, unnatural social groups, temperature fluctuations, noise levels and possibly either the predictability or unpredictability of the environment and daily routine. Individuals will vary in their ability to adapt to their environment. It is possible to use both the behaviour of an animal and physiological measures as complementary approaches in the assessment of the welfare of an animal in its captive environment and whether or not their behavioural needs are catered for. Several definitions have been given in the scientific literature for the word 'welfare' (e.g. … "the state of an individual as regards its attempts to cope with its environment"; Broom, 1986), however this variation in the definition of welfare has resulted in differences in research methods and interpretation of results (Duncan and Fraser, 1997). Fraser *et al.* (1997) suggested that animal welfare should be used as a 'bridging concept' which "…links scientific research to the ethical concerns that the research is intended to address". They proposed that animal welfare research needs to take into account concerns about the biological functioning and subjective experience of the animal as well as natural-living concerns.

1.4.1 Physiological assessment

Animals show both short-term and long-term physiological responses to challenging situations. The nervous system and the endocrine system are involved in communication and co-ordination both within an animal and between an animal and its environment. Cues from the environment such as visual, olfactory and auditory cues cause messages to be sent via neurons in the form of nerve impulses. These impulses are very fast, however they operate over a short time period. Sensory (or afferent) neurons carry messages to the brain and motor (or efferent) neurons carry impulses from the brain to muscles and glands (Fraser and Broom, 1990, p46). Gaps between neurons are called synapses and transmission across these synapses can be either electrical or chemical (Eckert *et al.*, 1988, p145). Electrical transmission is simpler and faster and is often used when the speed of the response is vital, e.g. escape from a predator

(Goodenough *et al.*, 1993, p156). Chemical transmission is more common and is slower than electrical transmission. During chemical transmission, impulses cause the release of chemical messages (neurotransmitters) at the presynaptic terminals of the neurons. These neurotransmitters activate receptors on postsynaptic cells to evoke additional electrical patterns, allowing information to be passed between neurons. The transmitter will continue to activate the postsynaptic cells as long as it remains in the presynaptic terminals. There are four types of neurotransmitters, namely acetylcholine, biogenic amines (e.g. dopamine and serotonin), amino acids and neuropeptides, with the majority of neurones using only one transmitter substance. Each has a specific effect on other neurons or effector organs. Acetylcholine, for example, is important in the autonomic nervous system, whereas the biogenic amines are important in the sympathetic nervous system (Fraser and Broom, 1990, p49).

Messages sent via the endocrine system are in the form of hormones and are slower but longer lived, thus prolonging their effect. These messages are able to reach every cell in the body via the bloodstream (Manning and Dawkins, 1992, p95). During short term responses to the environment such as a sudden threat or emergency situation the animal prepares for 'fight or flight' by secreting adrenaline. Adrenaline causes changes such as increased heart rate and more rapid breathing and diverts the blood to the muscles from the alimentary canal (Manning and Dawkins, 1992, p81). When a stressful situation persists the adrenal glands produce a variety of glucocorticoids which act on the liver causing increased glucose to be released into the circulation and therefore increasing the blood glucose levels to make energy available to the muscles (Eckert *et al.*, 1988, p300).

Although physiological measures can be useful, there are problems associated with their use. One of the problems is that obtaining the sample can be difficult and stressful for the animal, particularly wild animals (Duncan and Poole, 1990). Obtaining the sample can itself influence the findings, with the animals showing the above short-term responses to prepare for fight or flight, for example, changes in heart rate from activity cannot be distinguished from changes due to emotional responses (Broom and Johnson, 1993, p93). As well as the process of actually obtaining the measurements, the timing of the measurements is also important and can have a huge effect on the findings. Some measures may increase almost immediately and decrease quickly (e.g. heart rate), whereas others may take some time to occur and if exposed to chronic stress animals may habituate (Mason and Mendl, 1993). Although glucocorticoid levels can be a useful measure, their use also raises concerns, as the basal level of glucocorticoids shows a diurnal rhythm (Eckert *et al.*, 1988, p300). Levels can also vary due to courting behaviour, mating and active food acquisition (Fraser and Broom, 1990, p259). Care must therefore be taken when interpreting the levels of glucocorticoids found. Knowledge of the biology of the species is required in order to identify the type of responses being shown (Broom and Johnson, 1993), as responses may differ between species, as well as between animals of the same species but different ages, sexes or with different previous experience (Mason and Mendl, 1993). A further problem of using physiological measures is in their interpretation. This has to be done through analogy with humans, by measuring the human physiological responses to a situation where they are suffering and comparing this with the responses of animals (McFarland, 1989, p35).

1.4.2 Behavioural assessment

Behavioural responses to challenging situations can also be short-term or long-term. Short-term responses may be changes in posture or flight, whereas long-term responses may include the development of stereotypic behaviour. The main advantage that behavioural assessment of welfare has over the physiological measures is that it can be non-invasive and assessment can be carried out without influencing the animals and their behaviour (Duncan and Poole, 1990). Simple observations can determine any changes in posture, inability to carry out normal movements, avoidance of an aspect of the environment, flight, changes in the 'normal' behaviour of an individual, lack of maintenance behaviours such as grooming and the performance of abnormal behaviour and displacement behaviour. As with physiological measures, knowledge of the natural behaviour of the animal is also required when using behaviour to assess welfare, for example, vocalisation by an individual of one species may be of more concern than vocalisation from another. Similarly, some species naturally freeze in response to threat whereas others do not and such a response would be of more concern in some species than others (Mason and Mendl, 1993).

1.4.2.1 Observations in the captive environment

Assessment of the welfare of animals and the suitability of their captive environment has often been made using observations of the species in the wild or in semi-natural conditions which can be compared to observations of the animals in captivity (e.g. Stolba and Wood-Gush, 1989; Lehmann, 1991; Veasey *et al.*, 1996). This is particularly useful for zoo animals whose behaviour in captivity is likely to be closer to that of their wild counterparts than the behaviour of domestic species to their ancestors. Cage/pen observations can provide much information on the behaviour of an animal and how it uses different areas or features of the environment and this can be used to compare behaviour between different types of housing or between different groups of animals. Such observations do not however tell us how the animal perceives the environment or how important different resources are to them. The lack of performance of a behaviour by captive animals which wild animals or animals in semi-natural conditions would perform could be due to the animal adapting to its captive environment due to genetic differences which have arisen as a result of captive breeding or the behaviour may be strongly dependent on external stimuli and in the absence of such stimuli the animal is not motivated to perform the behaviour (Dawkins, 1988). There is therefore a risk of the behaviour being interpreted incorrectly due to assumptions being made about how the animal perceives the environment based on human perception.

1.4.2.2 Preference tests

An alternative to simple observations as a means of investigating the environment of an animal is to 'ask' the animal. This has been carried out using preference tests, which allow the animal the choice of different types of a resource (e.g. floor surface) or between having a resource and not having it. The option chosen most often or for the longest duration is assumed to be the preferred option. Tests for aspects of the environment such as floor surface may be carried out using a cage with a different floor type in each half (e.g. Hughes and Black, 1973) where the animal can easily and quickly move from one type to the other. They may also be in the form of a T-maze where the animal has to make a more obvious choice between the resources, moving away from one type of the resource to gain the other (e.g. Dawkins, 1977).

Preference tests have been widely used for a wide range of species, mainly farm and laboratory animals, and to test preferences for a wide range of resources. Studies into the preferences of laboratory animals have mainly focused on the rodent species. Such studies include the preferences of rats for cage heights and light intensities (Blom *et al.*, 1995) and for different types of flooring (Van de Weerd, 1996), which found that they showed a preference for wood shavings and paper bedding over sawdust and wire mesh. Studies of the preferences for mice have investigated the characteristics of soiling sites (Sherwin, 1996) and nesting material and nest boxes as sources of environmental enrichment (Van de Weerd, 1998a, 1998b). Preference tests have also been carried out to investigate preferences of laboratory rabbits for social contact (Held *et al.*, 1995) and of meat production rabbits for floor type (Morisse *et al.*, 1999).

Preference tests tell us what proportion of time the animals choose to spend with each of the options available to them, however, they only have a limited number of options available to them and the resource taken as the preferred one may not be so if the available options were different. The only sacrifice the animals have to make in choosing the preferred resource is not spending time with the other options, which may not be much of a sacrifice if the other options are ones the animals do not want. If the animals have to make a significant sacrifice to gain a resource a better indication may be made of the importance of a resource.

1.4.2.3 Strength of preferences

An addition to this simple type of test is to impose a cost on the animal gaining a resource. In these operant tests the animal 'operates' on the environment to achieve the desired consequences. These tests allow the study of the way in which the performance of a behaviour affects the likelihood of the behaviour occurring again (Kilgour *et al.*, 1991). This type of experiment was originally reported within the psychology literature as investigations into learning and memory, but such experiments have now been adapted to be used for practical investigations. Such tests show preferences for certain types of a particular resource but do not provide a quantitative scale for the strength of motivation for different resources (Fraser and Matthews, 1997). Motivation has been defined as the strength of the tendency to engage in a behaviour when taking into account not only internal but also appropriate external factors (Toates, 1986) and as the system within the brain that induces behavioural and psychological changes and determines which changes occur and when (Broom and Johnson, 1993). When an organism is motivated to behave in a certain way, this behaviour is terminated when a goal (which is usually of biological significance) is achieved. Motivated behaviour may therefore be characterised as guided by its consequences and is related to some end point linked with biological requirements of the animal (Wong, 2000).

It was proposed some time ago that consumer demand theory from human microeconomics could be combined with the performance of tasks to assess the importance of resources to animals (Lea, 1978; Dawkins, 1983, 1988). This approach would allow the motivation for resources to be titrated against the motivation for a resource such as food, which is necessary for survival and therefore a resource animals will be likely to work very hard for. In human economics, demand varies according to the price of the resource and the available income and resources may be described as having an elastic or inelastic demand depending on how consumers respond to changes in the price of a commodity (see Figures 1a and 1b). A commodity or resource is said to have an inelastic demand if the cost for that resource is paid and the preference for that resource is still apparent when the cost is increased (Lea, 1978). If the demand for a resource decreases as the cost increases the demand is said to be elastic. If an animal is strongly motivated to perform a behaviour or obtain a resource and is unable to do so in its captive environment it will suffer (Dawkins, 1983, 1988, 1990).

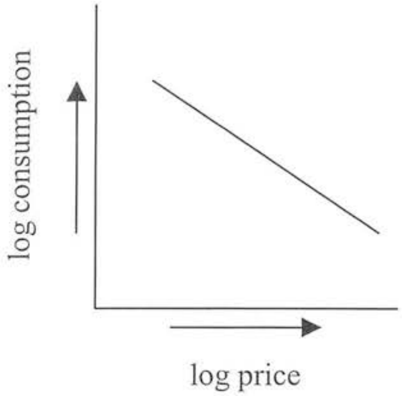

Figure 1a Elastic demand
Consumption of the resource decreases as the price increases

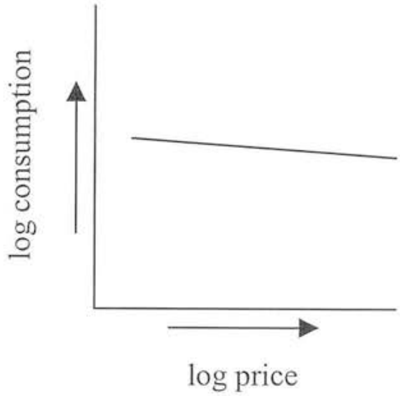

Figure 1b Inelastic demand
Consumption of the resource does not decrease as the price increases

Consumer demand studies originally involved imposing a cost in terms of a repeated task, for example, lever or bars presses. The animals had to perform a certain number of presses in order to obtain a pre-determined quantity of the reward, and usually only one resource was offered at a time. Such studies have been used to assess motivation of hens for straw and feathers (Gunnarsson *et al.*, 2000), of mice for addition space (Sherwin and Nicol, 1997) and of pigs for social contact (Matthews and Ladewig, 1994), as well as food motivation in sheep (Jackson *et al.*, 1999) and pigs (Lawrence *et al.*, 1988, 1989; Lawrence and Illius, 1989). Such an approach has also been used to assess the aversiveness of cage dusting to hens (Rutter and Duncan, 1992).

This approach has, however, been said to be over-simplistic as animals are likely to experience several simultaneous motivations which cannot be satisfied under such experimental conditions (Sherwin, 1996). A further problem with such studies is that the short-term choices may not reflect the long-term preferences (Dawkins, 1988) as it has been found that hens about to lay have such a strong motivation for a nest that they will enter trap-nests even when this means they will have no food or water for the next 24 hours (Duncan, 1978). Short term tests such as this may not be suitable for measuring certain types of motivation, for example, ones that may depend on a circadian rhythm and therefore motivation may be stronger at certain times of the day. Hens that have just dust-bathed are likely to be less motivated to perform a task to be able to do so than a hen which has been prevented for dust-bathing. It has also been found that the animals may not be able to associate the task they are expected to perform with the reward they gain after the performance of the task, for example hens have been found to be unable to learn to peck a key or operate a foot treadle to gain access to litter (Dawkins and Beardsley, 1986). This can lead to the assumption that an animal has low motivation for a resource, which may not be the case. Dawkins (1988) suggested that perhaps requiring an animal to respond in a way more closely related to the response it would make in the wild would be more appropriate. Young *et al.* (1994) found that pigs responded significantly more often for food when they had to manipulate a 'paddle' (operated by rooting or chewing the device) than when they had to press a panel using the flattened end of their snout.

Consumer demand type approaches have been used to provide several resources at once, each of which has a cost imposed on gaining access to it. Allowing the animals to live in the apparatus enables them to prioritise their behaviour and choose the resources they want at different times in the day and allows them to choose how long to spend with each resource. Such studies have tended to use an obstacle between the animal and the resources which they have to overcome and which can be made more difficult or more aversive. Consideration has to be given to the fact that the obstacle chosen by humans as being most costly may not be perceived in the same way by the animal. By using near-natural obstacles, care has to be taken not to use obstacles which may be ones which the species would naturally come across during territory monitoring or exploration and may not be perceived as imposing a cost at all (Sherwin and Nicol, 1995).

One of the most commonly used obstacles is a weighted push-door, where the weight on the door can be increased to increase the cost imposed. This approach has been used for assessing the motivation of mink for resources such as hay, a water bath, a raised platform and enrichment objects (Cooper and Mason, 2000; Mason *et al.*, 2001), of rats for nest-boxes, nesting material and either a grid or solid floor (Manser *et al*, 1996, 1998) and of hens for perches (Olsson and Keeling, 2002) as well as measuring feeding motivation of hens (Petherick and Rutter, 1990) and sheep (Jackson *et al*, 1999). Other obstacles used include water baths (Sherwin, 1996; Sherwin and Nicol, 1996), an air-stream (Faure and Lagadic, 1994) and a narrow gap (Cooper and Appleby, 1993, 1997; Sherwin and Nicol, 1995).

There are several economic measures that can be made to assess the importance of resources. Two which were suggested by Dawkins (1983, 1988, 1990) were the elasticity of demand and the income elasticity. The elasticity of demand for a resource is the change in consumption or usage of a resource when made more costly (Mason *et al.*, 1998). The income elasticity concept involves manipulating the subject's income, thus altering the relative rather than the absolute price of resources (Mason *et al.*, 1997). Ng (1990) and Houston (1997) questioned the validity of the elasticity of demand as a measure and recommended the use of the consumer surplus. This has recently been supported by Kirkden *et al.* (in press). The consumer surplus is obtained by plotting the number of times the resource is obtained against the cost of obtaining the resource, and calculating the area under the curve. Further measures which can be used are the maximum price paid for a resource, which can readily be used in experiments where the animal has control over how long it chooses to spend with each resource (Mason *et al.*, 1998) and the total expenditure for a resource in a given unit of time.

1.4.2.4 Behavioural assessment of the preferences of rabbits

Little is known about the memory and learning capabilities of the rabbit. However, the main defence mechanism of the wild rabbit is to run for cover, therefore they must be capable of memorising the location of the burrows or bolt-holes. Wild rabbits have also been found to learn quickly to avoid electric fences used to protect crops (McKillip *et al*, 1993; McKillop and Wilson, 1999). Laboratory rabbits have been trained to use a treadmill to promote regular exercise in orthopaedics research (Oyen-Tiesma *et al.*, 1998), however, there have been few operant studies carried out on rabbits. One such study attempted to determine floor space preferences of farmed rabbits (Kienle and Bessei, 1993) and in another, rabbits were trained to operate a lever to receive a squirt of sucrose in an investigation into Pavlovian-instrumental interactions (Lovibond, 1983). Most of the studies on laboratory rabbit housing and welfare have therefore been based on cage observations and as yet little evidence is available to indicate that rabbits can be successfully trained to perform tasks in order to assess the importance of resources.

1.5 Conclusion

Male laboratory rabbits are always housed singly due to high levels of aggressive behaviour shown when housed socially. Female laboratory rabbits may be housed either singly or socially and although it is recommended that they are socially housed it is not always possible to house them in this way. Factors such as the nature of the experiments, space and money have to be taken into account as well as the needs of the animals. The method of housing used can affect the behavioural repertoire of the rabbits as well as affecting aspects of health such as respiration and bone strength. Comparisons of the behaviour of wild and domestic rabbits have found that despite hundreds of years of domestication they show similar behaviour in terms of feeding and resting patterns, social, reproductive and maintenance behaviour and responses to predators. These behavioural characteristics are likely to affect how domestic rabbits adapt to different types of housing. The suitability of an animal's environment can be assessed using behavioural measures to determine how motivated they are to gain a resource or to perform a behaviour. An animal may suffer in an environment that does not allow it to gain a resource of perform a behaviour that it shows a strong motivation for.

1.6 Aim and research outline

The aim of this research was to investigate laboratory rabbit housing, identify resources that may be important to rabbits and assess the motivation of rabbits for these resources in order to make recommendations for the housing of laboratory rabbits.

In order to determine which aspects of laboratory rabbit housing to investigate, it was important to gain up-to-date information regarding the way in which rabbits are housed within the laboratory industry. This was carried out by sending a questionnaire to the main pharmaceutical companies in the UK and visiting several sites to carry out home cage/pen observations of laboratory rabbits housed singly and in groups, in cages and in floor pens. The findings of the questionnaires and analysis of video observations, along with information gathered from discussions with named veterinary surgeons, laboratory technicians and laboratory rabbit breeders, were used to determine the main concerns of laboratory rabbit housing and therefore the areas which the research would focus on (Chapter 2). Following a thorough investigation of the literature on determining preferences and strengths of preferences of animals, a method for measuring motivation of laboratory rabbits for resources was devised and tested (Chapter 3). This approach of short-term tests was then used to measure the motivation of singly and socially caged rabbits for one of the aspects of housing raised as an area of concern in Chapter 2, namely social contact with a conspecific (Chapters 4 and 5 respectively). The approach devised in Chapter 3 and used in Chapters 4 and 5 was then adapted to carry out a longer term closed economy experiment to test the motivation of laboratory rabbits for social contact with a conspecific, access to a platform, food and an empty space (Chapter 6) and to rank these resources in order of importance. Cage observations were then carried out to determine the use of platforms within laboratory cages and the effects of social contact on platform use (Chapter 7). The findings of the experiments carried out were then discussed in terms of the implications for the welfare of rabbits in housing types currently used within the laboratory industry and practical recommendations were made regarding laboratory rabbit housing which, from the findings of the research, are likely to improve laboratory rabbit welfare (Chapter 8).

Chapter 2 – Current situation within the UK pharmaceutical industry

Abstract

In order to decide which aspects of laboratory rabbit housing and welfare to investigate a questionnaire was sent to the members of the Pharmaceutical Housing and Husbandry Steering Committee (PHHSC) which included the main pharmaceutical companies within the UK. Information collected included the breeds and numbers of rabbits used (based on the previous year, 1998), how they were housed and factors affecting the type of housing used. It was found that over 90% of the rabbits used were New Zealand White and that more than two thirds of all rabbits used were female. Male rabbits were always housed singly due to problems with severe aggression, however females were housed either in single cages, in small groups in social cages or in groups in floor pens. Often the nature of the experiments and their associated constraints were the determining factors in how female rabbits were housed. Based on the questionnaires, five companies were chosen to visit to observe female New Zealand White rabbits in each type of housing. Video analysis highlighted the factors that had most influence on rabbit behaviour and this was used to determine the aspects of housing that required further investigation, along with further information gained from discussions with the PHHSC members, laboratory technicians and the two main laboratory rabbit suppliers in the UK. Similar concerns regarding laboratory rabbit housing were raised from all sources of information, with the main issues being the provision of visual and physical contact with conspecifics and the physical environment, particularly the provision of platforms within cages.

2.1 Introduction

Research has been carried out to investigate the effects of various enrichment devices on laboratory rabbit behaviour (e.g. Brooks *et al.*, 1993; Hansen and Berthelsen, 2000; Lidfors, 1997) as well as the success and effects of social enrichment (e.g. Held *et al.*, 1995; Huls *et al.*, 1991; Whary *et al.*, 1993). Research into social enrichment has mainly focused on female rabbits, as males are notoriously difficult to house socially due to aggressive behaviour (e.g. Bigler and Oester, 1996), and problems with aggression have even been found with castrated male rabbits (Raje and Stewart, 1997). Studies carried out on housing of laboratory rabbits have generally had the aim of determining the behaviour of the rabbits in the different types of housing and the advantages and potential problems associated with each of them (e.g. Batchelor, 1991; Gunn and Morton, 1995; Gunn-Dore, 1997; Podberscek *et al.*, 1991). There has however been little focus on how the findings relate to the industry in terms of the number of rabbits housed in the different ways, and the practicalities of the different housing types in relation to the constraints of the procedures being carried out.

Studies have shown that previous experience can have a significant effect on how an animal reacts to situations later in life and this has been found in many domestic species (e.g. domestic chicks - Jones and Waddington, 1992; cattle - Boissy and Bouissou, 1988; sheep - Romeyer and Bouissou, 1992; and cats - McCune, 1995), including rabbits (Jezierski and Konecka, 1996; Kersten *et al.*, 1989; Podberscek *et al.*, 1991; Pongracz *et al.*, 2001). This may have implications on how rabbits adapt to changing from one type of housing to another, either when moving from the breeder to the laboratory or for experimental reasons within the laboratory. Research carried out on laboratory rabbit housing has tended to overlook this.

In order to determine the aspects of laboratory rabbit housing that might be most problematic for the rabbits, knowledge of the methods currently used to house rabbits within the pharmaceutical industry was required. To achieve this the main pharmaceutical companies in the UK were consulted to gain up-to-date facts and figures regarding numbers of rabbits used, breed, sex, experimental uses and types of housing. The gathering of information was carried out in three parts:

Part 1 – a questionnaire was composed and sent to chief technicians and/or named veterinary surgeons within eleven pharmaceutical companies to gain background information

Part 2 – on analysis of the questionnaires, selected companies were visited to video rabbit behaviour in different types of housing

Part 3 – further information was gained from discussions with technicians during the laboratory visits and from members of the funding committee (PHHSC – see section 2.2) during meetings and via e-mail; information on housing used by laboratory rabbit breeders was gained by visiting one of the main breeders in the UK and telephoning the other

Part 1 – Questionnaire

2.2 Methods

A questionnaire (Appendix 1) was designed and sent by post to eleven members of the Pharmaceutical Housing and Husbandry Steering Committee (PHHSC), which composed of the main pharmaceutical companies in the UK at the time (Astra Charnwood, Glaxo Wellcome Research and Development, Hoechst Roussel Vet, Merck Sharpe Dohme, (NIMS) Novartis, Pfizer, Rhône-Poulenc, Roche Products Ltd, Sanofi Research, SmithKline Beecham and Zeneca Pharmaceuticals). Both open and closed questions were used to gather information from the previous year (1998) on the rabbits used (e.g. numbers, breeds), the source of the rabbits, how they were housed, any perceived problems with particular housing methods, type and duration of experiments and any constraints of experiments which may affect how the rabbits are housed. The questionnaire used by the Toxicology and Welfare Working Group (Hubrecht, 1995) was used as a basis for the questionnaire, with modifications made in order to gather additional information. The responses from the questionnaires were analysed using descriptive statistics.

2.3 Results

Of the eleven questionnaires sent out, all were returned although only seven were fully completed as four of the companies had not housed rabbits within the previous year and had housed few in the past. Questionnaires were filled out either by chief technicians or the named veterinary surgeon, or both. As it was important that the information was as up-to-date as possible, only the information from the seven companies that had housed rabbits in the previous year was analysed. The following information was gathered.

2.3.1 Characteristics of the rabbits

Breeds and numbers used

The breeds and numbers of rabbits used are shown in Table 2.1. The total number of rabbits held on site at any one time ranged from 12 to 500, with a mean of 178 (SD ± 163). Of the total number of rabbits used 69% were female and 31% male.

Breed	Number used Total number	 Mean (SD)	 Range	Percentage of total rabbits
New Zealand White (NZW)	4010	573 (SD ± 497)	100-1500	91
Dutch	250	125 (SD ± 106)	50-200	6
Other (e.g. Half lop)	158	53 (SD ± 46)	8-100	3

Table 2.1 Questionnaire results on breeds and numbers used giving the mean, standard deviation (SD), range and percentage of total rabbits for each breed

Reasons given for using the NZW included i) the consistency of the quality of the animals, ii) strains are well established with extensive background data on pharmacology, iii) larger breeds makes obtaining blood samples easier due to their large ear veins and iv) large breeds of rabbits have the reputation of being more docile than some of the smaller breeds. Reasons given for using the Dutch rabbit included i) extensive background data, ii) the pigmentation and iii) when the use of large rabbits is not of any benefit to the type of experiment being undertaken, using the smaller Dutch rabbit means that smaller cages can be used.

Source

Of the two main suppliers of laboratory rabbits in the UK, one was used by seven companies and the other by five (i.e. some companies used both). Six of the companies knew the housing systems used by the breeders and one did not. When asked if they were able to specify to the breeder what housing system they wanted the rabbits to have been raised in, four companies said they were unable to specify, one said they did not know and one said sometimes, if they ordered the rabbits far enough in advance. One company did not answer the question.

2.3.2 Housing

The information on all of the rabbits in all of the seven companies was grouped together to calculate the percentage of rabbits housed in each type of housing (Table 2.2).

Sex	**Housing type**	**Percentage**
Female	Single cages	46
	Social cages	12
	Social floor pens	42
Male	Single cages	82
	Single floor pens	18 (one company)

Table 2.2 Percentage of female and male rabbits housed in various types of housing

Reasons given for singly housing female rabbits included i) aggression during group housing (especially towards newly introduced individuals), ii) limited space and iii) experimental reasons. The experimental reasons given included studies involving topical drug administration fast turnover of animals in tissue harvesting which would disrupt social groups, the possibility of socially housed rabbits being stressed and absorbing their foetuses in reproductive toxicology studies, and to enable food and water consumption to be monitored in metabolism studies. The only reason given for singly housing male rabbits was aggression in social housing, even within sibling groups.

Cages

Six companies housed rabbits in single cages of seven different dimensions. The floor area ranged from 3000cm^2 to 5950cm^2 with the mean area being 4642cm^2 (SD $\pm$ 1009). The height of the cages used ranged from 47cm to 52cm with a mean of 48cm ($\pm$ 3). Two companies housed rabbits in social cages. One housed groups of three rabbits in cages with a floor area of 7688cm^2 and height of 52cm, giving a floor area of 2563cm^2 per rabbit. The other company using social cages did not specify the cage dimensions or group sizes.

Of the six companies that singly caged rabbits, four used cages that allowed visual contact between rabbits in adjacent cages. All six companies had cages positioned facing each other, one to two metres apart (mean 1.7m ± 0.4). None of the cages used allowed any physical contact between rabbits in adjacent cages.

Floor pens

Five companies housed rabbits in floor pens, each using pens with different floor area and different group sizes. One company housed rabbits in single floor pens of 8492cm². The mean group size of socially penned rabbits was eleven (SD ± 12.8) with a mean floor area of 4379cm² (± 1703) per rabbit.

Three of the five companies that housed rabbits in pens stated that rabbits were never introduced into an already established group. This happened only occasionally in the other two companies and attempts were made to reduce aggression by cleaning and disinfecting the pen first and by housing the new rabbit in a mesh isolation cage within the pen for two days. Attempts were made to match the rabbits for age and weight to try to increase their compatibility.

2.3.3 Husbandry

The husbandry for caged and floor penned rabbits was very similar.

Diet

All the rabbits were fed a pelleted diet, and almost all were fed hay (although one company stated only 'occasionally'). Two companies fed fruit/vegetables and one occasionally fed pet rabbit food to individuals with low food intake. Most of the rabbits were fed *ad libitum*. One company stated that the feeding regime depended on the type of study the rabbits were being used in.

Light intensity and regime

Light intensity ranged from 100 lux to 400 lux (mean 263, SD ± 95). The typical light regime was a 12 hour light:12 hour dark cycle. One company used a dawn/dusk facility with lights coming on/going off over a two minute period.

Platforms and bolt-holes

Five of the six companies that singly housed rabbits and all five of those that socially housed rabbits provided them with a platform/bolt-hole.

Enrichment

All rabbits were provided with some form of environmental enrichment regardless of whether they were housed singly or socially, in cages or in floor pens. These were listed as hay, observation ports, platforms, chew sticks (wood, grass), plastic tubing, wooden blocks, food supplements, '*Jingle balls*', '*Kong toys*', a metal ring clipped onto a bar of the cage door, a radio playing in the background and access to an exercise pen on a rotational basis. Deep litter bedding, cardboard boxes, tunnels, empty paper sacks, tree logs and hutches were also listed as enrichment for floor penned rabbits. Enrichment was usually continued throughout experiments.

2.3.4 Behaviour of rabbits in cages

Abnormal behaviours

Abnormal behaviours are reported in Table 2.3. Data are from the six companies that housed rabbits in cages and are based on observations during normal working hours, as none of the rabbits had been observed during the hours of darkness.

One company claimed that none of the behaviours in Table 2.3 were ever seen and another reported that they occurred in such small numbers (if at all) that it was difficult to state what percentage of their rabbits showed the behaviours. Hair chewing/plucking was shown mainly by pregnant females, which suggests that this was most likely to be part of nesting behaviour rather than an abnormal behaviour. Nose-sliding and kicking walls were reported to be shown only by male rabbits, however, kicking walls was shown when the rabbits were startled and was therefore not likely to be an abnormal behaviour. An additional behaviour reported by one company was scent spraying, but the percentage of rabbits showing this was not stated. None of the rabbits had been seen weaving. Few companies responded when asked at what times of the day they thought abnormal behaviours were likely to be most frequent, and the responses received were i) the start of the working day and ii) when there are no technicians in the room (as seen through an observation window in the door into the room).

Behaviour	Percentage of rabbits showing behaviour		
	Mean	SD	Range
Hair chewing/plucking	1.83	± 4.02	0-10
Circling	0.17	± 0.41	0-1
Nose-sliding (between bars)	1.67	± 4.08	0-10
Biting (bars, hoppers)	23.60	± 33.60	0-75
Kicking walls	10.00	± 20.00	0-50
Digging	14.5	± 29.90	0-75

Table 2.3 Mean, standard deviation (SD) and range of the percentage of rabbits showing behaviours previously seen in caged laboratory rabbits (e.g. Gunn and Morton, 1995; Lidfors, 1997)

Steps taken to reduce abnormal behaviours were reported as i) enrichment such as chew sticks (to reduce cage biting) and background music, ii) increased cage sizes, iii) platforms, iv) perspex partitioning, v) access to a floor pen and vi) increased handling. The one company that floor penned any singly housed rabbits did not observe any abnormal behaviour and believed that this was due to their system of housing.

2.3.5 Injury and disease

Overall, few injuries were reported. Bites and scratches were reported by three of the four companies that group housed rabbits in floor pens, in 2, 15 and 20% of rabbits. One floor penned rabbit suffered a broken back and three broke a leg. Coccidiosis and mucoid enteritis were reported in floor pens. The two companies socially caging rabbits reported that 10 and 20% of rabbits suffered from bites and scratches. One company reported that 2% of rabbits in cages suffered from hairballs. None of the rabbits suffered from sore hocks. A small number of cases of malocclusion were found in rabbits all three types of housing.

2.3.6 Type and duration of experiments

Rabbits were reported to be used for a variety of studies which were listed as tissue supply for cardiovascular work, antibody production, *in vivo* electrophysiology, reproductive toxicology, arthritis (knee-joint) model, vaccine potency tests and metabolism studies. The duration of experiments ranged greatly depending on the type of study, varying from one day (terminal experiments) to eight months.

The percentage of experiments requiring single housing ranged from 0 to 100% (mean 64%, SD ± 38). The duration of these experiments varied greatly with a mean of approximately six weeks.

Other constraints of experiments which could affect future design of housing systems were listed as requirements for topical drug administration, ease of frequent catching of animals, prevention of ingestion of non-dietary material, observational reasons, surgical modification and the use of radiolabelled compounds where only stainless steel cages could be used due to difficulties removing radiolabelled waste from plastic cages.

2.3.7 Additional comments

Only one additional comment was made, which was that the use of platforms in cages was thought to be the most useful form of enrichment.

2.4 Discussion

The questionnaire responses showed the most commonly used breed of rabbit in the pharmaceutical industry to be the New Zealand White, accounting for more than 90% of the total number of rabbits used. This was partly for experimental reasons such as the fact that their large ear veins makes the procedure of blood collection easier and partly due to their docile temperament making them easy to handle. There was found to be great variation within the industry in the number of rabbits used per year and the number held on site at any one time. Large variation was also found in the types of experiments rabbits were used for and in the duration of experiments.

Within the pharmaceutical industry male rabbits were always housed singly due to aggressive behaviour, which has also been found to be the case in other studies into housing of male rabbits (e.g. Bigler and Oester, 1996). The nature and duration of experiments were significant factors in determining how female rabbits were housed. Constraints of experiments, such as the fast turnover of rabbits due to tissue harvesting which would disrupt social groups, may mean that rabbits have to be singly housed. Rabbits may also have to be singly housed due the potential effects social housing may have on experiments, for example, stress may cause pregnant does to absorb their foetuses and females that are socially housed may be induced into false pregnancy (Huls *et al.*, 1991). These may be risks that cannot be taken in reproductive studies.

It was reported in the questionnaires that aggressive behaviour had been found between group housed female rabbits, causing bites and scratches to a number of individuals. It was said that in severe cases, rabbits may have to be removed from the group and one company reported having tried group housing in the past but changed back to single housing due to aggressive behaviour. It was also reported that when individuals were introduced into grouped together, attempts were made to match them for age and weight to increase compatibility. It has, however, been found in other species that social housing can be more successful if the animals differ in size. For example, pigs have been found to be less aggressive if the individuals within the group differ in weight (e.g. Rushen, 1987; Andersen *et al.*, 2000). It is possible that varying the age and size of rabbits within a group may reduce the level of aggression, however, it may be important that the rabbits within an experiment are of similar body weight, making this impractical.

Many studies have, however, been carried out into social housing of female rabbits (in groups of up to ten rabbits) and have found that female rabbits can often be successfully housed together once the hierarchy has been established and the group is stable (e.g. Stauffacher, 1986; Huls *et al.*, 1991; Love and Hammond, 1991). It has also been found that a social system for breeding does can be successfully established if carefully designed (Stauffacher, 1992). It is often assumed that it is the most subordinate animals in a group that are likely to suffer most from social stress and that social housing is therefore not acceptable due to the stress imposed on these individuals. It may, however, be that subordinates are able to avoid social stress whereas the dominant animal has to initiate interactions in order to maintain its status. A study into social stress of wild populations of dwarf mongoose and wild dogs found that glucocorticoid levels were higher in dominant individuals than in subordinates (Creel *et al.*, 1996). If this were also true of rabbits, social stress would have an effect on more than just the lowest ranking rabbits. Whary *et al.* (1993) found however that group housed rabbits did not differ significantly from singly housed rabbits in either physiological or immunological measures, hence the practical research performance of these rabbits (e.g. immune response, stress level, growth rates) was not significantly affected by their housing. Turner *et al.* (1997) similarly found that group housed does were suitable for raising antisera, one of the most common uses of laboratory rabbits.

The occurrence of abnormal or stereotypic behaviours was reported as being very low. It was noted that this was based on observations of the rabbits during normal working hours, when the lights were on. It has been found, however, that stereotypic behaviours in rabbits occur mainly at night, during the hours of darkness (Gunn, 1994). It may therefore be that the occurrence of abnormal behaviours reported in the questionnaires was misleadingly low.

Part 2 – Laboratory observations

2.5 Methods

The responses from the questionnaires were used to select five pharmaceutical companies to visit to observe rabbits in the different types of housing used within the UK pharmaceutical industry.

2.5.1 Subjects

It was decided from the responses of the questionnaires that only female New Zealand White rabbits would be observed, as the NZW was the predominant breed used and more than two thirds of the rabbits used were female. Focusing on these animals would make the findings of the observations applicable to as large a proportion of the rabbits used in the industry as possible. A total of 73 rabbits were filmed in single cages, social cages and group floor pens (Table 2.4). The social cages at Site 3 allowed the rabbits to gain access to two single cages via a small doorway at the height of the platforms. All of the rabbits were exposed to similar husbandry procedures (e.g. diet, feeding regime, light schedule) and all were provided with some form of environmental enrichment.

2.5.2 Protocol

Filming was carried out using a time-lapse video recorder (Panasonic AG-6024), a quad unit (Panasonic WJ-410) and up to four CCTV cameras (Panasonic WV-BP330/B), depending on the number of cages or size of the floor pen being filmed. Infra-red lights were used for recording at night. At each site the behaviour of the rabbits was initially recorded for a test period of approximately 24 hours. If, from watching the video tapes, the position of the cameras were found to be suitable and the lighting was sufficient for night-time observations the rabbits were filmed for a second 24 hour period. The initial 24 hour test period also served as a familiarisation period to allow the rabbits to habituate to the sight of the equipment and to ensure that the equipment was not positioned in a way which would interfere with the daily routine of the technicians.

Site		**1**	**2**	**3**	**4**	**5**
Single cages (17 rabbits)	Number Area (cm²) Height (cm) Platform	0	3 rabbits 3040 50 No	4 rabbits 5950 47 No	4 rabbits 5400 50 Yes	6 rabbits 4500 47 No
Social cages (6 rabbits)	Group size No. groups Area (cm²) Height (cm) Platforms	0	0	3 rabbits 2 7688 52 Yes	0	0
Floor Pens (50 rabbits)	Group size No. groups Area (cm²) Platforms	16 rabbits 2 120000 Yes	0	4 rabbits 2 14356 Yes	0	10 rabbits 1 30600 Yes

Table 2.4 Numbers of rabbits and housing at the five sites visited to film rabbit behaviour

2.5.3 Analysis

The behaviour of all of the 73 rabbits in cages and floor pens was recorded using instantaneous scan sampling (Martin and Bateson, 1993) every five minutes and recording the behaviours listed in Table 2.5.

Behaviour	**Description**
'Active'	
Ambulation	Moving around cage/pen, normal locomotary action not possible in cage
Chew/Lick/Manip Environment	Gnawing, biting, pulling or licking the environment e.g. walls, food hopper, water bottle, grid floor, cage bars
Chew/Lick/Manip Enrichment	Gnawing, biting, pulling or manipulating the sources of enrichment e.g. wooden blocks, hay, metal rings
Explore	Olfactory investigation of the environment
Hop	Rapid circling or leaping, shaking/twisting of body, flicking of head
'Resting'	
Lying alert	Lying down with eyes wide open, responding to the environment whilst relatively inactive
Sitting down	Differs from lying alert in that the forelimbs are tucked under the body
Sitting up	Forelimbs are straightened so the thorax and abdomen are clear of the floor, all paws remain on the floor
Stretched/sleeping	Lying stretched out, eyes may be closed, no other behaviours shown
'Grooming'	
Wash/Groom	Using the tongue or teeth on the fur, licking the forepaws, using the forepaws to wash face or ears
Allogroom	Washing/grooming another individual
'Platform/Bolt-hole'	
Platform	On raised area – platform/shelf/box etc
Bolt-hole	Hiding in an enclosed area e.g. under a platform, in a box
'Eat/Drink'	
Eat	Eating pelleted food from food hopper
Drink	Drinking from water bottle
'Other'	
Bar-biting	Gnawing/biting cage bars in a repetitive action
Chin marking	Rubbing the chin over the floor, walls, bars, cage/pen furniture
Paw/Dig	Corners of the cage/pen vigorously pawed at in a digging action
Rear	Both forepaws clear the floor (not including during washing)
Stretching	Forward extension of front paws with head tipped back

Table 2.5 Ethogram of rabbit behaviour, adapted from Gunn (1994)

The frequencies of behaviours were calculated and compared within and between different types of housing. Only the second 24 hour period at each site was analysed. The videos were analysed using The Observer software (Noldus Information Technology, 1994). Due to filming being carried out within different organisations, the type of housing system was not the only variable involved. Slight differences in husbandry (e.g. cleaning, handling, feeding times) as well as, for example, the number of rabbits in the room and position of cages/pens within the room may have had some effect, therefore statistical analysis was not carried out on the data.

2.6 Results

Figure 2.1 shows the mean daily time budgets for rabbits in single cages with and without a platform, in social cages and in floor pens (in groups of four, ten and sixteen rabbits).

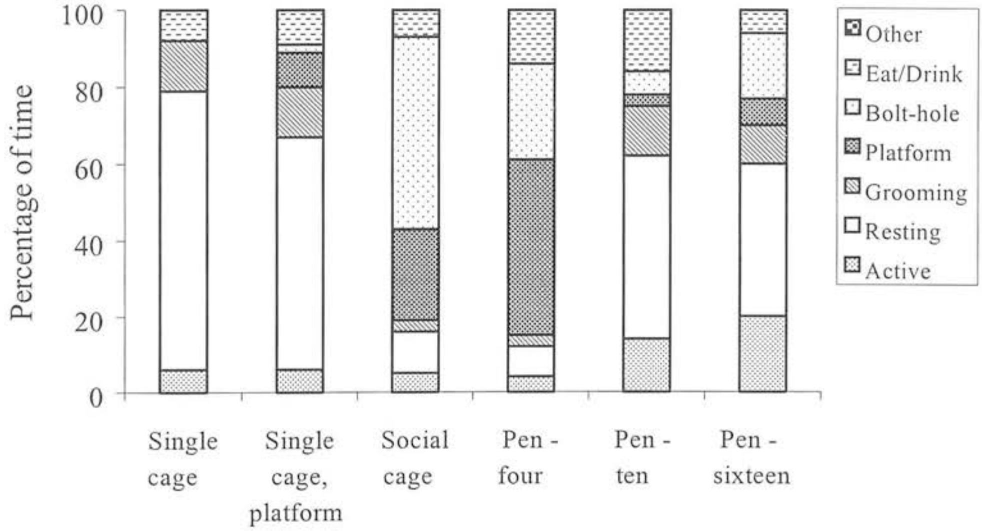

Figure 2.1 Mean time budget for rabbits housed in single cages without a platform, single cages with a platform, social cages and groups of four, ten and sixteen rabbits

2.6.1 Behaviour in single cages of different dimensions, without platforms

The behaviour of the singly caged rabbits at Sites 2, 3 and 5 (see Table 2.4) was compared to investigate whether or not the floor area of the cages had an effect on the frequency of behaviours shown. The cages at Site 2 ($3040cm^2$) were termed 'small', those at Site 5 (4500cm²) were termed 'medium' and the cages at Site 3 (5950cm²) were referred to as 'large'. In all three cage sizes the largest proportion of time was spent sitting down (small: 40%, medium: 36%, large: 41%), with only a small percentage of time spent ambulating (3%, 4%, 3%), sitting up (2%, 3%, 3%), stretched out (3%, 1%, 1%) and exploring (all 2%). The time spent lying alert increased with increasing cage size (11%, 23%, 28%). Rabbits in the small and large cages were provided with environmental enrichment in the form of wooden blocks and hay, and the rabbits in the medium cages were provided with a metal ring attached to one of the cage bars. Time spent chewing/licking/manipulating the enrichment objects decreased with increasing cage size (12%, 4%, 1%). Chewing/licking/manipulating the environment was only shown by two rabbits in the medium cages. Time spent eating and drinking was lower in the small and large cages than in the medium cages (10%, 15%, 8%). The first bar in Figure 2.1 ('Single cage') shows the mean time budget for the rabbits in the large cages.

2.6.2 Behaviour in social cages and floor pens with similar group sizes

Comparisons were made between behaviour of rabbits in social cages in groups of three (2992cm² per rabbit) and rabbits in floor pens in groups of four (3589cm² per rabbit), both of which were at Site 3 (see Table 2.4). The mean daily time budget for rabbits in both types of housing are shown as the third and fourth bars in Figure 2.1. The rabbits in the floor pens spent almost twice as much time on the raised platform areas compared to the rabbits in the social cages (46% and 24% respectively) and half as much time in the bolt-hole (25% and 50% respectively). None of the rabbits was seen to stretch out or allogroom in the social cages although both these behaviours were observed in the floor pens (each made up 1% of the total time). In the social cages a dominant rabbit was able to lie on the platform next to the doorway between cages and prevent the other rabbits from moving between cages. Such defending of space was not found in the floor pens as the pens were one continuous area and the platform was large enough for all of the rabbits to lie on it at once.

2.6.3 Behaviour in floor pens with different group sizes

Behaviours in groups of four, ten and sixteen rabbits were compared (Sites 3, 5 and 1). The mean daily time budgets for these three group sizes are shown in Figure 2.1 as the fourth, fifth and sixth columns respectively. The groups of four rabbits were provided with one large hutch as a platform, which covered approximately half of the floor area and allowed all rabbits to be on the raised area at the same time. The groups of ten and sixteen rabbits were provided with several small platforms that were only big enough for one or two rabbits and were not provided with enough platforms to allow all the rabbits to be on raised areas at the same time, allowing dominant individuals to defend these areas. Casual observations showed that in the groups of four, the rabbits spent much of the time huddled together on the top of the platform or all underneath the platform in the bolt-hole. In the group of ten, often five or six rabbits would lie together on the floor and on several occasions three rabbits shared a bolt-hole. In the groups of sixteen the rabbits were more widely distributed throughout the pen and tended to stay round the edges of the pen. The amount of ambulation increased with the increase in group size (groups of four=3%, group of ten=7%, groups of sixteen=15%) as did the amount of time sitting up (2%, 7%, 7%) and lying alert (1%, 11%, 12%) in front of the platform. The time spent sitting down was highest in the group of ten rabbits (4%, 27%, 19%). This was also seen in the time spent stretched out (1%, 3%, 2%), washing/grooming (2%, 13%, 10%) and exploring (1%, 6%, 3%). The time spent on the platform and in the bolt-hole were highest in the groups of four rabbits (Platform - 46%, 3%, 7%; Bolt-hole - 25%, 6%, 17%).

The lighting regime for the groups of sixteen rabbits included a dawn/dusk period, with lights coming on and going off gradually over a two minute period. Compared to the other group housed rabbits this group showed an obvious, yet gradual increase in activity at the onset of the dusk period whereas this was more delayed and then sudden when the lights went from off to fully on and vice versa.

2.6.4 Behaviour in cages with and without a platform

The rabbits in the single cages with and without platforms showed similar mean daily time budgets in terms of the time spent grooming, eating and drinking and being active (Figure 2.1, first and second bars). Although the rabbits in the cages with platforms were able to lie on the raised area and underneath it in a darkened area, this was shown for only a relatively small percentage of the daily time budget (9% and 2% respectively).

Time spent at the front and back of cage when there was a platform along one side of the cage (Site 4) was compared to a similar sized cage with no platform (Site 3). The platform gave the rabbits the opportunity to spend time on the floor at either the front or back of the cage and on the platform at either the front or back of the cage. The rabbits spent most of their time at the front of the cage (96%), both on the platform and on the floor compared to at the back of the cage (4%). In the absence of a platform, rabbits spent on average 78% of their time and only 22% at the back, regardless of whether or not there were rabbits in cages or pens opposite them. When platforms were positioned along the full length at the back of the cages, the rabbits spent on average only 15% of their time at the front of the cage and 85% at the back of the cage. The only cages with platforms positioned this way were those at Site 3 which were social cages, therefore social factors may have had an effect on platform use.

The single cages with platforms (Site 4) and the social cages with platforms (Site 3) were different heights (64cm and 47cm respectively). In the single cages there was more height above the platform as well as below it. Comparisons were made of the behaviours shown in the two types of cages, although in the lower cages the behaviour of the other rabbits may have had an influence. More time was spent on the platforms in the lower cages compared to the higher cages (31% and 10% respectively). Rabbits in the higher cages spent less of the time on the platform sitting down than they did in the lower cages (14% and 51% respectively), less time ambulating (2% and 6% respectively) and were less exploratory (4% and 10% respectively). They were also seen to rear, which was not shown in the lower cages and spent more time sitting up (26%, 11%) and washing/grooming (17%, 7%) in the higher cages than in the lower ones.

2.6.5 Abnormal behaviour

Abnormal behaviour, in the form of bar-biting, was shown by 41% of the singly caged rabbits, however each of these rabbits showed this less than 1% of the time. This behaviour tended to occur within the first two and last two hours of darkness. Pawing/digging was shown on a few occasions in the group of ten rabbits and by one singly caged rabbit, on the platform. The rabbits in the pens did however have a substrate to dig in which the caged rabbits did not have. When rabbits were manipulating the water bottle it was unclear on some occasions whether they were actually drinking or repeatedly biting the water bottle and on these occasions the rabbits were recorded as drinking. Although no repetitive biting of the water bottle was recorded, it may have occurred.

2.7 Discussion

Although the data could not be analysed statistically, it was apparent from the descriptive statistics that there were differences in the frequency of performance of certain behaviours that could be attributed to the type of housing. Rabbits in single cages with no platform spent a large proportion of time sitting down and spent little time ambulating or showing exploratory behaviour. They also spent little time fully stretched out regardless of the size of the cage, although the time spent lying down ('lying alert') increased as the cage size increased. From the discussions with technicians during laboratory visits (and responses in the questionnaires) it became apparent that hay tended to be provided to the rabbits as a form of environmental enrichment rather than as part of their diet. Hay has been found to reduce abnormal behaviours such as bar gnawing and excessive grooming (Lidfors, 1997; Berthelsen and Hansen, 1999), therefore when rabbits were seen manipulating hay during the behavioural observations it was recorded as 'chewing/licking enrichment objects' rather than as eating. The rabbits in the medium sized cages were not provided with hay and the time spent eating was highest in these cages. It would appear that when the rabbits in the smallest and largest cages were observed to be manipulating hay they may have actually been eating it (this was unclear from video tapes), and as hay was not available as part of the diet for the rabbits in the medium cages they may have been eating more pelleted food. The higher performance of eating and drinking in the medium cages could also have been due to the fact that when an individual was observed manipulating the water hopper this was recorded as drinking, although it was not obvious from the video tapes whether they were actually drinking or repeatedly biting the water hopper as it appeared on several occasions (which would have been recorded as 'chewing/licking environment'). It is possible that the lack of hay to manipulate in the medium sized cages caused these rabbits to direct oral behaviour towards the water hopper. It is also possible however, that the rabbits were actually drinking more, due to the increased dry pelleted food they were eating compared to the rabbits in the other cages. It appears that it is not just the size of the cage that affects the behaviour of the rabbits but also the cage environment, which has been suggested by other authors in relation to laboratory animals (e.g. Bantin and Sanders, 1989).

When rabbits were housed in small groups in both cages and in pens they were found to spend little time ambulating or resting on the floor in front of the platform and spent three-quarters of their time either on or underneath the platform (in the bolt-hole). The rabbits in cages spent two thirds of this time under the platform in the bolt-hole and a third on the platform whereas the rabbits in the pens did the opposite, spending one third in the bolt-hole and two thirds on the platform. The difference found between the time spent on and under the platform between the two groups could be due to the fact that cages have a relatively low roof which does not allow the rabbits to perform their full behavioural repertoire whilst on the platform (e.g. they are unable to rear up). The pens did not have a roof, allowing any of the behaviours the rabbits performed on the floor of the pen to be performed on the platform. Another difference in the structure of the pens and cages was in the size of the platform. The platform in the pens was slightly wider than in the cages and as well as allowing all of the rabbits to lie on the platform at once, this may have allowed the rabbits to lie more stretched out. During the behavioural observations, lying stretched out was not observed in the cages but was seen in the floor pens.

Unlike a floor pen, which is one open area, the social cages consisted of two standard cages linked via a small door which was accessed from on top of the platform. The rabbits could only move through the door one at a time and it was observed that if a dominant rabbit was lying on the platform near to the door that a lower ranking rabbit may avoid moving into the next cage or even onto the platform in direct visual contact with the dominant rabbit. This could influence the use of the platform by lower ranking individuals and the confined space may have meant that these rabbits avoided aggressive interactions by hiding in the bolt-hole. The fact that dominant rabbits were able to defend areas of the social cages highlights the importance of food and water being provided in both halves of the cages.

Differences in group size also affected the behaviour of the rabbits. The more rabbits there were, the more dispersed they seemed to be within the pen, although pairs or trios of rabbits were seen to huddle together. In the wild, rabbits live in small groups within a larger colony (e.g. Bell, 1983) and it may be that in larger groups the rabbits are less capable of recognising individuals and maintaining social bonds due to having fewer chance interactions with each other as they move around the pen. In the larger groups, dominant rabbits were seen to defend preferred areas on top of platforms, however this increased territorial behaviour may have been due to there being insufficient raised areas for all rabbits rather than being due to the social structure. The lack of platforms/bolt-holes is likely to be the reason why the rabbits in the larger groups appeared to spend less time on platforms and in bolt-holes compared to the smaller groups, rather than there being an effect of group size. There was an increase in ambulation and time spent sitting up and lying alert as the group size (and therefore pen area) increased. The increased ambulation appeared to be as a result of increased interaction between individuals rather than as a result of the increased floor area. Rabbits may have to be more alert in larger groups due to the increased chance of aggressive encounters. This could be the reason for the increase in sitting up and lying alert, as while in these resting positions the rabbits tend to be fairly vigilant. In the group of four rabbits the time spent on the floor in front of the platform was low as all rabbits were able to lie on/under the platform at once, which explains why the proportion of time spent sitting down, stretched out, washing/grooming and exploring was low in these groups. The groups of ten and sixteen rabbits were perhaps more comparable as in both these groups there was a limited number of platforms. From casual observations of rabbits in different situations, sitting down and stretched out appear to be relaxed postures and these were seen more frequently in the group of ten rabbits than in the group of sixteen. This was also true of the time spent washing/grooming and exploring. The rabbits in the group of ten spent less time both on and under the platform than the rabbits in the larger group. These relaxed postures, the performance of maintenance behaviours and exploration indicate a more stable and more suitable environment, which suggests that ten rabbits is a more optimal group size than sixteen.

Platforms in cages were used when they were provided, as found previously in studies carried out into laboratory rabbit housing (e.g. Hansen and Berthelsen, 2000; Stauffacher, 1992). Rabbits in single cages with no platforms spent more time at the front of the cage than they did at the back, however when housed with a platform at the back of the cage, rabbits spent the majority of their time at the back of the cage. This perhaps indicates that the rabbits preferred to be at the front of the cage, however their preference for the front of the cage was overcome by their preference for the platform. As these rabbits were housed socially there could however have been some influence of the other rabbits. When the rabbits had a platform at the side of the cage they again spent most of their time at the front of the cage where they could also be on the platform, again highlighting their preference for the front of the cage rather than the back. Possible reasons for this preference are that the front of the cage may have given the rabbits a better view of the room and the technicians, there is likely to be more ventilation at the front of the cage and perhaps the rabbits receive more olfactory cues when at the front of the cage.

It was found that additional height above and below the platforms affected the behaviour of the rabbits. The rabbits spent more time on the lower platforms and spent more time ambulating and exploring the environment whilst on the lower platforms compared to the higher platforms. This indicates that the higher platforms were perhaps too high and the rabbits did not feel secure enough to move about on them. The rabbits with the higher platforms appeared to be hesitant when jumping from the shelf onto the floor and often paced up and down several times. This could either be due to the height or the fact that these platforms had a solid, smooth surface rather than being dimpled like the lower ones, causing some rabbits to slip while jumping off. On the higher platforms (with the increased height above them) the rabbits spent more time sitting up and washing/grooming than they did on the lower platforms and were seen to rear, which suggests that there was insufficient space above the platforms in the lower cages for these behaviours to be performed easily. Again, however, the behaviour of the rabbits in the cages with less space above the platforms could have been affected by the behaviour of the other rabbits in the cage.

The observations of the rabbits in cages and pens, as well as casual observations, indicated that housing rabbits socially and increasing the complexity of the physical environment with the provision of a platform increased the overall activity levels of the rabbits, with singly housed rabbits with no platform spending much of their time inactive.

Part 3 – Additional information

2.8 Sources of information

In addition to the information gathered from the questionnaires and observations of rabbits in different types of housing, information was gained from discussions with the Pharmaceutical Housing and Husbandry Steering Committee (PHHSC) at meetings and via e-mail. Most of the representatives of the pharmaceutical companies were either the named veterinary surgeons or worked in the veterinary services department. The views of technicians were gained during the visits to the laboratories to record rabbit behaviour. Of the two main laboratory rabbits breeders in the UK, one was visited for a tour of the facilities and the other was contacted by telephone.

2.9 Information gathered

Discussions with representatives of the industry (PHHSC and technicians) revealed that there were several practical concerns and constraints which had to be taken into account when making any modifications to current housing.

2.9.1 Pharmaceutical Housing and Husbandry Steering Committee

One of the main concerns of the committee was the social environment of the rabbits and how best to cater for their social requirements. Where possible, rabbits were group housed in an attempt to cater for their social needs, although constraints of experiments often meant that this was not possible. As well as physical contact, concerns of the social environment included the provision of visual contact when experimental constraints meant that the rabbits had to be singly housed.

In addition to the social environment of rabbits, concerns were also raised regarding the physical environment. This included the provision of platforms within cages. It is said that platforms were originally introduced into cages as a way of increasing the floor space of the cages to comply with regulations, which meant the laboratories did not have to buy new cages. Although the original function of platforms was therefore not to increase rabbit welfare this is how they are now perceived, as rabbits have been found to make regular use of them. The committee expressed an interest in knowing the actual importance of the platform to the rabbits. Another physical aspect of the cage raised as a potential area of concern was whether solid walls or open/grid walls are preferred by rabbits, which ties in with the concerns over allowing visual contact between rabbits. The provision of environmental enrichment was raised as being of concern and the need for simple, effective devices. The possible effects on the rabbits of ultrasound from equipment was also raised as a concern.

The committee members expressed a view that any suggested changes for rabbit housing should not be so extreme as to require that current housing be discarded, as allegedly, economic constraints would not permit this. Any changes made to cages would have to take into account the dimensions of automatic cage washers to allow these to continue being used.

2.9.2 Laboratory technicians

As the technicians are the ones dealing with the animals on a daily basis, they were found to be extremely interested in and concerned about the welfare of the animals in their care and had similar concerns to the PHHSC members. It was discovered that one of the reasons for using the Dutch rabbit as opposed to the NZW was that these smaller rabbits could be housed in old cages that were too small for NZW rabbits according to recent regulations. The technicians reported that they prefer to work around rabbits that are group housed rather than rabbits housed in single cages and therefore gain more job satisfaction. As well as their own preference for group housing they described the rabbits as being 'happier' in groups than when housed singly and as being more active and showing much more interest in their environment. Some of the arguments which tend to be given against group housing such as difficulty in identifying and catching rabbits and increased work of cleaning out floor pens were said to be insignificant and did not outweigh the benefits of group housing.

2.9.3 Laboratory rabbit breeders

Visiting one of the main laboratory rabbit breeders in the UK highlighted several important aspects of rabbit housing prior to shipment to customers. It was found that their breeding females (up to two years of age) were housed in cages which were equipped with a nest box for four weeks out of five and these were also used by the does as platforms. The rabbits were provided with several types of enrichment (hay, cardboard tubes, paper), and the company perceived the process of birthing, lactating and mothering as a form of enrichment. Litters were weaned at five weeks of age and the females gave birth again approximately seven days later. The stud males were always singly housed. The males were provided with similar enrichment items as the breeding females, and mating, which occurred once or twice per week, was viewed by the company as being a source of enrichment. Following weaning at five weeks of age, the 'growing stock' were housed at the breeders until up to 20 weeks of age (most were sold by 13 weeks). They were housed in 'commune' cages in litter groups or same sex groups for approximately two weeks, then housed singly in cages. The rabbits were handled weekly, were fed hay periodically and were provided with cardboard tubes. Although this was the 'traditional' way of rearing the rabbits ongoing trials of group housing were being undertaken. Trials carried out with pair housed males and females found that they could be housed up to 12 weeks of age (the age at which they are usually sold) without major problems, although urine staining was common with the males. The use of enrichment reduced the incidence of fighting, and the pair housing did not adversely affect normal weight gains. Due to the success of these trials the company decided to house rabbits in larger groups of 20 in floor pens equipped with several sources of enrichment and they found that "the activities in the pen indicate that when group housed and compatible the animals like to share accommodation". One other benefit of the group housing was said to be that it served as a form of enrichment for the staff as well as the rabbits.

The breeders have several constraints which have to be taken into account and which may effect how they are able to house the rabbits. They have found differences in temperament between different strains of rabbits meaning that some strains are less likely to be successfully group housed, as they have a history of fighting. Group housing rabbits has the potential for rabbits to be injured, for example, bites and scratches, and although these may be minor for the rabbits, it is usually not acceptable to clients to receive 'damaged' animals. The breeders have a large amount of housing available due to the large number of rabbits they produce and therefore any changes to their housing, for example, the introduction of cage furniture may prove to be extremely costly. The fact that the rabbits are raised under sterile, pathogen-free conditions limits the type of enrichment they can be provided with, i.e. only items that can be irradiated.

The other main laboratory rabbit supplier in the UK reported that their rabbits were all housed in cages. The kittens were weaned at six weeks of age and split into same sex groups of six rabbits and housed in 'stock cages'. The groups were thinned out as the rabbits grew until they were pair housed at approximately ten to eleven weeks of age. When asked if they thought the way in which they housed their rabbits could affect how they adapted to different types of housing in the laboratories the reply was that this had not been considered.

2.10 Discussion

The committee members, technicians and breeders expressed similar concerns about rabbit housing, although some of the constraints imposed on breeders may differ from those of the laboratories. The main concern was the social environment of the rabbits and the provision of both physical and visual contact. The physical environment, i.e. what the rabbits were provided with, was also raised as being of concern, particularly with respect to platforms and environmental enrichment objects.

As animals are housed in laboratories for a specific reason, it is important that the housing does not interfere to too great an extent with the practical aspects of the procedures carried out and this has to be taken into account, as well as the requirements of the animals. It is therefore important that any changes to housing do not create problems for the technicians and that they are not overly expensive as the industry may be reluctant to make such changes.

2.11 General discussion

There have been many studies carried out into the housing of laboratory rabbits and there are found to be advantages and disadvantages of each, several of which were also reported by the industry. Two of the main concerns of single cages are the lack of physical contact (and often visual contact) with conspecifics and the prevention of 'normal' locomotary behaviour. Rabbits housed in cages have been found to have a significantly smaller bone diameter and greater deformation before bone breakage compared to rabbits housed in pens, as well as lower bone weight and breaking strength (Martrenchar *et al.*, 2001). Other concerns include intestinal disorders, thought to be due to stress (Jackson, 1991), respiratory diseases caused by a build up of ammonia due to poor ventilation (Wolfensohn & Lloyd, 1994) and the performance of stereotypic behaviours (e.g. Gunn & Morton, 1995). Housing rabbits individually means that they can be more easily monitored and identified and it is often said that they are easier to catch than when housed in a social group. It may, however, be that rabbits are easier to catch in group pens than in single cages. Caged rabbits may become aggressive when cornered in the back of their cage by a technician. Rabbits in a group are likely to gather in bolt-holes and under platforms when approached by a technician, and may be less aggressive when picked up if they have been able to respond in the instinctive way, by bolting for cover.

Housing rabbits in a group obviously allows them to have social contact with conspecifics, which increases their behavioural repertoire (e.g. Heath and Stott, 1990; Batchelor, 1999) and allows them to have more space. Allowing rabbits to have social contact, however, also leads to the potential for aggressive behaviour and perhaps injuries (e.g. Batchelor, 1991; Love and Hammond, 1991). Social housing is most successful if the group remains stable. Many of the studies carried out on group housing and whether or not it can be successful have focused on small groups, however, rabbits may also be housed in larger groups in laboratories and at the breeders.

From observations of laboratory rabbits in various types of housing within an industry situation it appears that several factors influence behaviour. These include the presence/absence of social contact, the presence of a platform as well as the height of the platform and the height of the space above it, the cage/pen size, group size and the type of social housing (i.e. social cage versus floor pen). It was found that housing rabbits socially had a clear effect on behaviour compared to singly housed rabbits. Although female rabbits are often housed socially, it is not always possible to house them this way due to experimental constraints. Some designs of cages allow rabbits to have visual and perhaps minimal tactile contact with rabbits in adjacent cages, however it is not know if the rabbits can gain any of the benefits of full social contact through such limited contact and it is therefore not known whether this type of contact is of value to the rabbits. Additions to the physical environment such as platforms increase the overall complexity of the environment and were found to have clear behavioural effects.

The questionnaires and discussions with members of the laboratory industry produced a large amount of up-to-date information on laboratory rabbits, their experimental uses and how this affects how they can be housed. The types of experiments the rabbits are used for appears to be an important factor in how rabbits are housed, particularly in terms of whether or not they are housed singly or socially. The committee and technicians raised similar concerns to those highlighted from the behavioural observations, with the main issue being social contact and the physical environment, particularly in cages. Constraints of space and money are also issues that have to be taken into account. It is, however, becoming increasingly accepted that housing can affect the results of an experiment (e.g. Birke, 1988; Knight, 2001). Physiological responses to challenging situations, for example, increased hormone levels (see Chapter 1, section *1.4.1*) may have an adverse effect on results. The requirements of the animals should perhaps, therefore, take priority in determining how laboratory animals should be housed, for experimental reasons, if not to improve animal welfare.

2.12 Conclusion

Despite the problems with single housing rabbits, it is often a requirement of the study that they are housed in this way (for example, during studies involving topical drug administration). Although group housing is often seen as the preferred option, there are also disadvantages to housing rabbit in this way, although they are mainly disadvantages for the technicians and for particular types of experiment rather than for the rabbits. Discussions with named veterinary surgeons and laboratory technicians, as well as behavioural observations of rabbits in different housing systems revealed than the two main areas of concern are the provision of social contact and the importance of a platform within cages. Further investigations will therefore concentrate on the housing of female New Zealand White rabbits, focusing primarily on social contact and secondly on platform use.

Chapter 3 – Testing a protocol for measuring motivation in laboratory rabbits

Abstract

Little is known about the learning capabilities of the rabbit and how rabbits perceive their environment. In order to determine the environmental priorities of laboratory rabbits a method of measuring motivation for resources was developed and tested. In order to determine the method that was most appropriate, the rabbits were initially given the opportunity to overcome three types of obstacle in a runway to gain visual and minimal tactile contact with a lower ranking rabbit. The obstacles used were a shallow water-bath with two depths of water, a fan with two air speeds and a push-door that could be weighted. All the rabbits went through the water bath at both depths of water and approached the fan at both air-stream speeds, therefore the water bath and fan did not appear to be seen as aversive enough to impose a cost at the water depths and air-stream speeds used. The water depths and air-stream speeds were not increased for practical and welfare reasons and these obstacles were therefore concluded to be unsuitable. The push-door, however, appeared to be perceived by the rabbits as a cost, as the rabbits were slower to move through the runway with the push-door in the runway compared with when there was no push-door in the runway. Weights were added to the push-door and these were increased to determine the maximum weight the rabbits would push through to gain contact with another rabbit. Although not significant, the rabbits tended to take longer to move through the runway as the weight on the push-door was increased and a point was reached where the cost outweighed the benefit and the rabbits did not push through the door. The push-door was concluded to be an appropriate obstacle to use for measuring motivation for resources. To ensure the rabbits were pushing through the door for the reward and not due to habit, the push-door approach was used to assess the motivation of the rabbits for a high incentive food reward (fresh vegetables) and for no reward (control), as well as for social contact. Although trends were found indicating that the rabbits were willing to push through heavier weights to reach the resources (social contact and food) than no reward, the differences in the

maximum weights pushed through were not significant; however, the sample size was small. The rabbits tended to push through the unweighted push-door to reach the end of the runway faster for the resources compared to the control and for food compared to social contact. The maximum weights pushed through were found not to be correlated with either the body weight of the individuals or their social rank.

3.1 Introduction

Although studies have been carried out into laboratory rabbit housing, little is known about how rabbits prioritise resources. In addition to home pen/cage observations, studies into resource requirements have mainly been carried out in the form of preference tests, where the animal is given a choice of resources and the one they spend most time utilising is seen as the preferred resource. Rabbits have been used in such tests to investigate preferences for cage flooring (Morisse *et al.*, 1999) and for either social contact or isolation (Held *et al.*, 1995). Preference tests however do not tell us the importance of the resource to the animal, as preference for one of two choices does not necessarily mean that the animal will suffer if provided with the less attractive of the two (Dawkins, 1983).

An alternative to preference tests is the use of motivational tests, a concept first introduced by Dawkins (1983), in which a cost is imposed on the animal gaining access to a resource or avoiding an unpleasant situation or stimulus. In such cost/benefit analysis the animal has to trade-off paying the cost with the benefit of the resource gained. It is assumed that animals will 'work' hardest (i.e. pay the highest price) for resources that are most important to them, such as food. The concept of a trade-off between cost and benefit is similar to the situation in the wild. For example, when a wild rabbit is feeding, the longer it spends out of cover the greater the risk of being predated on, therefore there is a trade-off between the time spent grazing (benefit) with the time spent being vigilant to avoid being caught by a predator (cost). The importance of such trade-offs was made clear by Dawkins and Krebs (1979 – as cited in Krebs and

Davies, 1993, p92), who stated, "A fox may reproduce after losing a race against a rabbit. No rabbit has ever reproduced after losing a race against a fox".

Motivational tests that have been carried out on other species have involved a variety of methods to impose a cost, which allow the cost of gaining the reward to be increased. These have taken the form of pushing on a lever/panel (e.g. Day *et al.*, 1996; Gunnarsson *et al.*, 2000), pushing through a weighted door (e.g. Manser *et al.*, 1996, 1998; Jackson *et al.*, 1999), pushing through a narrow gap (e.g. Cooper and Appleby, 1993) and overcoming a water bath (e.g. Sherwin and Nicol, 1996) or an air stream (e.g. Faure and Lagadic, 1994). During such studies animals have been tested in either open or closed economies. In an open economy the animals have access to the resource they are working for outside of the test environment (e.g. in their home cage/pen) and this can give misleading impressions of the importance of a resource as the animals may be less willing to work for a resource if they have access to it for free at other times (Mason *et al.*, 1998). It is usually recommended that animals are tested in a closed economy where they only have access to the resource within the test environment (e.g. Mason *et al.*, 1997).

There are some problems associated with motivational tests. An important problem is that the animals may not be able to associate the task they are expected to perform with the reward they gain after the performance of the task. Hens, for example, have been found to be unable to learn to peck a key or operate a foot treadle to gain access to litter (Dawkins and Beardsley, 1986). It has been suggested by Dawkins (1988) that requiring the animal to perform a more natural response to gain a reward may give more valid results. Young *et al.* (1994) found that pigs responded significantly more often for food when they had to manipulate a 'paddle' (operated by rooting or chewing the device) than when they had to press a panel using the flattened end of their snout. When investigating feeding behaviour of wild caught blue tits and coal tits, Partridge (1976) similarly found that the tasks that they were most successful at were those most similar to situations they come across in their respective habitats in the wild. Asking an animal to continually perform a task that is inappropriate for the reward was found by Breland and Breland (1961) to lead to what they termed 'instinctive drift'. The animals stopped performing the response they had been conditioned to perform and instead drifted towards performing the instinctive behaviour most closely related to the task they were being asked to perform. Consideration has to be given to the fact that the obstacle chosen by the experimenter as being most costly may not be perceived in the same way by the animal. Some obstacles may be ones which the species would naturally come across during territory monitoring or exploration and may not be perceived as imposing a cost at all (Sherwin and Nicol, 1995). It is also important that the experimental set-up is not perceived as frightening to the test animal. For example, a herd animal tested in social isolation is not likely to be willing to learn, or to work to eat if it feels threatened by the situation. In addition to methodological problems, the cost of equipment is often very high.

Due to the types of experiment discussed being vulnerable to error as a result of inappropriate experimental design, it is vital that the apparatus and methodology be carefully considered. The aims of this experiment were therefore to investigate whether or not rabbits could be trained to perform tasks, to identify the protocol and most appropriate obstacle to use in motivational tests to assess the importance of resources to laboratory rabbits and to test this protocol by measuring motivation for different resources. Following the review of the available literature on rabbits and motivational tests carried out on other species and discussions with several people with knowledge of rabbits, it was decided that trials would be carried out using a water bath, an air stream and a push-door as obstacles. Wild rabbits have been seen to avoid feeding during heavy rain and will compensate by increasing their feed intake prior to a storm (Thompson and Worden, 1956). It was assumed that the rabbits would find water aversive. Water baths have been successfully used to measure motivation of mice, using both increasing depth and width of water (Sherwin and Nicol, 1995; Sherwin and Nicol, 1996) and air-streams have been used successfully used with hens (Faure and Lagadic, 1994). In order for an obstacle to be suitable for measuring motivation for resources in future experiments it would have to be perceived as a cost by the rabbits, with a gradation seen in the effort required and time taken to overcome the obstacle as the cost of overcoming it was increased, and with a stage being reached where the cost outweighed the benefit of gaining the reward and the rabbits stopped pushing through the door. In Chapter 2 it was found that the provision of social contact was one of the issues of most concern regarding laboratory rabbit housing. As this would be one of the resources focused on and therefore the reward in the motivational tests in later experiments, social contact was used as the reward in the trials to find the most appropriate obstacle. Using social contact as the reward rather than food, which is perhaps the resource the rabbits would be most likely to work for, avoided the need to deprive the rabbits of food and therefore avoided any welfare issues in this early stage of the investigation. Using social contact as the reward raised the concern of the relative dominance status of the rabbits, as a rabbit may be more reluctant to work to approach a rabbit of higher rank than one of lower rank. The dominance status of the rabbits used was therefore established prior to the experiments. The obstacle chosen as being appropriate for measuring motivation for resources was then used to assess the motivation of the rabbits for a high incentive food reward and for no reward (control) to ensure that the rabbits were working for the reward and not as a habit.

Experiment 1

The aim of this experiment was to determine the most suitable obstacle to be used to impose a cost on rabbits gaining social contact with a lower ranking conspecific.

3.2 Methods

3.2.1 Animals, housing and husbandry

The experimental animals were seven female New Zealand White rabbits, between one and three years of age. Prior to the experiment, the rabbits were housed in a floor pen (approximately 4 x 5m) in a group of ten rabbits, which they had been in for several months. Two of the ten rabbits had to be removed from the study due to illness (unrelated to the study) and the lowest ranking rabbit was not used (see *3.2.2*).

Commercial rabbit pellets (STANRAB (P), Special Diet Services, UK), hay and water were available *ad libitum*. Carrots and cabbage were scattered around the pen daily. Several bolt-holes and environmental enrichment were provided. The light cycle was 13.5 hours light:10.5 hours dark and the temperature fluctuated 2°C both above and below the mean of 18°C. Rabbits were weighed on a weekly basis during routine health checks.

The rabbits were to be tested in a closed economy, therefore they were removed from the social group they were normally housed in and were housed singly in standard laboratory cages (71 x 56 x 46cm) for two days prior to, and throughout testing. The rabbits had been housed in these cages when their floor pen was cleaned and if they required veterinary treatment, therefore the cages were familiar to them. The rabbits were exposed to the same light cycle and room temperature while in cages as they were in the floor pen.

3.2.2 Assessment of dominance status

The dominance status of all ten rabbits in the group was determined from 30 hours of observations recorded on video tape using CCTV cameras (Panasonic WV-BP330/B), a quad unit (Panasonic WJ-410) and a time-lapse video recorder (Panasonic AG-6024). The observations were all made in daylight hours over five consecutive days, during the first and last three hours of the light cycle when the rabbits were most active. Dominance was established using the behaviours shown in Table 3.1 to decide the outcome of agonistic interactions. A dominance matrix was created using the outcome of interactions between each possible pairing of rabbits, placing the winner of most interactions between a pair above the loser. Each rabbit was paired with the next lowest ranking rabbit for experimental purposes, therefore the lowest ranking rabbit in the group was not used.

Behaviour	Definition
Attack	Rabbit runs aggressively at another with ears back in an attempt to bite another rabbit
Chase	Rabbit runs after another, may cause it to retreat
Nudge	Rabbit displaced another (perhaps from a favoured site) by physically pushing it out of the way
Retreat	Rabbit makes a running retreat from an aggressor
Sexual behaviour	Circling and mounting
Submission	May avoid contact with others by turning away, accepts the dominance of another rabbit by lying flat without any resistance or attempts to escape
Threat	Head lowered, ears laid flat as though about to attack

Table 3.1 – Ethogram of behaviours used to determine dominance within the group (Adapted from Gunn, 1994)

3.2.3 Apparatus and obstacles

The experimental apparatus was a solid sided wooden runway measuring 2.75m long, 0.4m wide and 0.6m high (Plate 3.1 and Figure 3.1). The floor was covered with wood chip paper and the top of the runway was covered with wire mesh (50mm). A wooden panel ('start box panel') could be slid vertically into the runway 0.4m from the start, creating a 'start box'. Approximately 15cm from the end of the runway a rectangle measuring 30cm x 25cm was cut out of the right side wall and was replaced with wire mesh (25mm). On the other side of the mesh panel was a small pen (1.5m x 1.25m) which a rabbit would be placed in during the experiment. The mesh panel would allow visual and minimal tactile contact between the rabbits. It was not possible for a rabbit in the runway to see into the pen until opposite the mesh.

Three types of obstacle were placed in the runway in turn, which the rabbits had to overcome to gain access to the end of the runway. These were a water bath, an air-stream and a push-door.

1. *Water bath* – plastic tray 75cm long, 32cm wide and 8.5cm high; the floor was scored using a sharp knife to roughen the surface to prevent the rabbits slipping

2. *Air-stream* – desk top fan (JAC S9D, 230mm, 50Hz, Get Plc., London, UK) with two settings for air speed
3. *Push-door* – cat flap (Cat Mate, Large cat flap, Pet Mate Ltd, UK) made of perspex, measuring 18cm x 19cm; the magnet at the bottom of the flap was removed so that there was no resistance when the rabbits tried to push through

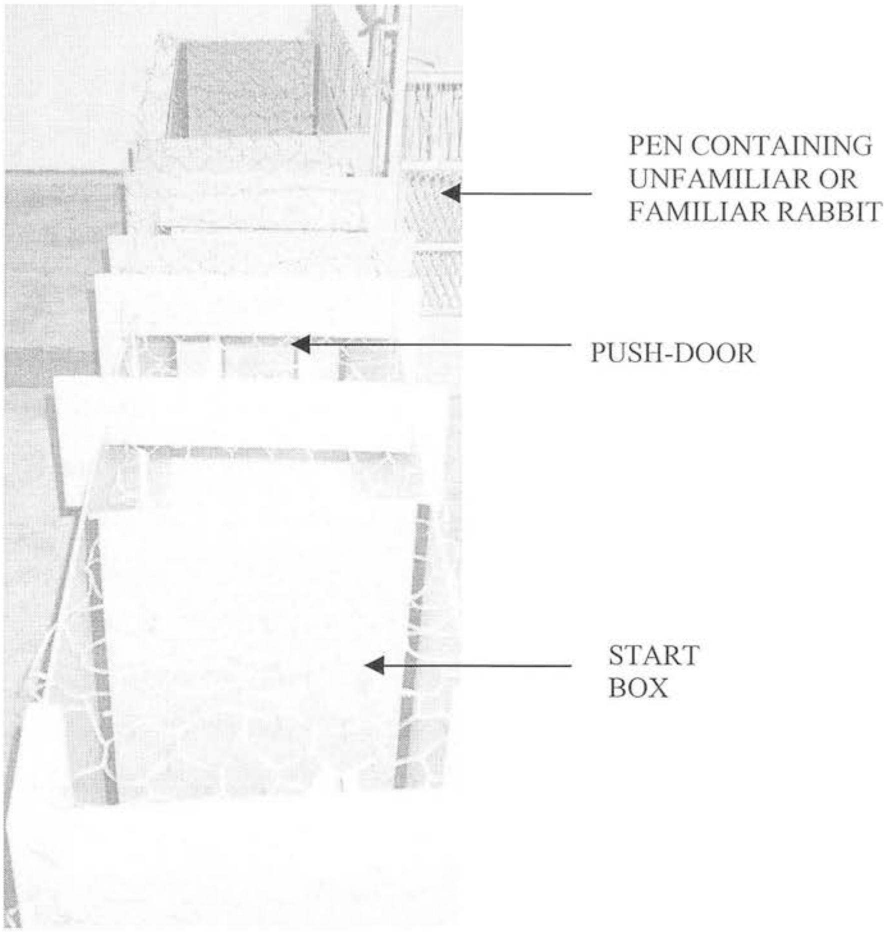

Plate 3.1 Experimental apparatus with the push-door in place

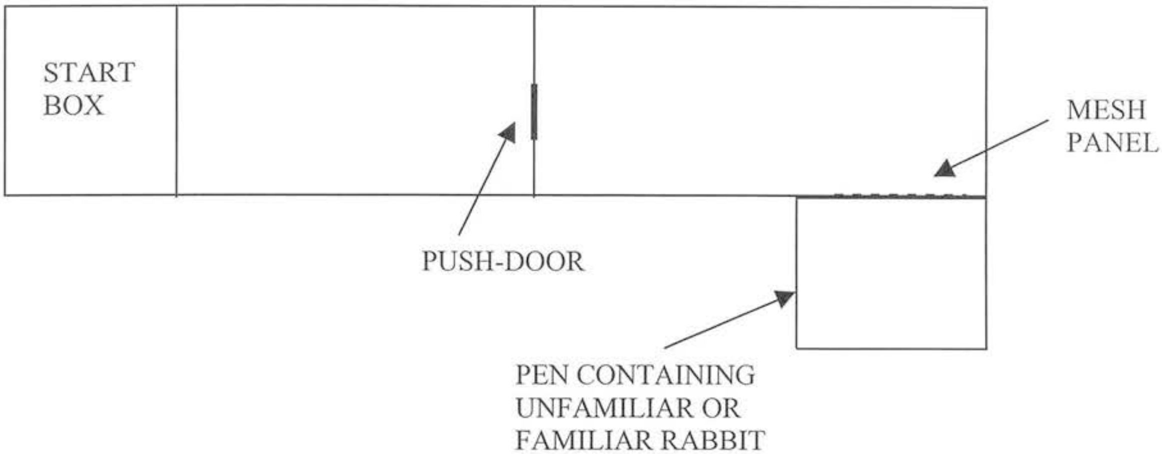

Figure 3.1 Experimental apparatus

3.2.4 Protocol

3.2.4.1 Familiarisation

The rabbits were familiarised with the runway and the obstacles over the course of a five day familiarisation period and were familiarised with only one obstacle per day. During familiarisation the lower ranking rabbit that each rabbit had been paired with was placed in the pen at the end of the runway.

Apparatus

Each rabbit was placed in the empty runway during four sessions over two days, for a total of approximately two hours.

Obstacles

The rabbits were then familiarised with the obstacles.

1. *Water bath* – The empty water bath was placed in the runway half way between the start box and the mesh panel (approximately half a metre from each). Although the water bath was slightly narrower than the runway there was not enough space for a rabbit to pass the water bath (approximately 4cm each side). Each rabbit was placed at the start of the runway. If they had not moved through the water bath within two

minutes they were encouraged through the water bath, with gentle nudging if required. Once through they were allowed to spend two minutes at the end of the runway in front of the mesh panel and the lower ranking rabbit. If at the end of the two minutes a rabbit was still at the end of the runway it was gently persuaded back through the water bath to the start of the runway. The familiarisation session was terminated when the rabbit had passed through the water bath four times in each direction.

2. *Air-stream* – The end wall of the runway was replaced with a panel with a circle the size of the fan cut out. The fan was placed on the floor behind it so that the rabbit could see the head of the fan but not the base and could therefore not reach the electrical cable. Each rabbit was placed in the runway for two 15 minute sessions (approximately two hours apart). The fan was not switched on to allow the rabbits to become familiarised with the sight of the fan alone. It was also necessary to familiarise the rabbits with the sound of the fan without actually being exposed to the air-stream so the end wall was replaced with the original solid wall and the fan placed behind this and switched on at full power. The rabbits were again placed in the runway for two 15 minute sessions. During familiarisation with both the sight and sound of the fan if the rabbits had not moved to the end of the runway within two minutes they were gently encouraged to the end. Positioning the fan right at the end of the runway meant that during testing the air-stream would get stronger the closer the rabbit got to the mesh panel and the rabbit in the pen.

3. *Push-door* – The push-door was fitted into a wooden panel which was fixed into the runway approximately half way between the start box and the mesh panel (just under 1m from each). The rabbits were trained to push through the door in four steps, with it initially being held fully open. Each rabbit was placed at the start of the runway. If they had not moved through the open door within two minutes they were encouraged through, with gentle nudging if required. Once through they were allowed to spend two minutes at the end of the runway in front of the mesh panel and

the lower ranking rabbit. If at the end of the two minutes a rabbit was still at the end of the runway it was gently persuaded back through the door to the start of the runway. The familiarisation session was terminated when the rabbit had passed through the open push-door four times. The same procedure was carried out with the push-door one quarter closed, half closed and three quarters closed.

3.2.4.2 Testing protocol

The testing protocol began two days after the familiarisation period. Before the obstacles were introduced into the runway the rabbits were placed in the empty runway and the time taken to leave the start box and reach the end of the runway to gain visual and minimal tactile contact with the rabbit in the pen was recorded. This would be used to assess the cost of the obstacle, as perceived by the rabbits. Testing using the obstacles began the following day. The order in which the obstacles were tested was varied between rabbits.

1. *Water bath* – The water bath was filled with water (25°C) to a depth of 2cm. Each rabbit was placed in the start box for five minutes, after which the start box panel was lifted to allow the rabbit access to the rest of the runway. If a rabbit had not left the start box within two minutes it was gently encouraged out. When the rabbit left the start box the clock was started. Several measurements were taken as the rabbit moved along the runway and through the water bath (Table 3.2). Once the rabbit had reached the end of the runway and gained the reward of visual and minimal tactile contact with another rabbit it was allowed two minutes in the runway before being removed and returned to its cage. The test was terminated after 20 minutes if the rabbit did not go through the water bath. The whole procedure was repeated the following day for those rabbits that had gone through the water bath, with the water depth being increased to 5cm.

2. *Air-stream* – The fan was placed at the end of the runway in view of the rabbits (see *3.2.4.1*) and was switched on at the lower of the two settings (approximately $2ms^{-1}$).

The same procedure was carried out as with the water bath with similar measurements being recorded (Table 3.2). The whole procedure was repeated the following day for those rabbits that had approached the end of the runway and therefore the fan, with the fan being switched to the higher setting (approximately 3 ms^{-1}).

3. *Push-door* – The above procedure was carried out with the push-door and the appropriate measurements taken (Table 3.2). Those rabbits that pushed through the unweighted push-door were tested again the following day with a 100gram weight added to the door. The door was weighted by means of small bags of sand stuck to back of the push-door (i.e. the side nearest the mesh panel). The original protocol was to test the rabbits with 0, 100 and 250grams on the push-door. As several rabbits pushed through the door weighted with 250grams, they were tested daily with weight increases of 100grams at a time to determine the maximum weight they would push through. When a rabbit did not push through the door within the maximum time allowed of 20 minutes the test was terminated and the last weight pushed through was taken as the maximum price paid for the resource.

3.2.5 Statistical analysis

Wilcoxon signed rank tests were used to compare the time taken to move to the end of the runway when no obstacle was present with the time to reach the end when each of the three obstacles was introduced, but before the cost was imposed (i.e empty water bath, sight/sound of air stream, unweighted push-door) to determine whether or not the obstacles themselves were perceived as a significant cost.

For the water bath and air-stream tests comparisons of the measurements in Table 3.2 were made between the familiarisation session (i.e. no water or air-stream) and the two experimental sessions using Friedman two-way analysis of variance tests and using a calculation for multiple comparisons between groups or conditions if any significant results were found (Siegel and Castellan, 1988). The same analysis was used to investigate any differences in the measurements in Table 3.2 for the push-door test, comparing results from when the door was unweighted, from when the first weight was added (100g) and from the last weight each rabbit pushed through.

Obstacle	Measurement	Definition
Water bath	'Start box'	Time taken for whole body to leave the start box
	'Time to water'	Time taken to reach the water bath (within approximately 5cm)
	'Time in water'	Time spent with all four feet in the water bath
	'No. of approaches'	Number of approaches made to the water bath before going through
	'Time to end'	Time taken to reach the end of the runway (i.e. the resource)
Air-stream	'Start box'	As above
	'Time to end'	As above
Push-door	'Start box'	As above
	'Time to door'	Time taken to reach the push-door (within approximately 5cm)
	'No. of approaches'	Number of approaches made to the push-door before going through
	'Time through'	Time in seconds for the whole body to move through the push-door
	'Time to end'	As above

Table 3.2 – Measurements taken during testing (times in seconds)

3.3 Results

3.3.1 Time to end of runway prior to cost being imposed

All of the rabbits moved to the end of the runway when there was no obstacle and when each of the obstacles was first introduced. No significant differences were found between the time to reach the end of the runway when no obstacle was present compared to when the empty water bath was put in and when the rabbits could see or hear the fan (Table 3.3). The rabbits were found to take significantly longer to reach the end of the runway when they had to push through the unweighted push-door compared to when there was no obstacle in the runway (Wilcoxon signed rank, $T=0.0$, $N=7$, $p<0.05$).

Obstacle	Time to reach end of runway
No obstacle	10.0 (5, 21)
Empty water bath	17.0 (12, 23)
Sight of fan	6.0 (4, 11)
Sound of fan	10.0 (6, 14)
Unweighted push-door	27.0 (13, 65)

Table 3.3 Time (in seconds) to reach the end of the runway with different types of obstacle (medians and inter-quartile ranges)

3.3.2 Water bath

All the rabbits went through both depths of water in the water bath to reach the end of the runway and gain visual and minimal tactile contact with a lower ranking rabbit. Comparisons of the time taken to move through the runway when the water bath was empty and had 2cm and 5cm of water in it found no significant differences (Table 3.4). Four of the seven rabbits however did not enter the water bath on the first approach when the water was 5cm deep.

	Time to leave start box	Time to water bath	Time spent in water bath	Time to end of runway
Empty water bath	2 (1, 3)	4 (2, 5)	5 (3, 14)	17 (12, 23)
2cm of water	1 (1, 2)	3 (3, 4)	3 (2, 4)	21 (16, 25)
5cm of water	3 (2, 7)	5 (3, 10)	2 (2, 3)	34 (15, 156)

Table 3.4 Time (seconds) taken to move through the runway when the water bath was empty, and with water 2cm and 5cm deep (median and inter-quartile range)

3.3.3 Air-stream

All the rabbits approached the fan at both air-stream speeds to reach the end of the runway and gain visual and minimal tactile contact with a lower ranking rabbit. There was found to be no difference in the time taken to leave the start box and reach the end of the runway when the rabbits could only see or hear the fan and when the fan was switched on at both speeds of the air-stream (Table 3.5).

	Time to leave start box	Time to end of runway
Sight of fan	1 (0, 4)	6 (4, 11)
Sound of fan	2 (2, 7)	10 (6, 14)
Approx. $2ms^{-1}$	1 (1, 2)	4 (4, 7)
Approx. $3ms^{-1}$	2 (1, 5)	6 (4, 7)

Table 3.5 Time (seconds) taken to move through the runway with only the sight/sound of the fan and at both air-stream speeds (median and inter-quartile range)

3.3.4 Push-door

The maximum weights the rabbits pushed through are shown in Table 3.7, with the results of Experiment 2. No significant differences were found between the unweighted door, the first weight (100g) and the last weight that each individual pushed through in the time taken to move through the runway (Table 3.6). There was however a trend showing a gradation in the time taken to push through the push-door and reach the end of the runway, with the time increasing as the weight on the push-door was increased.

	Time to leave start box	Time to push-door	Time through push-door	Time to end of runway
Unweighted	3 (2, 17)	5 (4, 18)	29 (10, 75)	30 (11, 79)
First weight	2 (2, 4)	5 (4, 7)	40 (15, 54)	42 (16, 55)
Last weight	3 (2, 6)	8 (4, 23)	41 (12, 115)	49 (14, 117)

Table 3.6 Time (seconds) taken to move through the runway when the push-door was unweighted, and with the first and last weights added (median and inter-quartile range)

3.4 Discussion

Wild rabbits have been seen to avoid feeding during heavy rain and will compensate by increasing their feed intake prior to a storm (Thompson and Worden, 1956). It was assumed that the rabbits would find the water bath aversive and therefore would not move through it unless the motivation for the reward was sufficient to overcome this aversion. The rabbits did not however appear to find the water bath aversive as all the rabbits moved through it, although just over half of the rabbits approached the water bath on more than one occasion before going through it when the water was 5cm deep. It is perhaps most likely that this hesitation was due to the previous experience with the water bath (i.e. 2cm of water) being unpleasant, rather than the rabbits perceiving the 5cm deep water as too deep. Whichever is true, contrary to expectation the water bath was not perceived as aversive enough to prevent the rabbits from overcoming the obstacle to gain the reward of social contact. This was also shown by the fact that the rabbits did not take significantly longer to move through the runway when the water depth was increased. It was therefore concluded to be an unsuitable method. The air-stream was also considered to be an unsuitable approach to use, as it also appeared not to be aversive to the rabbits due to the fact that all of the rabbits moved along the runway into the air-stream to gain the reward of social contact and did not slow down when the air-speed was increased. It was therefore concluded that at the depths and air-stream speeds used, neither a water bath nor a fan is an appropriate means of measuring motivation of rabbits for social contact. It is possible that they may be more suitable methods if the water depth and air-stream speed were increased, however these possibilities were not pursued for practical reasons.

Water baths have been successfully used to measure motivation of mice, using both increasing depth and width of water (Sherwin and Nicol, 1995; Sherwin and Nicol, 1996) and air-streams have been used successfully used with hens (Faure and Lagadic, 1994). The fact that these approaches appear to be unsuccessful for measuring motivation of rabbits supports the thoughts of Sherwin and Nicol (1995), that some obstacles which we may think of as being a cost to an animal may not be perceived by the animal in the same way. This also highlights the need to select an obstacle that is appropriate for the species and that the performance of a more suitable response to gain a reward may give more valid results (Dawkins, 1988).

The rabbits were quick to learn to push through a door in order to gain social contact and this approach allowed the cost of gaining the reward to be easily and practically increased. The push-door itself appeared to be seen as a cost, as the rabbits were significantly slower to move through the runway when they had to push through the unweighted push-door compared to when the runway was empty. Increasing the weight on the push-door did not affect the time taken for the rabbits to leave the start box and to approach the push-door. However, once they approached the push-door the rabbits were slower to push through the door, therefore increasing the overall time to move through the runway to gain social contact. Such gradation in the time taken to push through the push-door and reach the end of the runway as the weight on the door increased indicated that the weight was perceived by the rabbits as a cost, as did the fact that as the weight increased some of the rabbits stopped pushing through. The push-door approach was therefore concluded to be a suitable obstacle for measuring the motivation of rabbits for resources.

In the wild rabbits spend a large proportion of their day under ground in burrows (Mykytowycz and Rowley, 1958). As well as the main burrow rabbits also dig additional entrances to the main burrow known as bolt-holes, which they run to as their primary means of escape from a threat. These bolt-holes are often dug under bushes to camouflage them from predators. In areas of thick vegetation the rabbits may use the vegetation as a source of cover from predators rather than bolting underground (Kolb, 1991, 1994). Pushing through a small, weighted push-door could perhaps be equated to pushing through the undergrowth and narrow burrows and could therefore be a relatively natural behaviour for a rabbit. Despite this perhaps being a natural response, the increased time taken to reach the end of the runway as the weight on the push-door was increased indicates that it was also seen as a cost. The push-door approach has also been found to be successful for use with other species, for example, rats (e.g. Manser *et al.*, 1996, 1998), hens (e.g. Olsson and Keeling, 2002), sheep (Jackson, 1999) and mink (e.g. Cooper and Mason, 2000).

Experiment 2

The aim of this experiment was to carry out further investigations into the use of the push-door approach to determine the motivation of rabbits for resources. This was carried out using high incentive food items as a reward and with no reward at the end of the runway to investigate whether or not there were any differences in the maximum weights pushed through for different resources and to ensure that the rabbits were actually pushing through the door to gain the reward and not due to habit.

3.5 Methods

3.5.1 Animals, housing and husbandry

The rabbits were housed as they were prior to Experiment 1 (see *3.2.1*).

3.5.2 Apparatus and obstacles

The experimental apparatus and push-door described in *3.2.3* were used, with the mesh panel in the right side of the runway being replaced by a solid panel.

3.5.3 Protocol

3.5.3.1 High incentive food items

As this was still a relatively early stage in the overall investigation into using this approach it was decided that total food deprivation was not appropriate for welfare reasons. It was assumed that fresh vegetables were a commodity that the rabbits would perceive as favourable, as when fed them as part of their daily diet they tended to start eating them as soon as they were given. Similarly to when testing motivation for social contact the vegetables were removed from the rabbits' diet two days prior to and throughout testing.

The rabbits were re-familiarised with the apparatus with vegetables (one carrot and cabbage leaf) at the end of the runway. These were laid on the floor, as this was how they were fed to the rabbits in their home pen. The rabbits were placed in the runway without the push-door during two 15 minute sessions on two consecutive days. On the third day the push-door was placed back in the runway. The rabbits had to move through the open door and were allowed five minutes at the end of the runway before being encouraged back to the start. The same was repeated with the door three quarters closed.

The same testing procedure as described in section *3.2.4.2* was used, although the time at the end of the runway was increased to five minutes. Casual observations of the rabbits in their home pen found that feeding bouts tended to be short, often only one or two minutes long. It was however decided to be important to give the rabbits ample time to avoid interrupting feeding, as having bouts of behaviour interrupted may devalue the reward of gaining access to the resource (Mason *et al.*, 1997).

3.5.3.2 Control (no reward)

The same re-familiarisation process as described above for the high incentive food items test was carried out with nothing at the end of the runway. As well as showing whether the rabbits could learn that there was no reward this would also show how quickly a learnt behaviour could be extinguished. The testing procedure was carried out as above.

3.5.4 Analysis

The results of the trials in Experiment 1 where the rabbits were given the opportunity to push through the push-door to gain social contact were included in the analysis. The maximum weights pushed through to gain social contact, high incentive food items and no reward were compared using Friedman two-way analysis of variance. A Spearman rank correlation was carried out to determine any association between the maximum weights pushed through for resources and both body weight and social rank.

To give a further insight into the importance of these resources the simplest treatment (unweighted door) was used to determine any differences in the time taken to reach the end of the runway in the two resource tests and in the control. Comparisons were made using Friedman two-way analysis of variance.

3.6 Results

3.6.1 Maximum weights pushed through

The maximum weights the rabbits pushed through for the two resources and for the control are shown in Table 3.7. The medians are shown in Figure 3.2.

Rabbit	Social contact	High incentive food items	Control (no reward)
A	1050	550	350
B	550	450	0
C	550	550	100
D	100	100	0
E	650	450	0
F	0	100	100
G	450	750	650

Table 3.7 Maximum weights the rabbits pushed through (grams), shown in order of dominance with rabbit A being most dominant

Friedman two-way analysis of variance found no significant differences in the maximum weights pushed through to gain social contact, high incentive food items and no reward. The rabbits did however tend to work harder (i.e. to push through heavier weights) for the two resources compared to the control (no reward). Three rabbits pushed through heavier weights for social contact compared to high incentive food items and two pushed through equal maximum weights for the two resources (Table 3.7).

Individual variation in the maximum weights pushed through for social contact, for high incentive food items and in the control could not be explained by body weight (Spearman rank correlations, $r_s = 0.384$, $r_s = 0.532$, $r_s = 0.509$ respectively). There was also found to be no significant association with social rank (Spearman rank correlations, $r_s = -0.631$, $r_s = -0.055$, $r_s = 0.206$ respectively).

3.6.2 Time to reach the end of the runway

Although the rabbits tended to take longer to reach the end of the runway when there was no reward compared to when there was social contact or high incentive food items at the end of the runway (Figure 3.3), the differences between the three were not significant.

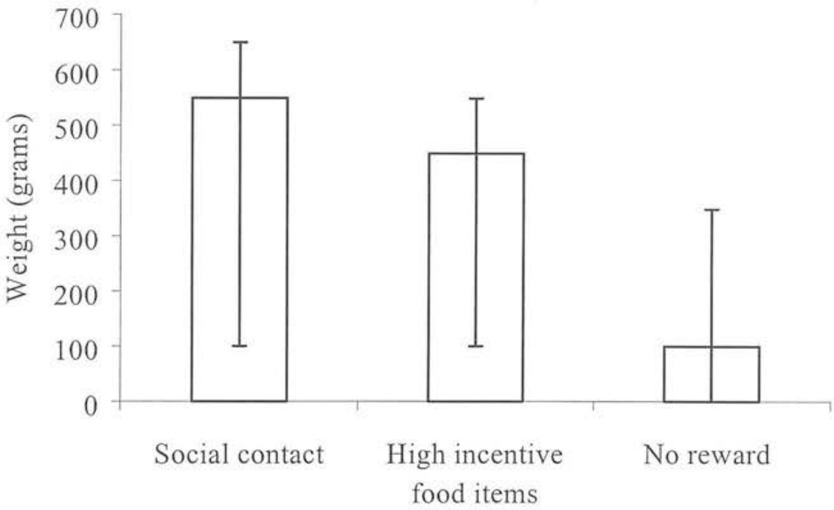

Figure 3.2 Maximum weights pushed through by the rabbits (medians and inter-quartile ranges)

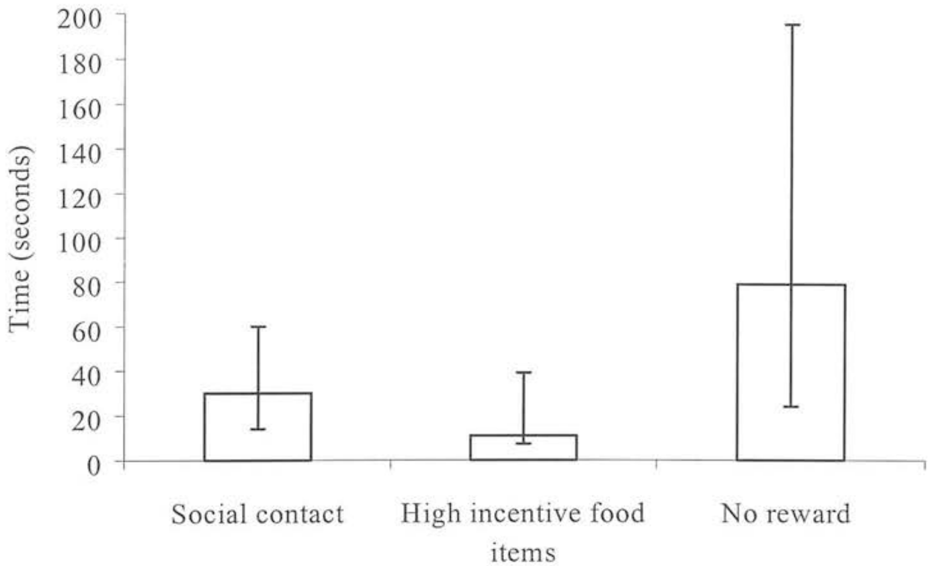

Figure 3.3 Time taken by the rabbits to reach the end of the runway (medians and inter-quartile ranges)

3.7 Discussion

Although the maximum weights pushed through in the control were lower than those pushed through for resources, they were not significantly different. This could be as a result of there being few animals used in the study, which was due to this being the early stages of investigation into the motivation of rabbits for resources. Despite this the push-door does appear to be a suitable method to use to measure the motivation of rabbits for resources. In the control, three of the seven rabbits did not push through the door when the first weight was added and two only pushed through the first weight. This indicates that some of the rabbits learnt quickly that there was no reward at the end of the runway and implies that others did not. It shows that the response the rabbits had learnt (i.e. to push through the door) was easily extinguished and had therefore not become a habit. Interestingly the two rabbits pushing through further than the first weight in the control (350g and 650g) were the highest and lowest ranked rabbits, which is similar to the finding that there was no association between social rank and the weights pushed through.

Although no relationship was found between the weights pushed through and the social rank of the rabbits, it is possible that this could be due to inaccuracy in the determination of the dominance status of individuals. Caution has to be exercised when interpreting dominance status and a common mistake is to take dominance status as a fixed trait of the animal. In actual fact the dominance status of an animal and relationships between individuals can change, often very quickly. Some reversals were found to occur, i.e. a subordinate rabbit winning an encounter with a rabbit usually seen as dominant to it. Wild female rabbits tend to have a clearly defined hierarchy (Mykytowycz, 1958) and domestic rabbits have been found to have a significant linear hierarchy (Stauffacher, 1986). Vastrade (1986) found that once a hierarchy has been established interactions may be rare, becoming obvious when competing at a feed hopper. The rabbits used in the current study had been in their group for more than a year and the group was therefore stable, meaning that not as many interactions were seen as there would be in an unstable group, nor were the interactions as aggressive. The rabbits were provided with several food hoppers, water troughs and bolt-holes, and hay was scattered around the pen so the rabbits did not have to compete for resources. The interactions of the rabbits in their home pen were often difficult to interpret as often if an aggressive dispute arose within the pen the dominant rabbit would very quickly diffuse the situation by chasing the rabbits involved. The rabbits used in this study had been used in a study over a year earlier when they were part of a group of nineteen rabbits, housed in the same pen as during the current study. Then, the assessment of the hierarchy was similarly discussed as perhaps being inaccurate, due to a low incidence of aggression (Nay, 1998). Although the current study found no association between social rank and motivation for resources, the possibility should perhaps not be disregarded. The lack of correlation between body weight and social rank was also found in sandy lop rabbits by Batchelor (1991), although there was a general trend for the heavier rabbits to have more dominant positions.

Although not significantly so, the rabbits tended to reach the end of the runway more quickly for the two resources than for the control, and for the high incentive food items compared to social contact. Despite this there was often a delay between the rabbits reaching the end of the runway and starting to feed. Although the rabbits had spent much time in the runway and would therefore be expected to be familiarised with it, wild rabbits naturally have to be vigilant while they feed as they risk being predated on and it was perhaps instinctive for them to behave in this way. After the maximum weights the rabbits push through for food, the time taken to reach the food is perhaps the next best measure of its perceived importance, rather than consumption. Similar to the maximum weights pushed through in the control, the rabbits showed variation in the length of time taken to push through the push-door and reach the end of the runway in the control, however they were slower in the control than when working for resources. The hesitation shown suggests that the rabbits were aware that there was no reward and therefore no real incentive for pushing through the door and perhaps they were moving through the runway as part of territory inspection.

3.8 Conclusion

This study has shown that rabbits can be trained to overcome certain obstacles and that they can learn the association between overcoming an obstacle and gaining a particular reward. The study was carried out to investigate a suitable method for measuring motivation of rabbits for resources. Using the criteria that the obstacle to be used in further experiments would have to appear to be perceived by the rabbits as a cost, and show a gradation in the effort required to overcome the cost as the cost increased, the push-door was concluded to be the most suitable obstacle.

An amendment will be made to the method used in Experiment 2 of this study to make results of future experiments more valid and more applicable to rabbits within the pharmaceutical industry. Since this was a welfare based study and in the early stages of investigation, laboratory rabbits already held within one of the University of Edinburgh's animal units were used, to reduce the number of animals used throughout the study. These rabbits were between two and three years old, which is older than many laboratory rabbits. Future experiments will therefore use rabbits which will be approximately 13-15 weeks old at the start of the experiment, which is the age at which most pharmaceutical companies take on rabbits from the suppliers. It is thought that younger rabbits may be more active during the tests.

Chapter 4 - Motivation of singly caged female rabbits for short periods of visual and minimal tactile contact with a conspecific

Abstract

Social housing of female laboratory rabbits is generally recommended. However, experimental constraints may require rabbits to be housed singly and in order to partially compensate for this, newer designs of laboratory cage allow visual and at times minimal tactile contact between rabbits in adjacent cages. It is not known, however, whether rabbits find such limited contact rewarding. This type of contact may even be negative, as rabbits may feel threatened by such close contact or be frustrated at not being able to respond to the presence of the rabbit. An experiment was therefore carried out to determine whether singly caged rabbits with only olfactory and auditory contact with rabbits in adjacent cages were motivated to gain short periods of visual and minimal tactile contact with a conspecific through a mesh panel. The methodology devised in Chapter 3 was used, allowing rabbits to push through a weighted door for access to a conspecific. The maximum weight the rabbits were willing to push through for contact and how long they took to gain access to the conspecific were measured to assess how important this was to them. Comparisons were made between how hard (i.e. maximum weights pushed through) and how quickly the rabbits worked for contact with an unfamiliar and a familiar rabbit. No difference was found in how hard the rabbits worked to reach the unfamiliar or familiar rabbit, although they pushed through significantly heavier weights for both than they did for no conspecific (control). The rabbits moved through the runway more quickly to reach the unfamiliar rabbit than they did for the familiar rabbit and spent longer in direct contact with the unfamiliar than the familiar rabbit. The findings indicate that short periods of visual and minimal tactile contact are rewarding for singly caged rabbits and it is therefore recommended that singly caged laboratory rabbits should be able to have such contact.

4.1 Introduction

In the wild, rabbits live in small social groups and may show severe aggression towards intruders of their territory, particularly during the breeding season (e.g. Bell, 1983). Comparisons between wild and domestic rabbits have found that domestic rabbits show a similar behavioural repertoire to wild rabbits (e.g. Stodart and Myers, 1964; Vastrade, 1986). In the laboratory situation, male rabbits have been found to be too aggressive to house socially and they are therefore always housed singly. Female rabbits, however, may be housed singly or socially. Several studies have been carried out into social housing of female rabbits in the laboratory, which have found this to be a viable method of housing (e.g. Heath and Stott, 1990; Batchelor, 1991; Love and Hammond, 1991; Stauffacher, 1992). Female rabbits have been found to choose to spend time with other rabbits when they are given the opportunity to do so (Huls *et al.*, 1991; Brooks *et al.*, 1993; Held *et al.*, 1995). Experimental constraints may mean that female rabbits have to be housed singly (see Chapter 2), although it is recommended that they should be group housed where possible (Home Office, 1989; Morton *et al.*, 1993).

One of the newer designs of laboratory rabbit cage (Tecniplast) allows singly caged rabbits to have limited social contact (see Chapter 5, Plate 5.1). The rabbits can see into the adjacent cages through a transparent door when they are on their platform at the back of the cage. A mesh panel at the front of the cage allows the rabbits to have visual and minimal tactile contact with rabbits in adjacent cages by rearing onto their hind legs. Contact between two rabbits relies on the relative position of the two individuals. If a rabbit moves underneath the platform it is out of sight of the rabbit in the adjacent cage. Rabbits in such cages do not therefore have full control over whether they have social contact and so may only be able to have short periods of social contact. It is possible that individuals perceive their cage as their own territory and if this is the case, having another rabbit in such close proximity may be seen as threatening. The only way a rabbit can avoid such contact may be to move underneath the platform. Not allowing rabbits to control the duration of social contact or to defend their cage territory from rabbits in such close proximity may be frustrating for the rabbits. The fact that rabbits can be successfully housed in groups and will choose to be with other rabbits when they have the opportunity to do so, indicates that social contact with conspecifics is something that rabbits do want. It has been assumed that allowing caged rabbits to have limited social contact is also something that rabbits want, however the rabbits do not necessarily perceive such contact in the way humans do. It is therefore important to investigate whether short periods of limited social contact are something that rabbits do want.

The importance of resources to animals has been assessed by imposing an increasing cost on gaining a resource to determine how hard they are willing to work for that resource (see Chapter 1, section *1.4.2.3*). Dawkins (1983) suggested incorporating measures from human economics into such tests of motivation, which would enable resources to be ranked in order of their importance. One such measure is the maximum price paid, which is simply the maximum cost the animal is willing to pay to gain a resource. Testing motivation in a closed economy (the animals only have access to the resource within the test environment) rather than an open economy (the animals have access to the resource out of the test environment as well as in it) is recommended as an open economy can lead to resources seeming less important than they actually are (e.g. Mason *et al.*, 1997). Such tests have been carried out allowing animals to work for only one resource at a time (e.g. Matthews and Ladewig, 1994) or for several resources at time, with a cost imposed on gaining each of them (e.g. Mason *et al.*, 2001). Depending on the resources chosen for the animals to work for, offering only one resource at a time may be more relevant to the applied situation. When offering animals the chance to work for more than one resource at a time it is important that the resources offered are chosen carefully to ensure they are neither substitutable or complementary as this can result in artificial measures of motivation. If two resources have a similar motivational basis, the consumption of one resource may decrease the motivation for the other. This can make the least preferred of two substitutable resources appear to be worthless, however deliberately using substitutable resources can highlight which aspects of resources animals are working for. For example, mink provided with a water bowl which allowed drinking but not swimming reduced their motivation for a water bath which allowed both (Mason *et al.*, 1999). This indicates that the mink were working to gain the water bath partly for drinking as well as for swimming. Resources are described as being complementary if the consumption of one resource increases the motivation for another resource, for example, mink have been found to choose the warmest, driest area possible after swimming (Mason, pers comm).

The aim of this experiment was to investigate whether singly housed rabbits would push through a push-door to gain short periods of visual and minimal tactile contact with another rabbit through a mesh panel, and whether they would persist in doing so when increasing weights were added to the push-door. This was carried out using the methodology devised in Chapter 3. A comparison was made between how hard the rabbits would work for contact with a rabbit that was unfamiliar to them and a rabbit that was familiar to them, as well as for no reward. The rabbits were tested in a closed economy and were only allowed to work for one resource at a time, as an unfamiliar and familiar rabbit would be likely to be substitutable resources. Various behavioural measures were taken throughout the experiment to determine how hard the rabbits were willing to work and how quickly they worked to reach the end of the runway.

4.2 Methods

4.2.1 Animals, housing and husbandry

The experimental animals were eight naïve, female New Zealand White rabbits which were approximately 14 weeks old and weighed between 2.20kg and 2.60kg (mean 2.40kg, SD ± 0.14) at the start of the experiment. The rabbits were singly housed in standard laboratory cages (see Plate 4.1) measuring 71 x 56 x 46cm (floor area 3976cm^2), in racks of six cages. The rabbits were in auditory and olfactory contact with the other rabbits, but had no visual or tactile contact. Commercial rabbit pellets (STANRAB (P), Special Diet Services, UK), hay and water were available *ad libitum*. Carrots and cabbage were fed daily. Environmental enrichment was provided in the form of wooden blocks, cardboard tubes and small plastic mesh balls (cat toys). The light cycle was 10 hours light:12 hours dark with an hour of dim light between the two periods ('dawn' and 'dusk'). The average minimum and maximum room temperature were 13°C and 17°C respectively (SD ± 3°C). The rabbits were weighed once a week during routing health checks.

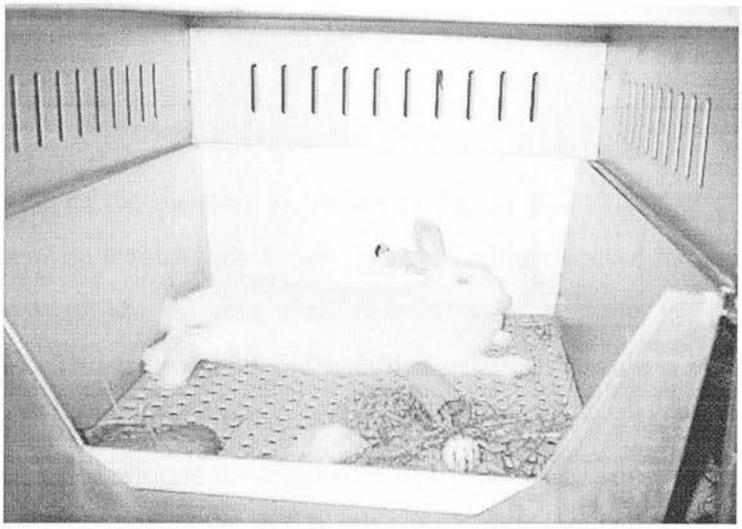

Plate 4.1 Standard laboratory rabbit cage used throughout the experiment

4.2.2 Apparatus

The experimental apparatus described and shown in Chapter 3 was used (see section *3.2.3*), with two amendments being made. The pen behind the mesh panel measured 40 x 40 x 40cm and the floor of both the runway and the pen were covered with dust-free shavings. The push-door described in section *3.2.3* was also used. Weights in the form of 4-inch long nails wrapped in tape were stuck to the back of the push-door during the tests.

4.2.3 Familiarisation with apparatus

The rabbits were familiarised with the runway with the push-door removed during five sessions totalling 120 minutes, with a mean duration of 25 minutes (SD ± 7.07). During familiarisation with the apparatus there was no rabbit in the pen at the end of the runway.

The rabbits were familiarised with the push-door over a three day period. During the initial period of 30 minutes the push-door was fixed open. On the subsequent two days the push-door was closed and the rabbits placed in the runway for 20 and 30 minutes respectively. The rabbits were observed from the next room through an observation window to ensure that they pushed through the closed push-door at least five times in each direction during both sessions. In addition, the rabbits were familiarised with the 'start box' of the runway on five separate occasions, for one minute each time.

4.2.4 Protocol

An overview of the protocol is shown in Figure 4.1. The test with the unfamiliar rabbit was of most relevance for singly caged rabbits housed in laboratory conditions, as rabbits tend not to have the opportunity to become familiarised with other individuals. This test was therefore carried out first to avoid the possibility of results being influenced by the familiar rabbit test. The control was always carried out last, as this was to investigate the extinction of the response of pushing through the push-door (see *4.2.4.4*). In addition, using this treatment order was as similar a protocol as possible to the experiments on socially caged rabbits, which had practical implications for the order in which tests were carried out (see Chapter 5, see section *5.2.5*). The order in which the individuals were tested was varied daily. After testing each rabbit in the experimental apparatus, any soiled shavings were removed. All tests were recorded on videotape using CCTV cameras (Panasonic WV-BP330/B), a quad unit (Panasonic WJ-410) and a time-lapse video recorder (Panasonic AG-6024) to enable more accurate measurements to be made.

Empty runway	**Unfamiliar Rabbit**	**Familiar Rabbit**	**Control (No reward)**	**Unfamiliar Rabbit**
0 weight (4 days)	**0 weight** (5 days) **Weights** (26 days)	**0 weight** (5 days) **Weights** (25 days)	**0 weight** (5 days) **Weights** (10 days)	**0 weight** (5 days)

Figure 4.1 Overview of protocol with duration of each test

4.2.4.1 Empty runway

Each rabbit was placed in the start box of the runway for 30 seconds, at which time the start box panel was raised to allow the rabbit access to the rest of the runway. The rabbits were allowed one minute in the runway after they had pushed through the push-door and moved opposite the mesh panel. This was carried out once a day for four days. The measurements in Table 4.1 were recorded and compared to the behaviour of the rabbits in the runway during the unfamiliar rabbit test (see section *4.2.4.2*) to investigate the immediate response of the rabbits to a conspecific.

Measurement	Definition
Time to mesh	Time taken to leave the start box, push through the door and move to a position opposite the mesh panel (at least the rabbit's head opposite the mesh)
Time to first approach push-door	Time taken to leave the start box and first approach the push-door (within 5cm)
First approach to through push-door	Time taken to push through the door after first approaching
Approach number	Number of approaches made to the push-door (within 5cm) before pushing through
Nudges	Total number of times the push-door was nudged before the rabbit pushed through
Push-door to mesh	Time taken to move to a position opposite the mesh panel after pushing through the door
Time spent opposite	Time spent opposite the mesh panel, of the one minute allowed after first moving opposite it (at least the rabbit's head opposite the mesh, i.e. in visual contact)
Time spent facing	Time spent facing the mesh panel, of the one minute allowed after first moving opposite it (opposite and facing the mesh)
Time in second half of runway	Time spent in the second half of the runway (after the push-door), of the one minute allowed after first moving opposite the mesh panel

Table 4.1 Measurements recorded during tests in the runway (times in seconds)

4.2.4.2 How hard will rabbits work to reach an unfamiliar rabbit?

A rabbit that was unfamiliar to the test rabbit was placed in the pen at the end of the runway. The procedure described in section *4.2.4.1* was carried out and the measurements in Table 4.1 were recorded. Five daily training trials were carried out to investigate whether or not the response times changed with successive trials. The unfamiliar rabbit used was changed every day so it remained unfamiliar to the test rabbit.

The procedure was repeated the day after the fifth training trial with a weight of 100grams on the push-door. The rabbits were tested daily with the weight being increased by 100grams each day until the rabbit did not push through the door within the maximum time allowed of ten minutes. The heaviest weight each rabbit pushed through was taken as the maximum price they were willing to pay to reach the unfamiliar rabbit. Weight increases of 100grams were used for all rabbits as they all had similar body weights at the start of testing.

4.2.4.3 How hard will rabbits work to reach a familiar rabbit?

Prior to this test the rabbits were assigned to experimental pairs and during daily 'familiarisation' sessions were placed in adjacent pens, allowing visual and minimal tactile contact between the individuals of a pair through a mesh partition. These sessions ranged in length from 45 to 60 minutes, totalling approximately 12 hours over the course of two weeks. After this time the pairs were assumed to be familiarised. The procedure described in section *4.2.4.2* was carried out.

4.2.4.4 Extinguishing the learnt response of pushing through the door for a reward (Control)

This was carried out to investigate how quickly the rabbits learnt that there was no reward (i.e. no rabbit) at the end of the runway. This would also ensure that pushing through the door to reach the end of the runway had not become a habit and that the rabbits were actually overcoming the cost of the weighted door to gain the assumed benefit of social contact with another rabbit. The same protocol as for the unfamiliar and familiar rabbit tests was carried out (see sections *4.2.4.2 and 4.2.4.3*).

4.2.4.5 Repeat trials with an unfamiliar rabbit

Five repeat trials with no weight on the push-door to gain access to an unfamiliar rabbit were carried out (as in section *4.2.4.2*). This was to investigate the findings of the control test (see section *4.3.5*) and to investigate whether rabbits would re-learn that there was a reward at the end of the runway.

4.2.5 Statistical analysis

The maximum weights the rabbits pushed through to gain contact with an unfamiliar rabbit, a familiar rabbit and for no reward (control) were compared using Friedman two-way analysis of variance. Multiple comparisons between groups or conditions were used to find where any significant differences lay (Siegel and Castellan, 1988).

The measurements recorded during the four trials when the runway was empty were compared using Friedman two-way analysis of variance. The median of these four trials was calculated for each measurement. These median values were then compared to the responses of the rabbits when the unfamiliar rabbit was first introduced (during the first training trial), to investigate their initial perception of the presence of a rabbit at the end of the runway. This was done using Wilcoxon signed rank tests. Comparisons were made between all five training trials to investigate any differences over time in how long the rabbits took to move through the runway to gain contact with the unfamiliar rabbit. This was carried out using Friedman two-way analysis of variance and multiple comparisons between trials to highlight where any significant differences lay. Friedman two-way analysis of variance was also used to compare the five training trials in the familiar rabbit test.

The data for the last training trial and all of the weighted trials in each of the three treatments was subjected to log transformation, with the exception of the time spent opposite the mesh panel which was not transformed as it was normally distributed. Each of the measurements recorded were analysed using a General Linear Model (GLM), blocking by rabbit and using the weight on the push-door as a covariate, to investigate any differences between treatments and any effect the weight on the push-door had on the measurements. Tukey tests were used to highlight where any significant differences between treatments lay. Analysis was carried out using Minitab 12.

4.3 Results

In both the unfamiliar and familiar rabbit tests all eight rabbits pushed through the push-door when it was unweighted and with at least the first weight of 100 grams attached. However, in the control test only three rabbits pushed through the push-door in all five trials when the push-door was unweighted and went on to push through at least the first weight on the push-door. The other five rabbits did not push through the unweighted push-door in any of the five trials. These five rabbits all approached the push-door, however, on no occasion did they nudge the push-door in an attempt to push through.

4.3.1 Maximum price paid

The median maximum price paid (i.e. heaviest weight pushed through) by each rabbit to reach an unfamiliar rabbit, familiar rabbit and no reward (control) are shown in Figure 4.2. Friedman two-way analysis of variance showed a significant difference between the three groups ($S=9.48$, $DF=2$, $p<0.05$). Multiple comparisons between groups or conditions found differences between both the unfamiliar and familiar rabbit tests compared to the control (both $z=10.5$, $p<0.05$). There was no significant difference in the maximum weights pushed through for unfamiliar compared to familiar rabbits. The maximum weight pushed through by a rabbit for social contact was 2.6 kg, which was approximately 76% of that individual's body weight. The minimum pushed through for social contact was 100 grams, which was only 3% of that individual's body weight.

4.3.2 Time to move through the runway in the unweighted trials

4.3.2.1 Empty runway

There were no significant differences between the four trials in any of the measures recorded. The median for each measurement was therefore calculated.

4.3.2.2 Introduction of the unfamiliar rabbit at the end of the runway

Comparisons of the measurements recorded when the runway was empty and when the unfamiliar rabbit was introduced in the first training trial found the only significant differences to be in the time spent opposite and facing the mesh panel. The rabbits spent significantly longer both opposite and facing the mesh panel when the unfamiliar rabbit was present than when the runway was empty (Wilcoxon signed rank $T=33$, $N=8$, $p<0.05$ and $T=35$, $N=8$, $p<0.05$ respectively).

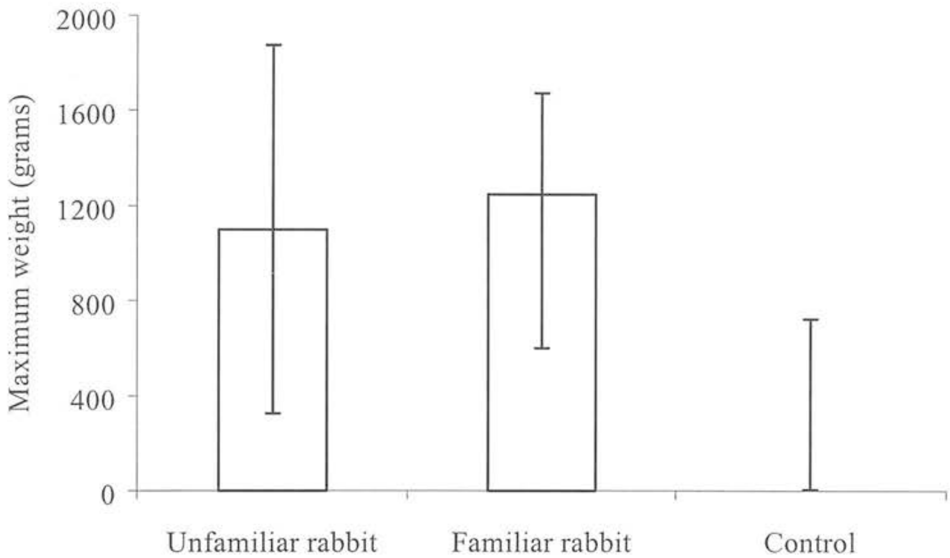

Figure 4.2 Median maximum weights pushed through to reach an unfamiliar rabbit, familiar rabbit and no reward (control), showing inter-quartile ranges

4.3.2.3 Five training trials with an unfamiliar rabbit

Comparisons between the five trials found a difference in the overall time taken to move through the runway to opposite the mesh panel ('time to mesh'; Friedman two-way analysis of variance, S=20.61, DF=4, $p<0.001$), with the rabbits moving through the runway more quickly by the second trial compared to the first ($z=14$, $p<0.05$). This overall decrease in the time to move through the runway between the first and second trials was due to the rabbits pushing through the door more quickly after first approaching (S=18.47, DF=4, $p<0.05$; $z=13$, $p<0.05$), which was in turn due to the rabbits approaching the push-door fewer times (S=19.38, DF=4, $p<0.05$; $z=15$, $p<0.05$) and nudging the door fewer times before pushing through (S=12.81, DF=4, $p<0.05$; $z=12.5$, $p<0.05$). There was no difference between the five trials in the time spent opposite and facing the unfamiliar rabbit.

4.3.2.4 Five training trials with a familiar rabbit

As with the training trials with the unfamiliar rabbit, comparisons between the five trials found a difference in the overall time taken to move through the runway to opposite the mesh panel ('time to mesh'; Friedman two-way analysis of variance, S=14.77, DF=4, $p<0.05$). The rabbits moved through the runway more quickly by the fourth trial compared to the first ($z=17.5$, $p<0.05$). This was due to the rabbits pushing through the push-door more quickly after first approaching (S=14.73, DF=4, $p<0.05$; $z=14.5$, $p<0.05$), which was due to the rabbits making fewer approaches to the door before pushing through (S=9.84, DF=4, $p<0.05$; $z=17$, $p<0.05$). There was no difference between the five trials in the time spent opposite and facing the familiar rabbit.

4.3.2.5 Five training trials with no reward (control)

During the five training trials with no rabbit at the end of the runway five of the rabbits did not push through the door within the time limit of ten minutes in any of the trials, with three rabbits pushing through the door in all five trials.

4.3.2.6 Repeat trials with an unfamiliar rabbit

Six of the rabbits pushed through the unweighted push-door in all five repeat trials with an unfamiliar rabbit, with the other two rabbits not pushing through during any of the five trials. For those rabbits that did push through, no significant differences were found between any of the five trials.

The first and last of the five repeat training trials with the unfamiliar rabbit were compared to the initial first and last training trials with the unfamiliar rabbit. No differences were found between the first training trials. In the fifth training trials however, the rabbits were significantly slower to move through the runway to the mesh panel in the repeat trials than in the initial trials (Wilcoxon signed rank T=21, N=6, $p<0.05$).

4.3.3 Time to move through the runway in the weighted trials

4.3.3.1 Time to mesh

There was found to be a difference between treatments in the overall time taken to move through the runway ('time to mesh'; GLM, $F_{(2,205)}=8.78$, $p<0.001$). The rabbits took significantly longer to reach the mesh panel in both the familiar rabbit test and the control compared to the unfamiliar rabbit test ($p<0.05$ and $p<0.001$ respectively).

The time to reach the mesh panel was also influenced by the weight on the push-door ($F_{(1,205)}$=21.37, $p<0.001$), with the time increasing as the weight was increased.

4.3.3.2 Time to first approach push-door

The rabbits were found to differ between treatments in the time taken to first approach the push-door ($F_{(2,221)}$=8.42, $p<0.001$), being slower to approach in the familiar rabbit test and control compared to the unfamiliar rabbit test ($p<0.05$ and $p<0.001$ respectively). The time to first approach the push-door increased as the weight increased ($F_{(1,221)}$=15.43, $p<0.001$).

4.3.3.3 First approach to through push-door

A difference was found between treatments in the time taken to push through the push-door after first approaching ($F_{(2,205)}$=5.94, $p<0.001$), with the rabbits being slower in the familiar rabbit test compared to the unfamiliar rabbit test. The time taken to push through the push-door after first approaching was also affected by the weight on the door ($F_{(1,205)}$=18.17, $p<0.001$), with the time increasing as the weight increased.

4.3.3.4 Approach number

The number of times the rabbits approached the push-door before pushing through was not affected by the treatment, however it was affected by the weight on the push-door ($F_{(1,206)}$=17.29, $p<0.001$), with the number of approaches increasing as the weight increased.

4.3.3.5 Number of nudges

As with the number of approaches to the push-door, the number of times the door was nudged before pushing through was not affected by the treatment, but was affected by the weight on the push-door ($F_{(1,206)}$=6.64, $p<0.001$), with the number of nudges increasing as the weight on the door increased.

4.3.3.6 Push-door to mesh

Neither the treatment nor the weight on the push-door affected the time taken to move in front of the mesh panel after pushing through the push-door.

4.3.3.7 Time spent opposite

Of the one minute allowed after the rabbits pushed through the push-door and moved opposite the mesh panel, the time the rabbits spent opposite the mesh panel differed between treatments ($F_{(2,205)}$=10.28, $p<0.001$). The rabbits spent longer opposite the mesh panel in the unfamiliar rabbit test than they did in the familiar rabbit test ($p<0.001$). The weight on the push-door did not affect the time spent opposite the mesh panel.

4.3.3.8 Time spent facing

Of the one minute allowed after the rabbits pushed through the push-door and moved opposite the mesh panel, the time the rabbits spent facing the mesh panel differed between treatments ($F_{(2,201)}$=8.78, $p<0.001$). As with the time spent opposite the mesh panel, the rabbits spent longer facing the panel in the unfamiliar rabbit test than they did in the familiar rabbit test ($p<0.05$). The weight on the push-door did not affect the time spent opposite the mesh panel.

4.3.3.9 Time spent in second half of runway

The time spent in the second half of the runway was not affected by either the treatment or the weight on the push-door.

4.4 Discussion

When an unfamiliar rabbit was first introduced at the end of the runway the rabbits spent significantly longer in front of and facing the mesh panel than they did before the rabbit was introduced. In the subsequent training trials the time to move through the runway decreased. The rabbits therefore chose to have visual and minimal tactile contact with a conspecific and chose to gain it more quickly suggesting that it was rewarding for them, as they might be expected to no longer push through the door or be slower in their responses if social contact was not rewarding.

When weights were added to the push-door the rabbits persisted in pushing through the door to gain social contact and pushed through heavier weights, i.e. paid a higher maximum price, to gain social contact than they did for no reward. The rabbits were slower to move through the runway as the weight increased, being slower to first approach the push-door and slower to push through the door after first approaching due to making more approaches and nudging the door more often before pushing through. This indicates that the weighted push-door imposed a cost on gaining social contact, yet the rabbits persisted in paying this cost. This indicates that visual and minimal tactile contact with conspecifics is something which rabbits want and is perceived by the rabbits as important to them. The current study has not, however, distinguished between whether the rabbits were working for both visual and tactile contact or for one more than the other. A survey of the UK pharmaceutical industry (see Chapter 2) found that just under half of female laboratory rabbits (46%) and all male laboratory rabbits were singly housed. Of the six companies singly caging rabbits, four used cages that allowed visual contact with rabbits in adjacent cages, however none allowed any physical contact. Cages tended to be positioned in rows opposite each other, one to two metres apart. The current study has found that rabbits will work for contact with rabbits in very close proximity, however, it is not known if visual contact at a distance of up to two metres is of value to rabbits.

There was no difference in the maximum weights the rabbits pushed through to gain contact with an unfamiliar rabbit or a familiar rabbit, although they moved through the runway to a position opposite the unfamiliar rabbit more quickly than they did for a familiar rabbit. The rabbits spent longer opposite and facing the unfamiliar rabbit than the familiar rabbit. No aggressive behaviour was seen, which suggests that the increased time spent in visual contact with the unfamiliar rabbit compared to the familiar rabbit was not due to territorial reasons. It is possible however that no difference was found in how hard rabbits would work for contact with an unfamiliar or familiar rabbit due to the fact that they did not perceive the familiar rabbit as being familiar to them. The rabbits were given visual and tactile contact with each other during familiarisation sessions, which obviously also provided olfactory communication. Rabbits in the wild scent mark their territory, using both anal and chin glands (Mykytowycz, 1962, 1968). Olfaction therefore plays an important role in recognising individuals as group members or intruders, and therefore whether they have to be driven away by the territory holders. Although the sense of smell of the rabbit is very well developed it is possible that scent is most important in recognising rabbits as familiar group members or unfamiliar rabbits, whilst physical contact is more important in the establishment and maintenance of relationships. Social interactions between female rabbits may begin with 'contact-making' behaviours (Stauffacher, 1986) such as nose-nose approaches, nose-body approaches or nose-anogenital approaches (Held *et al.*, 2001). Aggressive interactions tend to involve chasing, nipping, biting and scratching, with fur often being pulled out during such attacks. 'Contact-promoting' behaviours such as allogrooming (Stauffacher, 1986) are shown by rabbits once dominance is established and a rabbit may be seen pushing its head under another rabbit in order to be groomed (Gunn, 1994). It was not possible to allow the rabbits to have physical contact and therefore perform these social behaviours, as this would have had to be done very cautiously and gradually as rabbits can be very aggressive towards each other as they establish which is dominant over the other (Batchelor, 1991; Love and Hammond, 1991). As well as being very time consuming this has obvious risks of injury to the rabbits, potentially resulting in their removal from the study.

It is also possible that the results were affected by the order in which the treatments were carried out. In the familiar rabbit test the whole process of pushing through the runway to gain social contact may have been less novel to the rabbits as they had previously worked to gain contact with a conspecific in the unfamiliar rabbit test. This may have made the rabbits slower in their responses, although they still responded in a similar way as in the unfamiliar rabbit test in terms of pushing through similar maximum weights. Regardless of a possible effect of the treatment order, the rabbits persisted in pushing through up to 76% of their body weight to gain social contact and significantly heavier weights than they did for no reward.

During the control experiment when there was no rabbit in the pen at the end of the runway, it was found that only three of the eight test rabbits pushed through the door, even when it was unweighted. When further unweighted trials were carried out with an unfamiliar rabbit at the end of the runway most of the rabbits pushed through the door. As well as indicating that the rabbits were not pushing through the door due to habit, this suggests that olfactory and/or auditory cues from the unfamiliar/familiar rabbit were important during these tests. It appears that if a rabbit can hear and smell other rabbits, they want to have visual and/or minimal tactile contact with them. It is therefore possible that housing rabbits singly where they have strong olfactory cues from rabbits housed within close proximity, but are not able to see them could be frustrating for the rabbits.

Rabbits in the wild will defend their territory in aggressive interactions (Mykytowycz, 1958). It is possible that rabbits would perceive the experimental apparatus as their territory and therefore would be pushing through the door in order to inspect their territory and investigate the olfactory and auditory cues they received from the rabbit at the end of the runway. This may suggest that subordinate rabbits would not push through such heavy weights on the push-door as dominant rabbits. It was, however, not possible to assess dominance of the rabbits used as they were singly housed. However, the tests carried out in the runway were of short duration, only a maximum of ten minutes daily and this is unlikely to be long enough for the area to be perceived as a rabbit's territory.

In the wild, subordinate rabbits live on the fringes of home ranges and are able to avoid confrontation, however in the restricted space of a laboratory pen they are unable to move far away from the more dominant rabbits. It is thought that a group housing system may therefore be stressful for subordinate rabbits. Hurst *et al.* (1996) suggested that persistent aggressive interactions against rats that were unable to flee from aggressors were likely to cause social stress. Again, this would imply that subordinate rabbits would be unlikely to push through the door to gain social contact. However, physiological assessments of group housed rabbits have not found any differences between individuals and therefore any effect of social rank (Whary *et al.*, 1993; Turner *et al.*, 1997). Held *et al.* (1995) gave low-ranking rabbits a choice of whether or not they wanted to have social contact with other rabbits and found that they chose a large enriched group pen over a small, barren solitary pen. This indicates that any aversion that low-ranking rabbits may have to a group housing system was small enough to be clearly overridden by the small, barren solitary pen. It was however not possible to distinguish between whether the rabbits were choosing the enriched group pen for the social contact or for the physical aspects of the pen. As both ends of the runway used in the current study were identical other than the presence of an unfamiliar or familiar rabbit, the only choice the test rabbits had to make was between social contact or no social contact and it would seem that even timid rabbits (rabbits that would probably be subordinate in a social group) prefer to have social contact than to be without it.

It has been argued that short-term tests of motivation are not suitable for measuring an animal's motivation for a resource, as interrupting a bout may lower the value of the reward they are working for (Mason *et al.*, 1998). This has been particularly stressed for resources such as the opportunity to perform dust-bathing (Hutson, 1984) or nest-building as these are performed to a completion point. However Matthews *et al.* (1998) found that allowing hens to gain short periods of access to litter was a suitable way to quantify the value of the resource, as elasticity of the resource did not significantly differ with varying duration of access. Giving only short durations of access to a reward has also been used to measure motivation for food (e.g. Jackson, *et al.*, 1999) and for social contact (e.g. Matthews and Ladewig, 1994). Mason *et al.* (1998) criticised Matthews and Ladewig (1994) for only allowing pigs 15 seconds of social contact as a reward, with the only way of gaining more social contact being repeating the operant task. They compared this to being "Like trying to have a conversation with the phone repeatedly ringing…" and state that the interruptions may have devalued the behaviour and may explain why the pigs' demand for social contact was found to be only slightly less elastic than demand for an empty compartment. The rabbits in the current study, however, persisted in pushing through the weighted push-door and paying the cost of gaining only a short period of social contact. Jensen *et al.* (2000) used both pigs and calves to compare social behaviour when given short periods of social contact in succession compared to one continuous bout of social contact following 24 hours of social isolation. The pigs were found to show increased aggression when the social contact was subdivided and the authors suggested that if pigs were given short periods of social contact as rewards aggressive motivation rather than affiliative motivation may be measured. They found affiliative social motivation to be maintained in calves given short periods of social contact. It appears from the results of the current study that the rabbits were behaving more like the calves in the study by Jensen *et al.*, as the rabbits showed little aggression towards each other. This, and the fact that some of the rabbits were willing to pay two thirds of their body weight to reach the reward of social contact, suggests that short term motivational tests may be suitable for measuring social contact in rabbits.

4.5 Implications for laboratory rabbit housing

The results have implications for single housing of laboratory rabbits, suggesting that single housing should allow rabbits the opportunity to have both visual and tactile contact with other rabbits. The fact that rabbits showed no difference in how hard they worked for contact with an unfamiliar or familiar rabbit also has implications. It would seem that regardless of whether rabbits are always housed in the same cages next to the same rabbits, or are moved into different cages (perhaps for experimental reasons) it is still preferable for rabbits to be able to see the rabbits in the adjacent cages.

Chapter 5 – Motivation of socially caged female rabbits for short periods of visual and minimal tactile contact with a conspecific

Abstract

Rabbits have been found to choose to spend time with other rabbits when given the opportunity to do so and it is generally recommended that female laboratory rabbits should be housed socially where possible. Individuals may have to be removed from their social group due to injury or illness and it may be frustrating for a socially housed rabbit to be housed singly. Using a method previously used to measure motivation of singly caged rabbits for social contact (Chapter 4), the maximum weight socially housed rabbits were willing to push through for social contact after being separated from their cage-mate and how long they took to gain contact were measured to assess how important this was to them. Comparisons were made between how hard (i.e. maximum weight pushed through) and how quickly the rabbits worked for contact with an unfamiliar rabbit and their cage-mate. Comparisons were also made between how hard the rabbits worked for social contact when housed next to their cage-mate (i.e. only in olfactory contact) and when housed next to an unfamiliar rabbit (i.e. out of olfactory contact with their cage-mate). The rabbits were also given the opportunity to work for physical contact with their cage-mate. All of these treatments were compared with how hard the rabbits worked for no reward (i.e. no rabbit at the end of the runway). The rabbits were given daily sessions of up to one hour of contact with their cage-mate to ensure that they remained familiar. There was no difference in how hard the rabbits worked for visual and minimal tactile contact with an unfamiliar rabbit or their cage-mate. When housed next to their cage-mate (i.e. in olfactory contact) there was no difference in how hard the rabbits worked for social contact compared to the control, however they worked harder for social contact than in the control when housed next to an unfamiliar rabbit (i.e. out of olfactory contact with their cage-mate). In addition, the rabbits worked harder for visual and tactile contact with their cage-mate when housed

next to an unfamiliar rabbit than when next to their cage-mate. Olfactory contact with a former cage-mate appeared to be of value to the rabbits. Housing the rabbits with olfactory contact with their cage-mate appeared to de-value visual and minimal tactile contact. It seems that olfaction is therefore important in communication between rabbits. The dominance status of the rabbits in each pair was assessed, but there was found to be no difference between dominant and subordinate rabbits in the maximum weights pushed through in any of the treatments. The subordinate rabbits did however approach the mesh panel (and therefore the other rabbit) more quickly after pushing through the push-door and spent more time opposite the mesh panel compared to the dominant rabbits, suggesting that visual and tactile contact may be more important to subordinate rabbits. The motivation of the dominant rabbits to move through the runway may have partly been related to territory inspection as they appeared to be more bold in the runway, being faster to first approach the push-door and nudging the push-door more often. The findings suggest that if socially housed rabbits have to be removed from their social group they should be housed with visual contact with a conspecific and, if possible, with olfactory contact with a familiar rabbit.

5.1 Introduction

Singly housed rabbits have been found to choose to push through a push-door to gain access to visual and minimal tactile contact with a conspecific and continued to do so when a cost was imposed on gaining contact by adding increasing weights to the push-door (Chapter 4). Olfactory cues were found to have an important effect on the behaviour of the rabbits, in that several of the rabbits did not push through the unweighted push-door even once when there was no rabbit at the end of the runway and therefore no olfactory or auditory cues. As the rabbits were singly housed, any effect of dominance on the preference for social contact could not be established. The rabbits showed no difference in how hard they were willing to work for either an unfamiliar rabbit or a familiar rabbit (which they had been allowed visual and tactile contact with prior to the test). It was possible however, that, as the rabbits had not been familiarised by allowing full physical contact, a proper social bond had not been formed.

The aim of this experiment was to investigate whether socially housed rabbits would work to gain short periods of visual and minimal tactile contact with a conspecific. Comparisons were made between how hard and how quickly rabbits would work to regain contact with their cage-mate (familiar rabbit) and for contact with an unfamiliar rabbit. The importance of olfaction was investigated, by comparing how hard the rabbits worked for social contact when housed next to their cage-mate and next to an unfamiliar rabbit. When tested in an open economy, animals are able to access a resource they are being offered within the test situation for free at other times, whereas in a closed economy the resource is only available within the test situation. Testing the rabbits when provided with olfactory contact with their cage-mate and without it would indicate whether such contact devalues visual and tactile contact during the experiment, which would give an indication of how much value olfactory contact is. Comparisons were also made with how hard the rabbits worked for physical contact with their cage-mate and for no reward (control). It was not possible to allow the singly caged rabbits to work for the opportunity to have full physical contact with another rabbit due to the risk of aggression between newly introduced rabbits (Chapter 4). It was however possible to investigate the preference for full physical contact compared to visual and tactile contact with the socially caged rabbits, although only with their cage-mate. As suggested in Chapter 4, the dominance of individuals may affect their behaviour with regard to gaining social contact. The dominance status of the rabbits within their pairs was therefore assessed and comparisons of the cost paid for social contact were made between the dominant and the subordinate rabbits.

5.2 Methods

5.2.1 Animals, housing and husbandry

The experimental animals were fourteen naïve, female New Zealand White rabbits which were approximately 16 weeks old at the start of the experiment and weighed between 2.50kg and 3.75kg (mean 3.10kg, SD $\pm$ 0.37). The rabbits were housed in pairs in 'Tecniplast' interconnecting cages in racks of six cages. Each pair had access to two cages of floor area 5400cm², giving the rabbits a total floor space of 10800cm². Each cage was fitted with a platform, which provided a raised area of additional floor space of 2350cm^2 in each cage and a bolt-hole underneath. Access between the two cages was via a transparent sliding door at the height of the platform (Plate 5.1). The cages were 50cm in height, with approximately 30cm above the platform and 20cm below. A mesh panel at the front of the cage allowed the rabbits to see into the other half of the cage if they reared up onto their hind legs. The rabbits were in olfactory and auditory contact with other rabbits in the room, but did not have visual or tactile contact with them. Husbandry, diet and enrichment were the same as for previous experiments (see Chapter 4, section *4.2.1*).

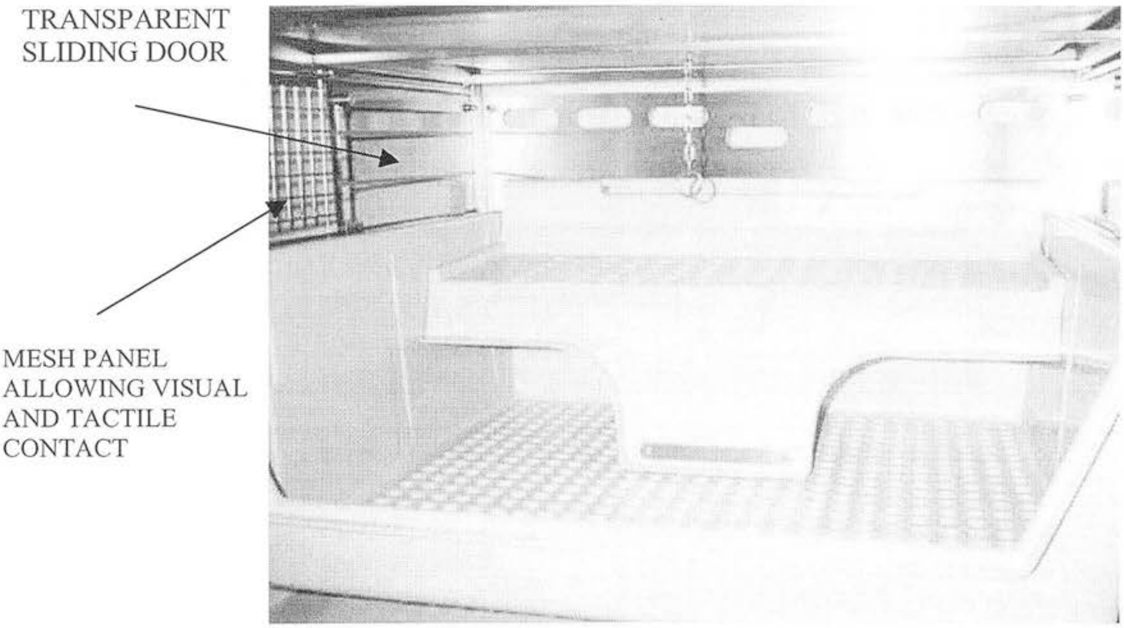

Plate 5.1 Interconnecting rabbits cages, showing the transparent door which can be opened to allow rabbits access between two cages and the mesh panel that allows visual and minimal tactile contact between rabbits

5.2.2 Assessment of dominance

The pairs of rabbits were separated from each other for 24 hours by closing the door between the cages and placing a board between the cages to prevent visual and tactile contact. After 24 hours the door was opened to allow the rabbits access to both cages and each other. The rabbits were filmed for 15 minutes immediately after the door was opened and the behaviours in Table 5.1 were recorded. This was carried out on four occasions, with three days between each.

For each pair the total number of dominant and submissive behaviours shown throughout the four 15 minute sessions was calculated. The proportion of the dominant and subordinate behaviours shown by each individual was calculated. The rabbit showing the largest proportion of dominant behaviours was assumed to be the dominant rabbit of the pair.

Behaviour	**Definition**
Dominant	
Attack	Rabbit runs aggressively at another with ears back in an attempt to bite it
Bite	Rabbit bites another when stationary
Chase	Rabbit runs after another and may cause it to retreat
Nudge	Rabbit displaced another (perhaps from a favoured site) by physically pushing it out of the way
Sexual behaviour	Circling and mounting
Threat	Head lowered, ears laid flat as though about to attack
Submissive	
Retreat from threatening behaviour	Rabbit makes a running retreat from an attack or chase
Retreat from non-threatening behaviour	Rabbit makes a retreat from a non-threatening approach by another rabbit
Submission	Either the rabbit avoids contact with another by turning away or accepts the dominance of another rabbit by lying flat without any resistance or attempts to escape

Table 5.1 Dominant and subordinate behaviours used to assess dominance of the individuals of each pair of rabbits (adapted from Gunn, 1994)

5.2.3 Apparatus

The apparatus used was the same as that used in Chapter 4 (see section *4.2.2*). The apparatus was modified for the physical contact test (see section *5.2.5.5*). The mesh panel at the end of the runway was removed and replaced with an unweighted one-way push-door, which could only be opened from the runway. Behind the push-door was a pen measuring 90 x 90cm, made of the same materials as the runway with the same floor surface.

5.2.4 Familiarisation with apparatus

Familiarisation with the runway and push-door was the same as in Chapter 4 (see section *4.2.3*), with the exception of the duration of the familiarisation sessions with the push-door. Each session (one with the push-door open and two with it closed) was reduced from 30 minutes to 15 minutes, as in Chapter 4 the rabbits all pushed through the door the required five times within the first few minutes of each session.

5.2.5 Protocol

An overview of the protocol is shown in Figure 5.1. The order of the tests shown was the same for all rabbits, due to practical constraints of separating rabbits and moving rabbits between racks of cages for certain treatments (see sections *5.2.5.2* and *5.2.5.4*). Four days prior to the tests and throughout the tests, the pairs of rabbits were housed separately. In order to ensure that cage-mates remained familiar with each other they were allowed a period of between 30 minutes and one hour of physical contact each day after testing, during all of the tests. Allowing the rabbits to have contact meant that they were not tested in a fully closed economy, which risked the possibility of the rabbits learning that they would have 'free' social contact after being in the runway and therefore reducing how hard they were willing to work for social contact. To reduce the effects of this, the time between the daily trial and the period of physical contact was varied at random each day, from 30 minutes to four hours. The order in which the rabbits were tested was varied daily. After testing each rabbit in the experimental apparatus any soiled shavings were removed. All tests were recorded on videotape using CCTV cameras (Panasonic WV-BP330/B), a quad unit (Panasonic WJ-410) and a time-lapse video recorder (Panasonic AG-6024) to allow for more accurate measurements to be made.

Housing	Next to cage-mate with olfactory contact (30-60 minutes daily "re-familiarisation" with cage-mate)				Next to unfamiliar rabbit, no olfactory contact with cage-mate, (30-60 minutes daily "re-familiarisation" with cage-mate)		
Stimulus	Empty runway	Unfamiliar Rabbit	Familiar Rabbit	Control (No reward)	Unfamiliar Rabbit 2	Familiar Rabbit 2	Physical contact (cage-mate)
Test regime	0 weight (4 days)	0 weight (5 days) Weights (6 days)	0 weight (5 days) Weights (6 days)	0 weight (5 days) Weights (7 days)	0 weight (5 days) Weights (8 days)	0 weight (5 days) Weights (9 days)	0 weight (5 days) Weights (9 days)

Figure 5.1 Overview of protocol with housing and duration of each test

5.2.5.1 Empty runway

Each rabbit was placed in the start box of the runway for 30 seconds, after which the start box panel was raised to allow the rabbit access to the rest of the runway. The rabbits were allowed one minute in the runway after they first moved opposite the mesh panel. This was carried out once a day for four days. The measurements in Table 5.2 were recorded and compared to the behaviour of the rabbits in the runway during the first training trial of the unfamiliar rabbit test to investigate the immediate response of the rabbits to a conspecific (see section *5.2.5.2*).

5.2.5.2 How hard will rabbits work for visual and minimal tactile contact when housed in olfactory contact with their cage-mate?

The pairs of rabbits were housed separately by closing the sliding door between the two cages. A board was placed between the two cages to prevent visual and tactile contact between cage-mates. The procedure in section *5.2.5.1* was carried out with an unfamiliar rabbit in the pen at the end of the runway (Unfamiliar 1) and again with a familiar rabbit (cage-mate) in the pen (Familiar 1). The measurements in Table 5.2 were recorded. The unfamiliar rabbit used was changed every day so it remained unfamiliar to the test rabbit.

The procedure was repeated the day after the fifth training trial with a weight attached to the back of the push-door. The body weights of the rabbits varied by over 1kg at the start of the experiment, therefore the weight on the push-door was varied according to each rabbit's body weight, with the first weight being 3% (approximately 100grams). The rabbits were tested daily with weight being increased by 3% of body weight each day (to the nearest 50grams). This was continued until the rabbit did not push through the door within the maximum time allowed of ten minutes. The heaviest weight each rabbit pushed through was taken as the maximum price they were willing to pay to reach the unfamiliar or familiar rabbit.

Measurement	Definition
Time to mesh	Time taken to leave the start box, push through the door and move to a position opposite the mesh panel (at least the rabbit's head opposite the mesh)
Time to first approach push-door	Time taken to leave the start box and first approach the push-door (within 5cm)
First approach to through push-door	Time taken to push through the door after first approaching
Approach number	Number of approaches made to the push-door (within 5cm) before pushing through
Nudges	Total number of times the push-door was nudged before the rabbit pushed through
Push-door to mesh	Time taken to move to a position opposite the mesh panel after pushing through the door
Time spent opposite	Time spent opposite the mesh panel, of the one minute allowed after first moving opposite it (at least the rabbit's head opposite the mesh, i.e. in visual contact)
Time spent facing	Time spent facing the mesh panel, of the one minute allowed after first moving opposite it (opposite and facing the mesh)
Time in second half of runway	Time spent in the second half of the runway (after the push-door), of the one minute allowed after first moving opposite the mesh panel

Table 5.2 Measurements recorded during tests in the runway (times in seconds)

5.2.5.3 Extinguishing the learnt response of pushing through the door for a reward (Control)

This was carried out to investigate how long the rabbits took to learn that there was no reward at the end of the runway. This would also ensure that pushing through the door to reach the end of the runway had not become a habit for the rabbits and that they were actually overcoming the cost of the weighted door to gain the assumed benefit of social contact with another rabbit. The same protocol as for Unfamiliar 1 and Familiar 1 was carried out.

5.2.5.4 How hard will rabbits work for visual and minimal tactile contact when housed without olfactory contact with their cage-mate?

The pairs of rabbits were housed separately in single cages in different racks within the same room. This prevented rabbits having close olfactory contact with their cage-mate, which they had when housed in adjacent cages. Each rabbit was housed next to an unfamiliar rabbit, with the sliding door closed and a board placed between the two cages to prevent visual and tactile contact. The protocol in section *5.2.5.2* was carried out with both an unfamiliar rabbit (Unfamiliar 2) and their cage-mate (Familiar 2) in the pen at the end of the runway. As Unfamiliar 2 followed the control, the rabbits were re-learning the response of pushing through the door for a reward.

5.2.5.5 How hard will rabbits work to gain physical contact with their cage-mate?

The rabbits were housed as in section *5.2.5.4*. The runway was modified as described in section *5.2.3*. A familiarisation session was carried out in the runway with no rabbit in the pen at the end of the runway. The rabbits were allowed to explore the modified apparatus until they had pushed through the second push-door, after which they were allowed one minute in the pen. Each rabbit was then tested with their cage-mate placed in the pen at the end of the runway. The test rabbit was able to push through the first push-door and then through the second door to gain physical contact with its cage mate. The second push-door was always unweighted as it was only used to provide a means of allowing the test rabbit into the pen without allowing its cage-mate to come out of the pen into the runway. The same procedure used in the other tests of five unweighted trials and adding weights until the rabbits no longer pushed through was used.

5.2.6 Statistical analysis

The maximum weights the rabbits pushed through in each treatment were compared using Friedman two-way analysis of variance. Multiple comparisons between groups or conditions were used to find where any significant differences lay (Siegel and Castellan, 1988). For each treatment, the maximum weights pushed through by the dominant and subordinate rabbits were compared using Wilcoxon signed rank tests. Comparisons were made between treatments for the dominant and subordinate rabbits separately, using Friedman two-way analysis of variance. The body weights of the dominant and subordinate rabbits were compared at the end of each treatment, using Wilcoxon signed rank tests to investigate any differences in body weight that could have affected the maximum weights pushed through.

The measurements recorded during the four trials when the runway was empty were compared using Friedman two-way analysis of variance. The median of these four trials was calculated for each measurement. These median values were then compared to the responses of the rabbits when the unfamiliar rabbit was first introduced (during the first Unfamiliar 1 training trial), to investigate their initial response to the presence of a rabbit at the end of the runway. This was done using Wilcoxon signed rank tests. Comparisons were made between the five training trials for each treatment to investigate any differences over time in how long the rabbits took to move through the runway. This was carried out using Friedman two-way analysis of variance and multiple comparisons between trials to highlight where any significant differences lay.

The data for the last training trial and all of the weighted trials in Unfamiliar 1, Familiar 1, Unfamiliar 2, Familiar 2 and the control was subjected to log transformation, with the exception of the time spent opposite and facing the mesh panel which were not transformed as they were normally distributed. Each of the measurements recorded were analysed using a General Linear Model (GLM), blocking by rabbit and using the weight on the push-door as a covariate, to investigate any differences between treatments and any effect the weight on the push-door had on the measurements. Tukey tests were used to highlight where any significant differences lay. The physical contact test was not included in this analysis as the procedure used in that test differed slightly from the other tests. Further analysis using GLM was carried out to investigate any effects of dominance on the measurements recorded, this time blocking by pair and dominance. Analysis was carried out using Minitab 12.

5.3 Results

5.3.1 Assessment of dominance

One rabbit within each pair was always clearly dominant over the other rabbit in each of the four observation periods. Dominant rabbits performed 93% (SD ± 9.77) of the dominant behaviours seen and subordinates performed 99% (SD ± 1.89) of the subordinate behaviours seen.

5.3.2 Responses

In both Unfamiliar 1 and Familiar 1, all of the rabbits pushed through the push-door in the five unweighted training trials and with at least the first weight on the push-door. In Unfamiliar 2, Familiar 2 and the physical contact test, all of the rabbits pushed through the push-door in the training trials and all but one pushed through at least the first weight on the push-door. In the control, four of the rabbits gave up pushing through the push-door before the end of the five training trials, with two not pushing through by the third trial and two by the fourth trial. The other eight rabbits pushed through at least the first weight on the push-door.

5.3.3 Maximum weights pushed through

The maximum weight pushed through by any individual for social contact when housed next to their cage-mate (Unfamiliar 1 and Familiar 1) was 600grams, which was 15% of that individual's body weight. The maximum pushed through by an individual for social contact when housed next to an unfamiliar rabbit was 1050grams (24% of body weight). In the control and physical contact test the maximum weights pushed through were 800grams (18% of body weight) and 950grams (24% of body weight). The median maximum weights pushed through by each rabbit in each of the treatments are shown in Figure 5.2.

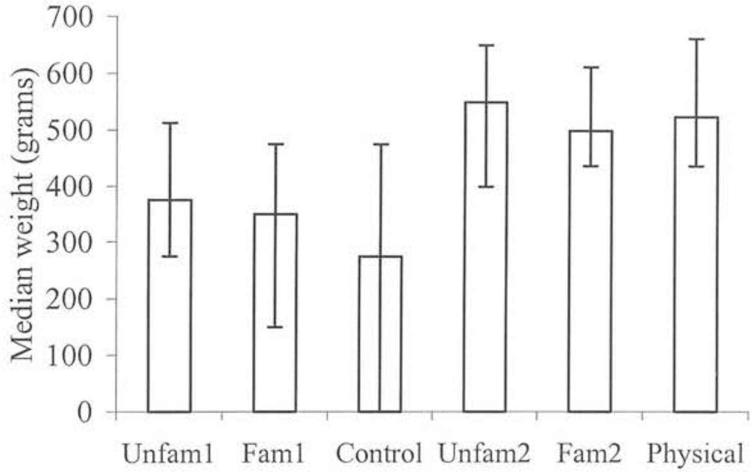

Figure 5.2 Median maximum weights pushed through in each treatment, showing inter-quartile ranges (Unfam = Unfamiliar, Fam = Familiar, Physical = Physical contact)

There was found to be a significant difference in the maximum weights pushed through in the different treatments (Friedman two-way analysis of variance, $S=18.76$, $DF=5$, $p<0.005$). Multiple comparisons between groups found that the maximum weights pushed through in Unfamiliar 2, Familiar 2 and in the physical contact test were significantly higher than the control ($z=26$, 26.5 and 29.5, $p<0.05$), although they did not differ from each other. The maximum weights pushed through in the physical contact test were significantly higher than those pushed through in Unfamiliar 1 and Familiar 1 (both $z=24$, $p<0.05$). The rabbits pushed through significantly heavier weights in Familiar 2 than in Familiar 1 ($z=21$, $p<0.05$).

The maximum weights pushed through were also analysed to compare the dominant and subordinate rabbits of each pair. There were found to be no significant differences between the dominant and subordinate rabbits in any of the treatments (see Figure 5.3). The inter-quartile range bars however indicate that there was more variation in the maximum weight pushed through by subordinate rabbits than dominant rabbits. The subordinate rabbits showed no significant difference between treatments, but the dominant rabbits did show a treatment difference (Friedman two-way analysis of variance, $S=18.27$, $DF=5$, $p<0.05$). The dominant rabbits pushed through heavier weights in Familiar 2 and in the physical contact test compared to Unfamiliar 1 ($z=16.5$ and 19 respectively, $p<0.05$), Familiar 1 ($z=16.5$ and 19 respectively, $p<0.05$) and the control ($z=15.5$ and 18 respectively, $p<0.05$). No differences in body weight between dominant and subordinate rabbits were found at any stage.

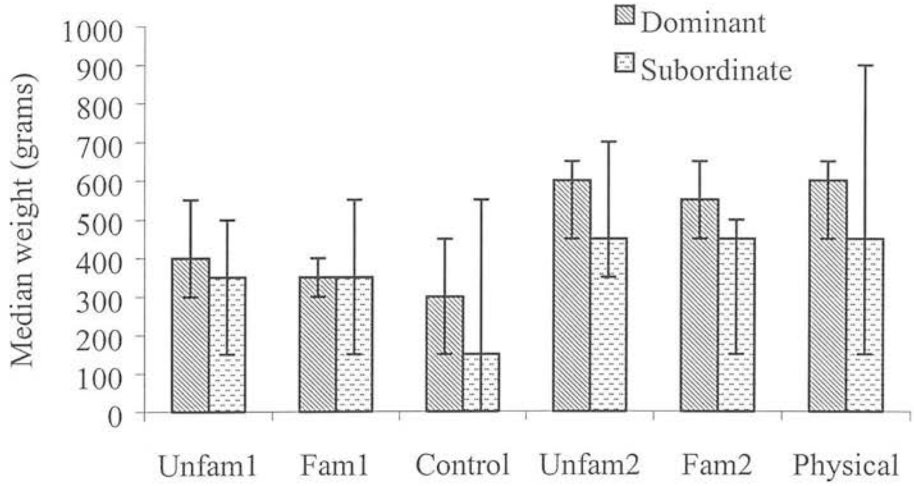

Figure 5.3 Median maximum weights pushed through by dominant and subordinate rabbits in each treatment, showing inter-quartile ranges

5.3.4 Time to move through the runway in the unweighted trials

5.3.4.1 Empty runway

Analysis of the data recorded found that there were no significant differences between the four trials in any of the measures recorded. The median for each measurement was therefore calculated.

5.3.4.2 Introduction of the unfamiliar rabbit at the end of the runway

When a rabbit was first placed in the pen at the end of the runway during the first Unfamiliar 1 training trial, there was no significant difference in the overall time taken to reach the end of the runway. However, once the rabbits had pushed through the push-door they were significantly slower to approach the mesh panel when the rabbit was first introduced compared to when there was no rabbit (Wilcoxon signed rank, $T=57$, $N=14$, $p<0.05$). The rabbits also spent significantly longer in the second half of the runway (Wilcoxon signed rank, $T=84$, $N=14$, $p<0.05$) and longer both opposite and facing the mesh panel (both Wilcoxon signed rank, $T=105$, $N=14$, $p=0.001$) when the unfamiliar rabbit was introduced.

5.3.4.3 Five training trials - Unfamiliar 1

There was found to be a difference in the time taken to move through the runway to the mesh panel (Friedman two-way analysis of variance, $S=22.76$, $DF=4$, $p<0.001$). The rabbits moved through the runway more quickly in the second trial compared to the first trial ($z=21.5$, $p<0.05$). This was found to be due to the rabbits first approaching the push-door more quickly ($S=12.62$, $DF=4$, $p<0.05$; $z=24$, $p<0.05$), pushing through the push-door more quickly after first approaching ($S=18.08$, $DF=4$, $p=0.001$; $z=18$, $p<0.05$) and moving from the push-door to opposite the mesh panel more quickly ($S=13.81$, $DF=4$, $p<0.05$; $z=22$, $p<0.05$). Pushing through the push-door more quickly after first approaching was due to the rabbits nudging the push-door fewer times before pushing through ($S=12.09$, $DF=4$, $p<0.05$; $z=20$, $p<0.05$).

5.3.4.4 Five training trials - Familiar 1

No significant differences were found between the five trials in any of the measurements taken.

5.3.4.5 Five training trials - Control

No significant differences were found between the five trials in any of the measurements taken.

5.3.4.6 Five training trials - Unfamiliar 2

During this phase of re-learning that there was a rabbit at the end of the runway, the rabbits did not show a significant difference between trials in the overall time taken to move through the runway to a position opposite the mesh panel. However, by the third trial the rabbits pushed through the door significantly faster after first approaching it than in the first trial (Friedman two-way analysis of variance, S=11.99, DF=4, $p<0.05$; z=21.5, $p<0.05$) and by the fourth trial approached the door significantly fewer times before pushing through than in the first trial (S=19.04, DF=4, p=0.001; z=18.5, $p<0.05$).

5.3.4.7 Five training trials - Familiar 2

The only significant difference was in the amount of the one minute of time allowed that was spent opposite the mesh panel (Friedman two-way analysis of variance, S=19.01, DF=4, p=0.001). In the second and third trials the rabbits spent significantly longer opposite the familiar rabbit (their cage-mate) than they did in the first trial (z=22.5 and 18.0, $p<0.05$) although this was not sustained in the fourth and fifth trials.

5.3.4.8 Five training trials - Physical contact

The only difference shown in the time taken to move through the runway was in the time to push through the push-door after first approaching (Friedman two-way analysis of variance, S=11.60, DF=4, $p<0.05$). The rabbits took significantly longer to push through the door in the fifth trial compared to the first and second (z=19.5 and 20.5, $p<0.05$).

5.3.5 Time to move through the runway in the weighted trials

The results of the General Linear Models are shown in Table 5.3. For each of the measurements recorded there was a difference between individuals, with the exception of the number of approaches made before pushing through the push-door ('Approach number'). The weight on the push-door had an effect on each of the measurements, with all of the time measurements increasing as the weight on the push-door increased. The number of approaches made before pushing through and number of nudges to the door before pushing through also increased as the weight on the push-door increased.

Measurement	Individual	Weight	Dominance	Treatment	Treatment differences
Time to mesh	** $F_{(13,272)}$=5.32	** $F_{(1,272)}$=95.42		* $F_{(4,272)}$=3.82	C > U1, F1, U2 All *
Time to first approach push-door	** $F_{(13,339)}$=19.4	* $F_{(1,339)}$=8.65	* $F_{(1,345)}$=5.27	** $F_{(4,339)}$=5.47	C > U1, F1, both ** F2 > U1, F1, both *
First approach to through push-door	** $F_{(13,265)}$=2.83	** $F_{(1,265)}$=99.7		* $F_{(4,265)}$=4.06	C > F1*, U2**, F2*
Approach number		** $F_{(1,272)}$=78.9			
Number of nudges	** $F_{(13,273)}$=5.42	** $F_{(1,273)}$=119.3	* $F_{(1,279)}$=4.76	* $F_{(4,273)}$=2.93	U1 > U2*, F2**
Push-door to mesh	** $F_{(13,258)}$=7.19	* $F_{(1,258)}$=12.28	** $F_{(1,264)}$=13.84		
Time spent opposite	** $F_{(13,272)}$=6.15	* $F_{(1,272)}$=9.31	* $F_{(1,278)}$=6.89		
Time spent facing	** $F_{(13,272)}$=6.05	* $F_{(1,272)}$=6.05			
Time spent in second half of runway	** $F_{(13,272)}$=2.95	** $F_{(1,272)}$=23.82			

Table 5.3 Results from General Linear Models, showing differences between individuals, effects of weight and dominance and differences between treatments for each measurement taken (* = $p<0.05$, ** = $p<0.001$; U=Unfamiliar, F=Familiar, C=Control)

Treatment differences were found in the overall time taken to move through the runway to the mesh panel, the time to first approach the push-door and the time to push through the door after first approaching, with the rabbits being slowest in the control. There was also a difference between treatments in the number of times the push-door was nudged before the rabbits pushed through. Dominance affected four of the measurements, with the subordinate rabbits being slower than the dominant rabbits to first approach the push-door, but then moving opposite the mesh panel after pushing through the door more quickly and spending more time opposite the mesh panel than the dominant rabbits. The dominant rabbits nudged the push-door more times before pushing through.

5.3.6 Physical contact versus visual/tactile contact

During 11% of the total number of trials carried out, rabbits pushed through the weighted push-door and moved to a position opposite the second push-door where they could gain visual contact with their cage-mate through the perspex push-door, but did not push through the second unweighted door to gain physical contact. Eight of the fourteen rabbits showed this response, with five of these rabbits only showing this once. The maximum number of trials this was shown in by one rabbit was five. Of the eight rabbits, five were subordinate and three were dominant.

5.4 Discussion

When the rabbits were housed out of olfactory contact with their cage-mate (i.e. in a different rack of cages) and were housed next to an unfamiliar rabbit, but were given up to one hour of contact with their cage-mate each day, they did not show any difference in the weights they were willing to push through to gain visual and minimal tactile contact with either their cage-mate or an unfamiliar rabbit. However, the rabbits pushed through significantly heavier weights than they did when there was no rabbit at the end of the runway, in the control. When the rabbits were housed in olfactory contact with their cage-mate but were prevented from having physical or visual contact with them (for approximately 23 hours a day), they again did not show any difference in the weights they were willing to push through to gain visual and minimal tactile contact with their cage-mate or with an unfamiliar rabbit. In addition, the maximum weights pushed through for contact with a conspecific did not differ significantly from those the rabbits pushed through when there was no rabbit at the end of the runway. As short periods of contact with their cage-mate was allowed under both these conditions, this suggests that olfactory contact with their cage-mate was sufficient to reduce the value of and therefore the demand for visual contact.

The maximum weights pushed through to reach an unfamiliar rabbit did not differ according to how the rabbits were housed. However, when housed out of olfactory contact with their cage-mate, the rabbits pushed through significantly heavier weights for visual contact with their cage-mate than they did when they were housed in olfactory contact with their cage-mate. This suggests that olfactory deprivation increases the strength of the rabbits' preference for visual and minimal tactile contact with a familiar rabbit, indicating that olfaction plays an important role in communication between rabbits. It was similarly found in Chapter 4 that olfactory cues influenced how the rabbits responded in the test apparatus, and was it suggested that olfactory contact may be important in the recognition of familiar rabbits. Olfaction is known to be the most important sense for the wild rabbit (e.g. McBride, 1988). Rabbits in the wild defend territories and scent mark their territories using glands under the chin and in the anal region (Mykytowycz, 1962, 1968). They deposit faecal pellets on dung-hills (Mykytowycz, 1968), or latrines (Thompson and Worden, 1956), around the warren area and spray areas with urine during aggressive disputes (Mykytowycz, 1968). Interestingly, it was noted during casual observations of the rabbits in their cages that when they were first housed next to an unfamiliar rabbit (with no visual contact), more than half of the rabbits began using the area on top of the platform nearest to the adjacent cage as their latrine area. This was continued throughout the time that they were housed next to the unfamiliar rabbit.

When rabbits were given the opportunity to work to gain physical contact with their cage-mate they worked significantly harder than they did for no reward and for visual contact when housed with olfactory contact with their cage-mate. There was no difference in the weight the rabbits were willing to push through for physical contact compared to visual contact when housed out of olfactory contact with their cage-mate. This again indicates the importance of olfactory contact between familiar rabbits and suggests that following olfactory deprivation with a familiar rabbit, rabbits are equally motivated for visual and physical contact. The importance of visual contact was also indicated by the fact that during the physical contact test, there were occasions when the rabbits paid the cost of pushing through the weighted push-door but did not push through the second unweighted door to gain physical contact. These rabbits were therefore paying the cost for visual contact through the push-door. These findings are similar to the suggestion in Chapter 4, that olfactory contact may be important in the recognition of familiar rabbits, and that physical contact may be more important for establishing new relationships between rabbits rather than for maintaining existing ones. However, when familiar female rabbits are separated and re-introduced (even after a matter of hours) they are usually seen to initially behave almost as though they are unfamiliar and re-establish dominance and bonds very quickly. This may be done through 'contact-making' behaviours (Stauffacher, 1986) such as nose-nose approaches, nose-body approaches or nose-anogenital approaches (Held *et al.*, 2001); or through 'contact-promoting' behaviours such as allogrooming (Stauffacher, 1986). Aggressive interactions may be seen and may involve chasing, nipping, biting and scratching, with fur often being pulled out during such attacks. The initial period of re-introduction can therefore be used to assess the dominance status of individuals, as was done in this experiment. It is possible, however, that this re-establishment of dominance and social bonds is the reason that the rabbits did not always push through the second push-door to gain physical contact when they had the opportunity to do so, although it might be expected that it would be the subordinate rabbits that responded in this way and this was not always the case.

The maximum weights the rabbits pushed through were found to be lower than those pushed through by singly caged rabbits in Chapter 4. The median maximum weights pushed through by the singly caged rabbits in Chapter 4 were 1100grams for an unfamiliar rabbit and 1250grams for a familiar rabbit, which were approximately 30 and 35% of the body weight of the rabbits. In the current study, the socially caged rabbits pushed through a median maximum weight of 550grams for an unfamiliar rabbit (Unfamiliar 2) and 500grams for their cage-mate (Familiar 2), which was less than 15% of the body weight of the rabbits. This suggests that singly housed rabbits are more motivated for social contact than socially housed rabbits. However, this may also be due to the fact that the singly housed rabbits were tested in a fully closed economy (i.e. were unable to gain visual and tactile contact with a conspecific other than in the experimental set-up), whereas the socially housed rabbits were not tested in a fully closed economy. In order to ensure that the socially housed rabbits remained familiar with their cage-mate and therefore perceived contact with their cage-mate in the same way throughout the experiment the rabbits were unavoidably given short periods of social contact each day following the behavioural tests. It is possible that the rabbits learnt that they were able to gain social contact out-with the experimental set-up and were therefore not prepared to pay the cost. Despite this, the rabbits did push through the weighted push-door to gain social contact and differences between treatments were found, indicating that the rabbits perceived the treatments differently and responded accordingly. It appears that although a short period of physical contact each day may affect how motivated rabbits are for further contact, it does not completely satisfy the need for social contact. As in Chapter 4, there may also have been an effect due to the order in which the tests were carried out. There was however no overall pattern of increase or decrease in responses as the tests were carried out, and the weights were seen to decrease (although not significantly) from working for social contact to working for no reward and increase again when working for social contact. Therefore, again, the rabbits were responding according to the rewards.

As well as the maximum weights animals will push through to gain a reward, the time taken to do so can give an indication of how the animals perceive the reward. In the current experiment, when an unfamiliar rabbit was first placed in the pen at the end of the runway the rabbits tended to be hesitant to approach the mesh panel after pushing through the push-door. However, they spent more time opposite and facing the mesh panel than they had then there was no rabbit in the pen, indicating that social contact with an unfamiliar rabbit was perceived as a reward rather than being aversive. The rabbits were found to move through the runway to reach the mesh panel more quickly during subsequent training trials. The differences were not as notable the second time the rabbits had to learn that there was a rabbit at the end of the runway, i.e. during the training trials with an unfamiliar rabbit after the control trial.

During the weighted trials, the only significant differences in the overall time taken to move through the runway to opposite the mesh panel were between the control and other treatments. The time to move through the runway was not affected by whether the rabbit in the pen was unfamiliar or familiar, which supports the findings of the analysis of the maximum weights pushed through, which found no differences between how hard rabbits worked for unfamiliar or familiar rabbits. This was also true of the time taken to push through the push-door after first approaching. The increasing weight on the push-door affected several of the measurements recorded, causing the rabbits to be slower in the overall time taken to move through the runway, slower to first approach the push-door, slower to push through the door after first approaching and caused the rabbits to make more approaches to the push-door and to nudge the push-door more times before pushing through. This indicates that the weighted push-door was imposing a cost on the rabbits gaining social contact, yet they persisted to overcome this cost for the reward. The push-door was similarly perceived as a cost by the singly housed rabbits in Chapter 4.

A further suggestion made in Chapter 4 was that since dominant rabbits defend their territories in the wild (e.g Bell, 1983), it may be expected that the dominant rabbits in the experiment would be more motivated to 'inspect' the experimental apparatus than subordinate ones, and may therefore push through heavier weights that subordinate rabbits in order to do so. It was also suggested that subordinate rabbits might prefer to avoid social contact, a second reason for expecting that dominant rabbits might push through heavier weights than subordinates. In contrast to these predictions, no differences were found in the maximum weights pushed through by the dominant rabbits compared to the subordinate rabbits in any of the treatments, even in the test for physical contact where it might be expected that subordinate rabbits would avoid the initial period of the re-establishment of dominance and the possibility of aggressive interactions. Dominance was, however, found to have an effect on several measurements taken as the rabbits moved through the runway. The subordinate rabbits were slower to first approach the push-door than the dominant rabbits, although once they had pushed through the push-door the subordinate rabbits move opposite the mesh panel more quickly than the dominant rabbits, and once in front of the mesh panel, the subordinate rabbits spent longer opposite it than the dominant rabbits did, suggesting that the visual contact was more rewarding to the subordinate rabbits than to the dominant rabbits. Although the subordinate rabbits did not differ between treatments in the maximum weights pushed through, it is possible that they were pushing through the push-door for different reasons. The subordinate rabbits may have been seeking social contact for security and the benefit of increased vigilance, as although they had been in the runway many times, this was still an area other than their home cage and therefore a less 'secure' environment. The subordinate rabbits showed more variation in the weights pushed through during all of the treatments which may indicate that some rabbits were more confident in the runway than others. The behaviour of the dominant rabbits in the runway could be thought of as being more bold than that of the subordinate rabbits as they first approached the push-door more quickly than subordinate rabbits and once they approached the push-door they nudged the door more times before pushing through than the subordinate rabbits. They also showed a difference between treatments. The motivation of the dominant rabbits to push through the push-door may have been partly related to territorial behaviour. Although there was no difference in the maximum weights the dominant and subordinate rabbits pushed through in the control, there appears to be more variability in the weights pushed through by the subordinate rabbits, whereas the dominant rabbits have been more similar in their responses. This also could suggest that the dominant rabbits were partly motivated to explore the environment regardless of whether there was a social reward at the end of the runway.

5.5 Implications for laboratory rabbit housing

The findings have implications for the laboratory situation. As well as having been found to work to gain visual contact, rabbits appear to derive considerable value from olfactory contact with a former cage-mate in an adjacent cage. This suggests that if a socially housed rabbit has to be removed from its social group, perhaps due to injury or illness, another rabbit should also be removed from the group and housed in an adjacent cage. It would be recommended that visual and tactile contact should be possible between cages as this has been found to be important to rabbits, in both the current chapter and Chapter 4. Removal of a rabbit from the group may result in that rabbit having to be removed from the experiment and therefore the removal of a second, healthy rabbit from the group and therefore the experiment may not be possible. If this were to be the case, the removed rabbit should be housed next to an unfamiliar rabbit if possible. Under such circumstances the rabbits should be allowed to have visual and tactile contact with each other, as it has been found that a rabbit housed without olfactory contact with a familiar rabbit is more motivated for visual and tactile contact than a rabbit housed with olfactory contact with a familiar rabbit. As olfaction has been found to be important, it is possible that placing bedding material from the social group in the tray underneath the cage may provide the rabbit with olfactory cues from familiar rabbits.

Chapter 6 – The importance of aspects of the cage environment to female laboratory rabbits

Abstract

Although previous experiments (see Chapters 4 and 5) demonstrated that rabbits were willing to work to gain social contact, the importance of social contact in relation to other resources was not established. In addition, some designs of cage allow rabbits to gain social contact with rabbits in adjacent cages whilst they are on a platform. It is not known whether the rabbits in these cages are using the platform primarily as an area to rest upon or as a means of gaining social contact, or both. Rabbits were therefore given the opportunity to work for these two aspects of the platform separately, i.e. visual and minimal tactile contact with a conspecific and a raised platform. The resources were offered within two of the arms of a plus-shaped apparatus (resource cages), with food and nothing (control) in the other two. The resource cages could only be accessed via push-doors that could be weighted. The experiment was a closed economy set-up, therefore the resources could only be gained within the experimental apparatus. The rabbits were housed in the apparatus for the duration of the experiment. Following a familiarisation period of free access to the resource cages, a 250gram weight was attached to the push-doors into the cages. The weight was increased by 250grams every two days. This was continued until the rabbits had not pushed into the food cage for 20 hours. Two economic measures were used to assess the relative importance of the resources: the maximum price paid (i.e. the maximum weight pushed through) and the total expenditure per day (cumulative weight pushed through in a day). It was found that food and social contact were of equal and most importance to the rabbits and the importance of the platform differed according to the measure used, being as important as food and social contact in terms of the maximum price paid, but being less important according to the total expenditure per day. It was found that the rabbits altered their daily time budget as the weights increased, decreasing the number of visits made to cages and increasing the mean duration of visits. Analysis of the resource use in the social contact cage found that the rabbits chose to spend over a third of their time not in direct visual contact with the rabbit. This indicates that in addition to visual

contact being important, being in close proximity to and in close olfactory contact with another rabbit whilst not necessarily in visual contact with them was also important. In the platform cage, the rabbits spent the majority of their time in front of the platform, rather than on or under it, suggesting that the feature of the platform that was most important to the rabbits was the fact that it provides a means of escape if the rabbit feels threatened. It is recommended from the findings of the experiment that singly housed female rabbits should have visual contact with conspecifics as they valued this as highly as food, but they should also be able to avoid contact. It is also recommended that cages should be fitted with a platform.

6.1 Introduction

In the field of economics, theories and equations have been established in order to investigate the importance of goods to individuals and the population as a whole and what effect an increase in the cost of the goods will have on the amount demanded. Incorporating such consumer demand theories and human economic measures into studies of the preferences of animals was suggested some time ago (Lea, 1978; Dawkins, 1983). This method of assessing preferences allows the strength of the preference to be measured and therefore the importance of the resources to the animals to be established. This enables the resources to be ranked in order of priority. Resources which an animal persists in gaining when the cost increases are often described as having an inelastic demand, whereas resources for which an animal stops paying the cost as the price increases may be described as having an elastic demand (e.g. Lea, 1978). Dawkins (1983, 1988, 1990) suggested that if an animal is strongly motivated to obtain a resource or perform a behaviour which its captive environment does not permit, it is likely to suffer as a result.

Such studies of motivation for resources may offer the animals either one resource at a time, or several resources together, each of which has a cost imposed on gaining them. Depending on the resources chosen for the animals to work for, offering only one resource at a time may be more relevant to the applied situation and avoids the problems of resources being substitutable or complementary (see below). However, there is a possibility that the animals may show an artificially high level of motivation for the resource, simply because it is the only resource they have been offered. Offering more than one resource allows the animals to re-schedule their behaviour and alter the time spent with resources as the cost of gaining them increases. As well as the costs animals are willing to pay for resources, such re-scheduling of visits to resources and time spent with resources can also give an indication of the importance of the resources (e.g. Sherwin and Nicol, 1995, 1996; Cooper and Mason, 2000). As mentioned in Chapter 4, when offering animals the chance to work for more than one resource at a time it is important that the resources offered are chosen carefully to ensure that they are neither substitutable or complementary as this can result in artificial measures of motivation. If two resources have a similar motivational basis, the consumption of one resource may decrease the motivation for the other. For example, mink provided with a water bowl which allowed drinking but not swimming reduced their motivation for a water bath which allowed both (Mason *et al.*, 1999). This indicates that the mink were working to gain the water bath partly for drinking as well as for swimming. Substitutable resources can therefore be used deliberately to highlight which aspects of the resources the animals are working for. Resources are described as being complementary if the consumption of one resource increases the motivation for another resource, for example, mink have been found to choose the warmest, driest area possible after swimming (Mason, pers. comm.).

Some designs of laboratory rabbit cages allow the rabbits to gain social contact with rabbits in adjacent cages whilst on the platform in the cage. This may be part of the reason why rabbits in cages are seen to use platforms. The aim of this experiment was to assess how motivated laboratory rabbits were for visual contact with a conspecific and a platform (two aspects of a laboratory rabbit cage). Providing social contact and a platform separately would allow whether the rabbits were using the platform to lie on a raised area or for social contact to be established. Also, the need to re-familiarise the pairs of rabbits for a short period each day during the experiment in Chapter 5 meant that the rabbits were not tested in a fully closed economy. There was evidence to suggest that this weakened the demand for social contact during the tests. Since there has been found to be no difference in how hard rabbits worked for contact with an unfamiliar and familiar rabbit (Chapters 4 and 5), singly housed rabbits were used in the current experiment and social contact was only available in the experimental apparatus i.e. the rabbits were tested in a fully closed economy. This was achieved using a plus-shaped apparatus allowing access to four resource cages via weighted push-doors. Increasing costs were imposed on gaining access to these resources and on gaining access to food and an empty space. Although it was established in Chapters 4 and 5 that rabbits were willing to pay costs to gain social contact, the relative importance of social contact compared to other resources had not been established. It is widely accepted that food is a useful resource to use in such studies to titrate other resources against, as it is a resource required for survival. Providing the rabbits with an empty cage would ensure that throughout the experiment the rabbits were not entering the resource cages either as part of territory inspection or to access the additional space, rather than to gain access to the resources within the cages and would act as a baseline. Using a resource that the rabbits were likely to work hard for (food) and one that was expected to be of low value allowed the importance of social contact and a platform to be determined in relation to such resources. The relative importance of the resources was assessed using two different economic measures - the maximum price paid for each resource and the total expenditure per day for each resource. Investigations were made into the number of visits made to the resource cages, the total time spent with each resource and the mean visit duration, and how these were affected as the rabbits re-scheduled their behaviour in accordance with the increasing cost of gaining access to the resources. The behaviour of the rabbits in the social contact and platform cages was investigated to give an indication of how the rabbits used the resources once they had paid the cost to gain access to them.

6.2 Methods

6.2.1 Animals, housing and husbandry

The experimental animals were eleven naïve, female New Zealand White rabbits, which were between 22 and 27 weeks old at the start of the experiment and weighed between 2.95kg and 3.95kg (mean 3.50kg, SD ± 0.32). The rabbits were singly housed in standard laboratory rabbit cages with a floor area of 5400cm^2 and height of

50cm (Tecniplast, six cage interlinked rack – see Chapter 5, Plate 5.1). The raised platforms normally fitted in these cages were removed. A board was placed across the wire mesh panel between cages and the transparent door that enables two cages to be interlinked to prevent visual and tactile contact between rabbits in adjacent cages. The rabbits were however in olfactory contact. The rabbits were housed like this for between six and twelve weeks prior to the start of the experiment, as the experiment was carried out in three replicates with four rabbits being tested at one time. Husbandry, diet and enrichment were the same as for previous experiments (see Chapter 4, section *4.2.1*).

6.2.2 Apparatus

Four wooden plus-shaped (+) sets of apparatus were built (Figure 6.1). The four arms of each set of apparatus were termed 'resource cages' and the area in the centre termed the 'home cage'. The home cage and three of the four resource cages measured 71 x 55cm (area = 3905cm^2) and were 61cm high. The fourth resource cage measured 71 x 64cm (area = 4544cm^2) and was 81cm high (see section *6.2.3*). Throughout the apparatus the floor surface was dimpled plastic, similar to that of a typical laboratory cage. The whole apparatus was covered with wire mesh.

Each resource cage contained a different resource, which was only available within that cage and could only be accessed from the home cage through a one-way perspex push-door measuring 18 x 19cm (Cat Mate, Large cat flap, Pet Mate Ltd, UK). The only way the rabbits could exit each resource cage was via a second (identical) one-way push door. The position of the resources within the apparatus was varied between the four sets of apparatus. Each set of apparatus was used three times to enable all eleven rabbits to be tested and were thoroughly cleaned between groups.

6.2.3 Resources available

The resources available to the rabbits in the resource cages were:

i. food

ii. visual and minimal tactile contact with a conspecific

iii. a platform

iv. an empty cage

The home cage was the only place where water was provided. This was to ensure that the rabbits returned to the home cage where they could choose to enter any of the resource cages, rather than staying in the food cage and also ensured that no cost was imposed on the rabbits gaining water. Each rabbit was provided with the water bottle from its own laboratory cage.

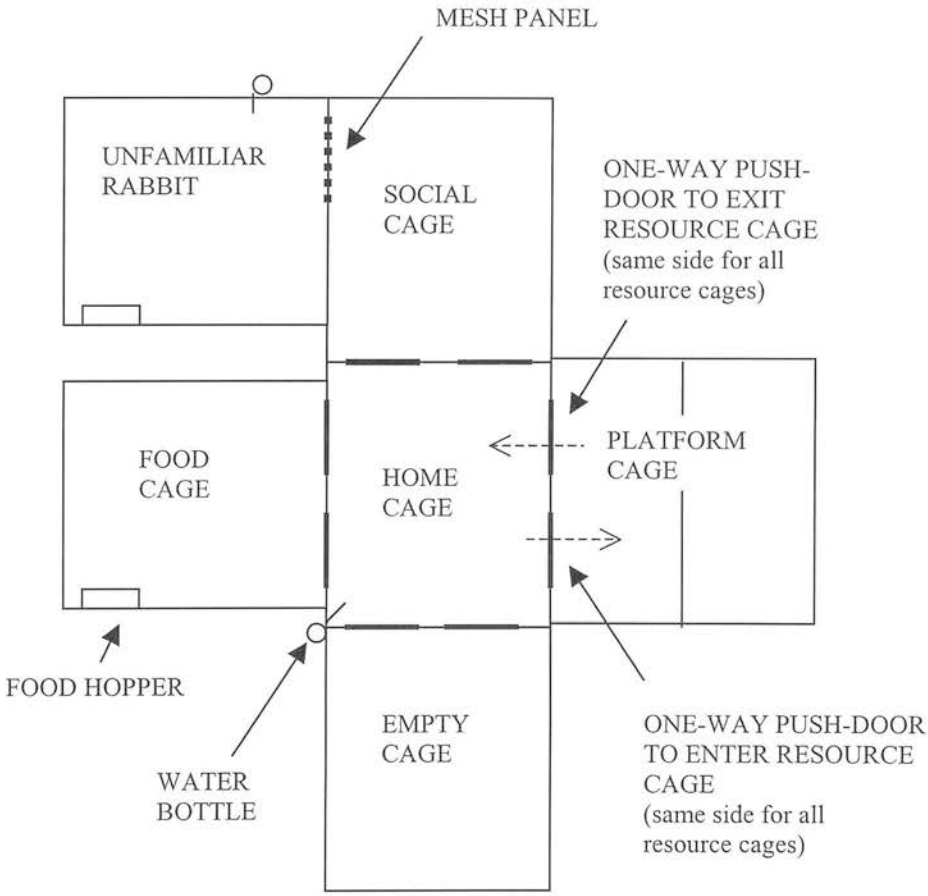

Figure 6.1 One of the four plus-shaped sets of apparatus showing the resource available in each resource cage

i. Food

The rabbits were provided with their usual diet of *ad libitum* commercial rabbit pellets and hay in one of the three smaller resource cages. The food was provided in the same way as in the rabbits' usual laboratory cage, with the pellets in a food hopper identical to that in their cage and hay on the plastic floor. The rabbits were not fed carrots and cabbage as they had been in their cages prior to the experiment. This was because the carrots and cabbage tended to be at least partially eaten as soon as they were given, even when there was pelleted food and hay available at all times and were therefore high incentive items. Providing a resource that is likely to be completely consumed means that after consumption of the resource the rabbits would not gain the same reward every time they paid the cost of pushing into that cage and this may have affected their motivation. In addition, not all rabbits in the laboratory are provided with fresh vegetables as part of their diet (see Chapter 2) and not including them in the experiment meant that the reward in the food cage was more applicable to the industry situation.

ii. Visual and minimal tactile contact with a conspecific

In another of the three smaller resource cages, a rectangle measuring 30 x 20cm was cut out of one of the side walls 5cm from the back wall and was replaced by a mesh panel (mesh size 2.5cm). On the other side of the mesh panel was a pen measuring 71 x 65cm and 61cm high. A rabbit, which was unfamiliar to the test rabbit at the start of the experiment, was placed in this pen. The same rabbit remained in the pen for the duration of the experiment. The rabbit was provided with its normal diet of commercial rabbit pellets and hay at the end of the pen furthest from the mesh panel and was provided with water and the enrichment objects from its laboratory cage. The rabbit was not provided with carrots and cabbage, to avoid the possibility of their smell causing the test rabbits to push into this cage to try to reach them rather than for social contact. Although it was likely that the test rabbit would be able smell the commercial rabbit pellets, this type of food was available to the test rabbit within the apparatus (food cage), and the pellets do not appear to be such a high incentive food as carrots and cabbage (see above).

iii. Platform

The larger of the four resource cages contained a platform taken from a Tecniplast laboratory rabbit cage. Platforms in cages provide additional floor space as well as a darkened, semi-enclosed area underneath. Although this cage was larger than the others were, the platform covered approximately half of the floor area, therefore although the rabbits could use the floor area underneath the platform, this cage did not provide a continuous open space of larger area than any of the other resource cages. The platform was approximately 20cm high, therefore the height of this resource cage was increased by 20cm to make the height above the platform the same as the height above the floor in the other resource cages.

iv. Empty cage

The final resource cage available to the rabbits had nothing in it and was used as a baseline, or control.

6.2.4 Familiarisation with apparatus and resources

For the first hour that the rabbits were in the apparatus the push-doors into each resource cage were fixed open allowing the rabbits free access to the cages to become familiar with the layout of the apparatus and the resources available. The exit doors all remained closed, giving the rabbits the opportunity to investigate a closed push-door. After one hour the push-doors into the resource cages were closed. If a rabbit failed to push through a closed push-door into any of the resource cages within the first hour of the doors being closed, a long piece of wood was used to nudge the door open to encourage the rabbit to investigate and push through. Similarly, if on the first occasion that a rabbit pushed into a resource cage it failed to exit the cage within an hour (i.e. appeared to be trying to leave the resource cage, nudging the one-way push-door into the resource cage) the exit door was nudged open to encourage the rabbit to leave. The rabbits were closely observed until they were seen entering and leaving the resource cages freely.

6.2.5 Protocol

6.2.5.1 Free access

The rabbits were initially allowed a period of free access to all four resources (i.e. push-doors were unweighted) to ensure that they had thoroughly explored the apparatus and the resources available. The rabbits were allowed to explore the apparatus until a reduction in exploratory behaviour was seen and the rabbits had shown consistency in their behaviour for two consecutive days, i.e. to ensure they had settled into a stable time budget. This was determined using the number of visits per day to each resource cage, total duration of visits to each resource cage (as percentage of time) and mean duration of visits to each resource cage (in minutes). The experiment was continued to the next stage (see section *6.2.5.2*) when the difference in number of visits between the two previous days was less than five for each resource cage, the difference in the total duration of time in each resource cage was less than 10% for each cage and the difference in the mean duration of visits of cages was less than 10 minutes for each cage. A maximum of seven days was allowed.

6.2.5.2 Cost imposed

On the day following the period of free access, plastic boxes weighing 250grams were bolted to the push-doors into each of the four resource cages to impose a cost on gaining access to the resources. The rabbits were given 48 hours of access to all the resource cages with this weight on the push-doors. After this time, a 250gram weight was placed in each of the four boxes, making the weight on the push-doors 500grams. The weight on the push-doors was increased by 250grams every 48 hours. The exit doors from each of the resource cages was always unweighted.

The termination of the test was based on how hard the rabbits would work for food, and the determination of the criterion to be used was based on the welfare of the rabbits. Rabbits may suffer from gut impaction if they do not feed for prolonged periods therefore one of the University of Edinburgh Assistant Named Vets and the Home Office were consulted when deciding upon this criterion. According to the Home Office, the maximum period of time food can be withheld from rabbits without a project licence is 24 hours. It was considered to be unnecessary to wait for 24 hours from the last visit to the food cage, therefore the test was terminated when the rabbit had not pushed through the weighted push-door into the food cage for 20 hours.

6.2.5.3 Husbandry

At the same time every morning the rabbits were removed from the apparatus and placed in their laboratory cage for approximately one hour to allow for the cleaning of the apparatus and the replacement of food and water, for both the test rabbits and the rabbits used for providing social contact. Whilst in their cages the rabbits did not have access to any of the resources from the apparatus (i.e. food, platform, social contact) to ensure that the rabbits were tested in a fully closed economy. The use of dimpled floors in the resource cages and home cage meant that urine and faeces were collected in trays underneath and these were emptied every two to three days (as is typically done within the pharmaceutical industry). The pelleted food left in the food hopper each day was weighed to monitor the food intake of the rabbits. The rabbits were weighed and given a health check once a week. After the husbandry procedures had been carried out the rabbits used for providing social contact were returned to their pens first, then the test rabbits were returned to the home cage of the apparatus.

6.2.5.4 Data collection and handling

The behaviour of the rabbits in the four sets of plus-shaped apparatus was recorded on videotape using CCTV cameras (Panasonic WV-BP330/B) mounted on brackets directly above each set of apparatus, a quad unit (Panasonic WJ-410) and a time-lapse video recorder (Panasonic AG-6024). Infra-red lights were used to record behaviours during the dark hours of the light cycle. The behaviour of the rabbits in their laboratory cages had previously been recorded, therefore the rabbits were familiar with the lights.

Each day the number of visits the rabbits made to each cage and the duration of each visit were recorded from the videotapes. The mean duration of visits to each cage was calculated, as was the overall duration of time spent in each cage (as a percentage of total time). Investigations were made into resource use within the social contact and platform cages. For each rabbit, video analysis was carried out on the last ten visits to each of these resource cages before the first weight was added, the first ten visits after the first weight was added and the last ten visits made when weights were added. Continuous recording was used to determine how the rabbits positioned themselves within the cage. The position of the rabbits within the social contact cage was noted as being either 'near to' the other rabbit (i.e. at the back of the cage, in the half opposite the mesh panel) or 'away from' the other rabbit (i.e. at the front of the cage). If the rabbit was positioned towards the centre of the cage its position was determined by where its head lay, i.e. if two thirds of its body was in the front half of the cage and its head in the back half, it was recorded as being in the back half of the cage, near to the other rabbit. In the platform cage, the rabbits were recorded as being on the platform, under the platform or in front of the platform.

6.2.6 Statistical analysis

The number and duration of visits made to resource cages on the last day before the weights were used as the data for the unweighted push-doors. For each weight, the mean of the number of visits made and total duration in each resource cage over the two days allowed were calculated. These were then used to determine the mean duration of visits to each resource cage at each weight.

The importance of the resources was assessed using the maximum price paid for each resource (maximum weight pushed through) and the total expenditure for each resource per day. The total expenditure was calculated by multiplying the number of visits made at each weight by the weight on the push-door (excluding the unweighted push-door). Comparisons were made between resources for both measures using Friedman two-way analysis of variance (blocking by rabbit) and, where significant results were found, multiple comparisons between treatments were carried out to indicate which resources were significantly different from each other (Siegel and Castellan, 1988).

Histograms of the number of visits, total duration and mean duration of visits to each resource cage showed that the data were not normally distributed. The number of visits and the mean duration of visits were therefore transformed using a log transformation to improve normality and the total duration of visits was subjected to arcsine transformation. Effects of the resource and the weight on the push-door on the number of visits, total duration and mean duration of visits were investigated using General Linear Models, blocking by rabbit and using the weight on the push-door as a covariate. Tukey tests were used to highlight where any significant differences lay. Data up to the sixth weight (1500g) only were used in analysis, as when the weight was increased to 1750g there were only two rabbits left in the experiment.

For the analysis of resource use within the social contact cage, Friedman two-way analysis of variance was used to compare the time spent near/away from the rabbit in the three samples (last ten unweighted visits, and first and last ten weighted visits) to investigate any differences in resource use as the weight on the push-doors increased. The three samples were then grouped together and a Wilcoxon signed rank test carried out to investigate any difference in the time spent near to or away from the rabbit behind the mesh panel. Similarly, the position of the rabbits within the platform cage was analysed using Friedman two-way analysis of variance to compare the three samples for the time spent on, under and in front of the platform. The three samples were then grouped together and a Friedman two-way analysis of variance carried out to compare the time spent on, under and in front of the platform. Multiple comparisons were carried out to investigate where any significant differences lay. Analysis was carried out using Minitab 12.

6.3 Results

6.3.1 Free access

A reduction was found in the number of visits made to each cage between the first and second days in the apparatus. The mean number of visits to the food cage on the second day decreased by 25% (SD ± 4.87) of the number of visits made on the first day. The number of visits to the platform cage decreased by 52% (SD ± 14.26), the number of visits to the social contact cage by 26% (SD ± 7.36) and the number of visits to the empty cage by 64% (SD ± 7.92). Six of the rabbits were allowed the maximum time with the unweighted push-door of seven days, for four rabbits the first weight was added after six days as the number of visits they made to resource cages and time spent within them had been consistent over two days, and for one rabbit the first weight was added after five days.

6.3.2 Maximum price paid for resources

The test was terminated when the rabbits had not eaten for 20 hours, regardless of whether they were still pushing through into other resource cages. Only one rabbit paid a higher maximum price for food than for any other resource and one rabbit paid a higher maximum price for the platform than they did for food. Three of the rabbits paid an equal price for food and social contact, one paid an equal price for food and the platform and three paid an equal price for food, social contact and the platform. One rabbit paid an equal price for food and the empty cage and one paid an equal price for all four resource cages. The median maximum price paid (i.e. maximum weight pushed through) by the rabbits for the different resources are shown in Figure 6.2.

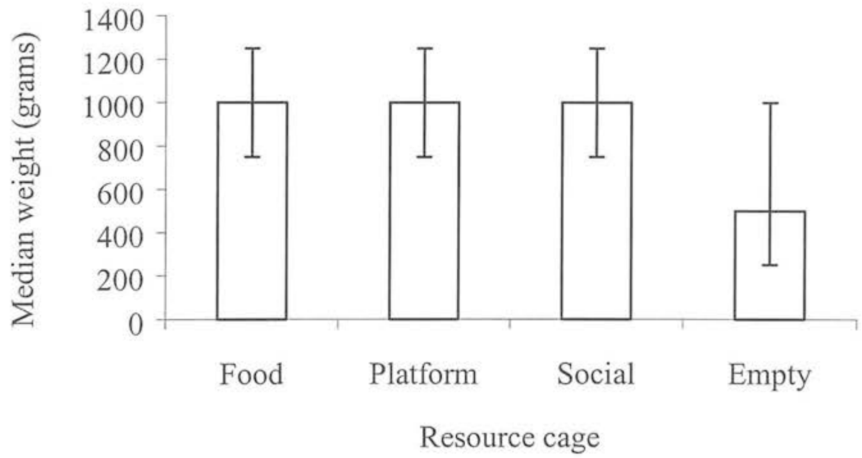

Figure 6.2 Medians of the maximum price paid by the rabbits to gain access to each resource cage, showing inter-quartile ranges

A significant difference was found in the maximum price paid for different resources (Friedman two-way analysis of variance, S=15.35, DF=3, $p<0.005$). The maximum price paid for food, the platform and social contact were all significantly greater than that for the empty cage (z=19.5, 11.0 and 13.5, $p<0.05$) but did not differ significantly from each other. Using the maximum price paid as a measure of the relative importance, the resources were ranked as food, social contact and a platform being of equal most importance and an empty cage being of least importance, although there was some variation between rabbits.

6.3.3 Total expenditure

The percentage of the total expenditure throughout the experiment that was spent on each resource by each of the rabbits is shown in Figure 6.3. The median total expenditure per day for the different resources is shown in Figure 6.4.

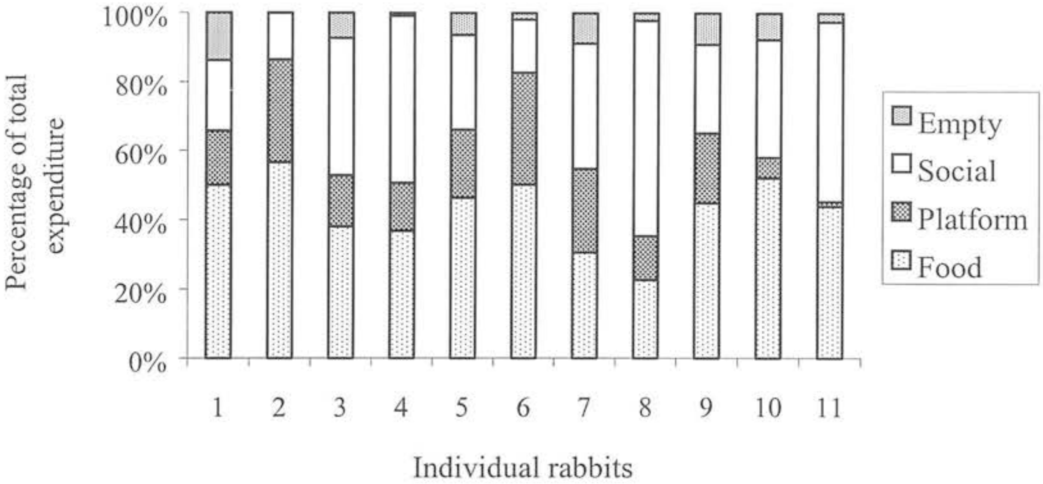

Figure 6.3 Percentage of total expenditure throughout the experiment that was spent on each of the resources by the individual rabbits

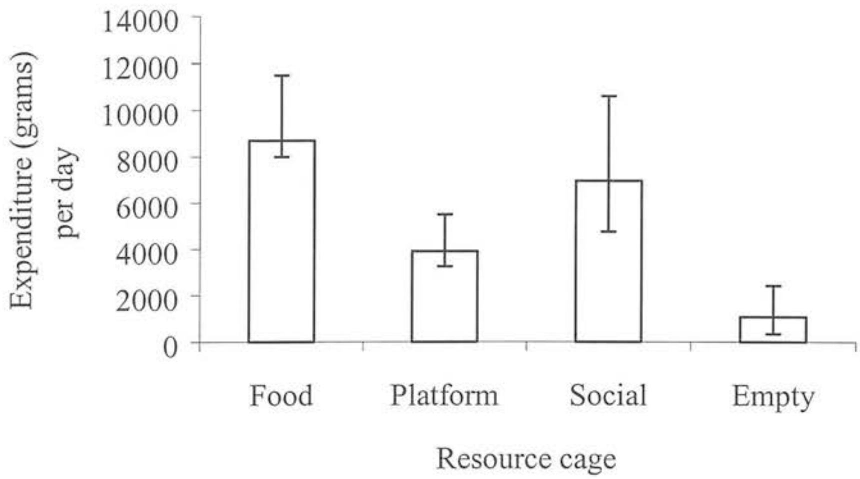

Figure 6.4 Median total expenditure per day (grams) for the different resources, showing inter-quartile ranges

A significant difference was found in the total expenditure per day for different resources (Friedman two-way analysis of variance, S=24.27, DF=3, $p<0.01$). The total expenditure per day for access to food was significantly greater than that for access to a platform and an empty cage (z=17 and 26, $p<0.05$). The total expenditure per day to gain access to social contact was also significantly greater than that paid to gain access to a platform and an empty cage (z=14 and 23, $p<0.05$). Using the total expenditure per day as a measure of the relative importance, the resources were ranked as food and social contact being of equal most importance and a platform and empty cage being of equal least importance.

6.3.4 Number of visits to resource cages

The mean of the total number of visits made to all of the resource cages throughout the experiment was 186 (SD ± 51). Most visits were made to the food cage (mean = 68 ± 15), followed by the social contact cage (mean = 67 ± 35) and the platform cage (mean = 36 ± 15), with the least number of visits being made to the empty cage (mean = 13 ± 8). The mean number of visits to each resource cage when the push-doors were unweighted (last day only) and weighted are shown in Figure 6.5.

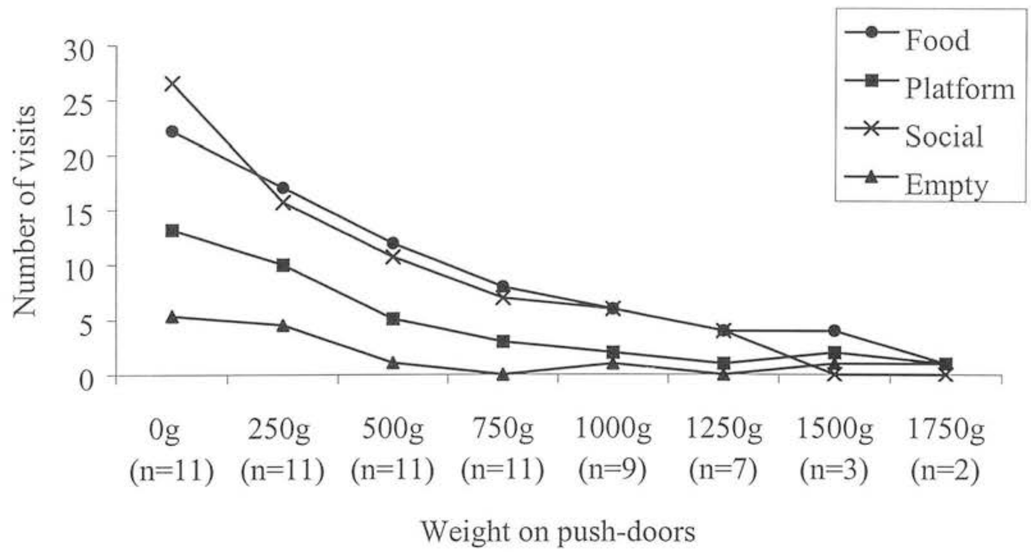

Figure 6.5 Mean number of visits to each resource cage when push-doors were unweighted and weighted up to 1750grams

There was a significant difference in the number of visits made to the different resource cages (GLM, $F_{(3,186)}=36.08$, $p<0.001$). The rabbits made significantly more visits to the food, social contact and platform cages than to the empty cage (all p<0.001) and more visits to the food and social contact cages compared to the platform cage (both p<0.001). The weight on the push-doors was found to have a significant effect on the number of visits made to resource cages ($F_{(1,186)}=161.96$, $p<0.001$), with there being a negative relationship between the weight and the number of visits made. The number of visits was found to be significantly different between individuals ($F_{(10,186)}=3.45$, $p<0.001$).

6.3.5 Total duration of time spent in resource cages and home cage

The mean time spent in each of the resource cages throughout the experiment is shown in Figure 6.6, with the time in each cage being converted to a percentage of the total time at each weight. Overall the most time was spent in the home cage (39%, SD ± 19), followed by the platform cage (27% ± 15), the social contact cage (17% ± 14), the food cage (14% ± 6) and the empty cage (4% ± 4).

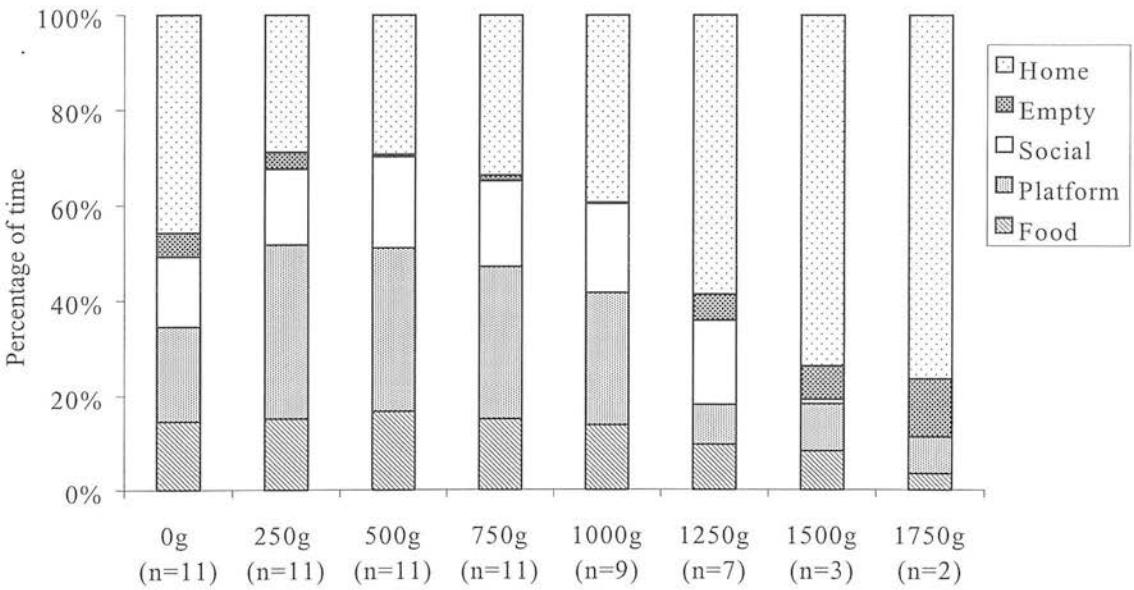

Figure 6.6 Mean percentage of time spent in each resource cage when push-doors were unweighted and weighted up to 1750g

There was found to be a highly significant difference in the total duration of time spent in the different cages (GLM, $F_{(4,295)}=13.47$, $p<0.001$). The rabbits spent significantly more time in the food, social contact, platform and home cages than they did in the empty cage (all p<0.001). The time spent in the home cage was also significantly greater than the time spent in the food and social contact cages ($p<0.05$). There was found to be an interaction between weight and treatment, with the total duration spent in different resource cages being affected differently by the weights on the push-doors ($F_{(4,295)}=4.00$, $p<0.005$). The time spent in the home cage increased significantly with increasing weights on the push-doors into the resource cages ($p<0.001$) and the total duration spent in the platform cage decreased with increasing weight ($p<0.05$).

6.3.6 Mean duration of visits to resource cages and home cage

There was found to be a highly significant difference in the mean duration of visits to the different resource cages (GLM, $F_{(4,241)}=11.90$, $p<0.001$). The mean duration of visits to the food, platform and social cages were significantly greater that those to the empty cage (all $p<0.001$), as was the mean duration of time spent in the home cage ($p<0.05$). The mean duration of visits to the platform cage was also significantly greater than those made to the food and social contact cages ($p<0.05$) and to the home cage ($p<0.001$). The weight on the push-door was found to have a highly significant effect on the mean duration of visits to resource cages ($F_{(1,241)}=81.06$, $p<0.001$), with the mean duration increasing as the weight increased (see Figure 6.7).

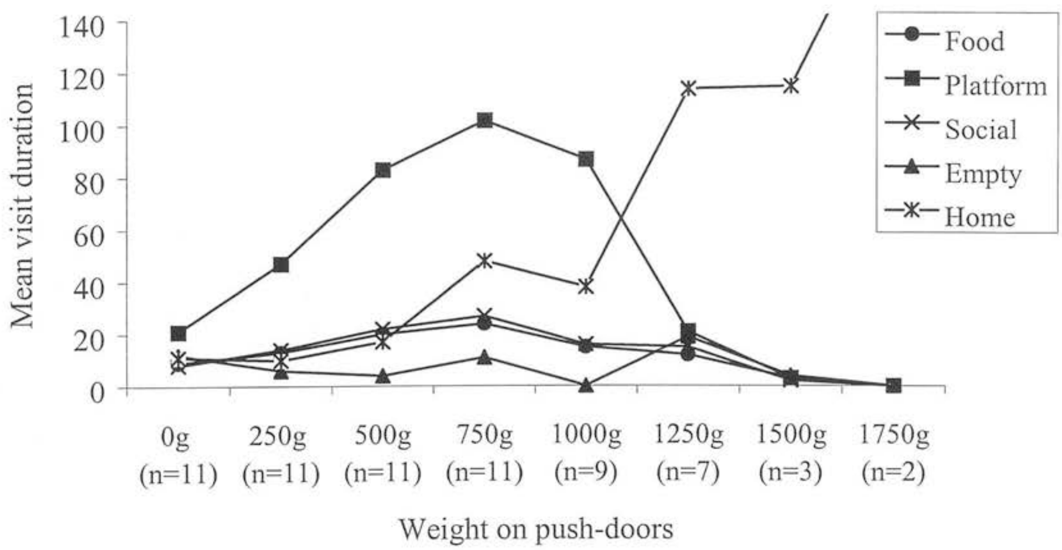

Figure 6.7 Mean visit duration (in minutes) to each of the resource cages (mean duration in home cage at 1750g = 193 minutes, not shown)

6.3.7 Behaviour in the social contact cage

The time spent near to and away from the rabbit behind the mesh panel did not differ depending on whether the push-door was unweighted, weighted with the low weights or weighted with the heavier weights. Of the time spent in the social contact cage, the percentage of time spent near to the rabbit and away from the rabbit are shown in Figure 6.8.

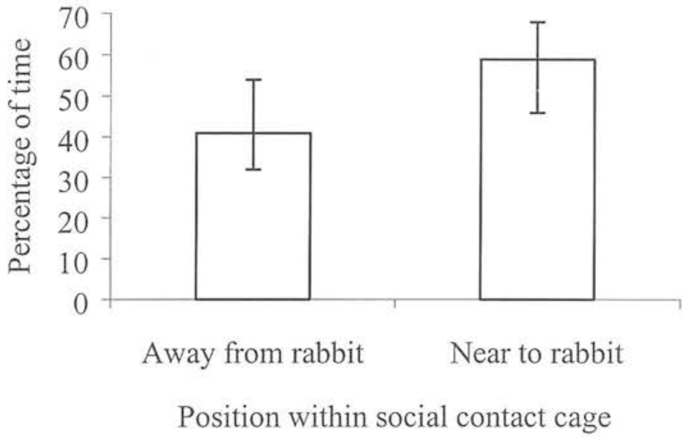

Figure 6.8 Median percentage of time in the social contact cage spent near to the rabbit and away from the rabbit, showing inter-quartile ranges

The rabbits spent significantly more of their time in the social contact cage near to the rabbit than they did away from it (Wilcoxon signed rank, N=32, T=372.5, $p<0.05$)

6.3.8 Behaviour in the platform cage

The time spent on, under and in front of the platform did not differ depending on whether the push-door was unweighted, weighted with the low weights or weighted with the heavier weights. Of the time spent in the platform cage, the median percentage of time spent on the platform, under the platform and in front of the platform are shown in Figure 6.9.

A significant difference was found in the position of the rabbits within the platform cage (Friedman two-way analysis of variance, S=40.11, DF=2, $p<0.001$), with more time being spent in front of the platform than either on or under the platform (z=39.5 and 47.5 respectively, $p<0.05$).

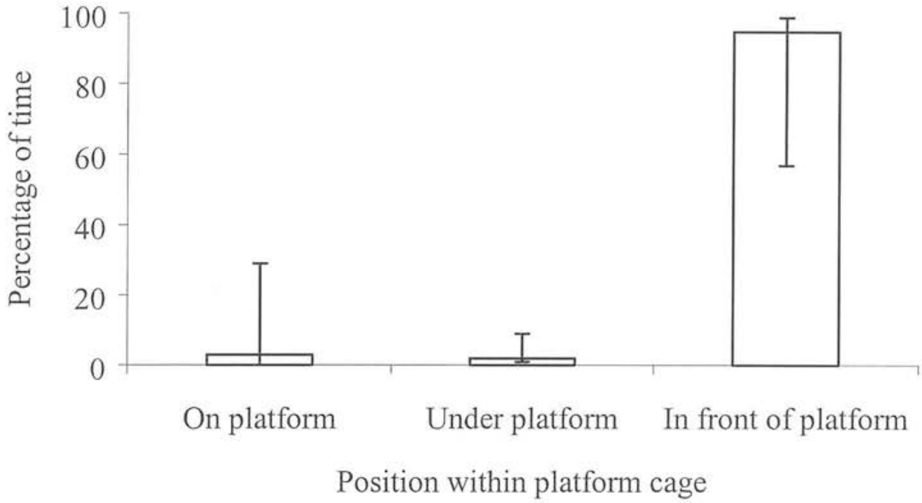

Figure 6.9 Median percentage of time in the platform cage spent on, under and in front of the platform, showing inter-quartile ranges

6.4 Discussion

Imposing a cost on gaining access to the resources showed that the rabbits were willing to pay a higher maximum price for food, a platform and social contact than they did for an empty space. There was no difference in the maximum price paid for food, a platform and social contact, indicating that these resources were of equal importance to the rabbits. Using the total expenditure per day as a measure of importance it was found that the rabbits paid more for food and social contact than they did for a platform and an empty space, indicating that social contact is as important to the rabbits as food and that a platform is less important. There are problems associated with both these measures of importance. The maximum price paid can understate the importance of resources and the total expenditure can overstate the importance. Using the maximum price paid, two resources can appear to be of equal importance despite the fact that one resource may only have been visited once at the maximum price and the other may have been visited many times. It does not take the frequency of gaining access to the resource into account. The total expenditure does take the frequency of gaining access into account, however it does not take the maximum price paid into account. This cumulative approach may give the same rank of importance to two resources when one has been accessed few times, but when the price is high, and the other has been accessed many times, but only when the price is low. It is therefore useful to use both, and when interpreting the findings it is important to also look at the number of visits made to resources and the visit durations. Both measures found that food and social contact were of equal and most importance and that the empty cage was the least important. The relative importance of the platform varied with the measure used. The fact that the empty cage was significantly less important than other resources in terms of both the maximum price paid and the total expenditure per day indicates that the rabbits were actually pushing through into the resource cages for the resource and not for the additional space or as part of territory inspection.

On several occasions, the maximum price paid that was recorded was not necessarily the absolute maximum price the rabbit was willing to pay for that resource. This was due to the test being terminated for welfare reasons, according to the feeding behaviour of the rabbits. The number of visits to the resource cages was very low at the last weight the rabbits pushed through for food, therefore prolonging the period between the last visit to the food cage and the termination of the test to allow the rabbits the opportunity to continue to gain access to other resources was not likely to have significantly altered the relative importance of the resources. Despite terminating the test in this way, social contact and a platform were found to be as important to the rabbits as food (depending on the measure used), which indicates that these are resources that the rabbits are highly motivated to gain access to. Whether or not they worked harder for another resource than for food would only increase the strength of the conclusion that the resource was important to the rabbits.

Increasing the weight on the push-doors resulted in a decrease in the number of visits made to resource cages and an increase in the mean duration of each visit. Increasing the weight had no effect on the total duration of time spent in the food, social contact and empty cages, but caused the time spent in the platform cage to decrease and the time in the home cage to increase. This re-scheduling of behaviour by altering the mean duration of visits to compensate for the decrease in the number of visits made to defend food and social contact again indicates that these two resources were of most importance.

The re-scheduling of behaviour when costs are imposed on gaining access to resources has been seen with other species such as mice (Sherwin, 1996; Sherwin and Nicol, 1996), mink (Cooper and Mason, 2000), hens (Cooper and Appleby, 1997) and doves (Larkin and McFarland, 1978). The animals are found to defend the resources of most value to them when the costs of gaining access to them increases. Resources which are defended most strongly can be ranked as being of most importance to the animals, and it is expected that under such circumstances food will be the most, or one of the most, strongly defended resources, as food is necessary for survival. Several studies have found that animals, as expected, show an inelastic demand for food, that is, they persist in paying the cost for food as the cost increases (e.g. Matthews and Ladewig, 1994; Sherwin and Nicol, 1995). In the current study, daily weighing of the amount of food eaten and weekly weighing of the rabbits found that the amount of food remained consistent throughout the experiment and that the rabbits either remained at a constant body weight or gained weight. The amount of food the rabbits ate was therefore not affected by the cost imposed, indicating that the consumption of the resource was consistent throughout the experiment. Altering of feeding behaviour depending on current circumstances has been seen in wild rabbits. Rabbits have been observed to alter their feeding behaviour from the usual, and at times 'casual', grazing pattern to feed 'particularly voraciously' before a storm (Thompson and Worden, 1956). This is perhaps done to avoid grazing in heavy rain, and this could be interpreted as the rabbits re-patterning their feeding behaviour due to the cost of feeding in the storm (of getting wet). Those rabbits were altering their feeding due to time-pressure, which was not the case in the current experiment as the rabbits were able to alter their time budget as they chose to. However, rabbits are a grazing, prey species, and whilst feeding they move continually with the need for vigilance frequently interrupting their grazing (e.g. McBride, 1988). This may be an instinctive response that, even in the experimental set-up where there are no threats, means that rabbits will not prolong feeding bouts in one area.

As the weights on the push-doors increased, although the number of visits made decreased and the mean duration increased, the rabbits were still seen to make several relatively short visits to the social contact cage rather than few long visits. This implies that it was the frequency of the visits to the social contact cage that was important rather than the length of the visits there. It is possible that the rabbits were visiting the social contact cage partly to inspect/defend their territory, hence the frequent visits. Rabbits in the wild will patrol and mark their territory frequently using faecal pellets, urine and secretions from their chin gland (Mykytowycz, 1962, 1968). Rabbits in the wild use latrines (mounds of droppings) as a means of territory marking (Thompson and Worden, 1956). These are often formed near to the burrow entrance and serve to inform non-resident rabbits that the area is occupied (Mykytowycz, 1968). Faecal pellets produced at latrines have a more intense odour than those produced randomly throughout the territory (Mykytowycz, 1968; Sneddon, 1991) and contain stronger pheromones that pass on information about the identity, age, sex and reproductive status of an individual (Bell, 1983). The age, sex and social status of rabbits have been found to affect their behaviour at latrines (Sneddon, 1991). Adult females have been found not to make regular visits, and when they do visit latrines they spend a greater proportion of time resting and grooming and are thought to be spreading the scent of the dominant males across their body. Young females (and males) appear to visit latrines mainly to investigate the odours. It was noted that during the experiment each of the rabbits in the pen behind the mesh panel tended to use the corner next to the mesh panel as their latrine area. The test rabbits almost exclusively used the social contact cage as their latrine area, and also tended to use the corner next to the mesh panel most frequently. During the experiment, when the weights on the push-doors were low all of the rabbits used only the social contact cage as their latrine and when the weights increased to over 1000grams the rabbits began to leave some droppings in other cages, usually the home cage. This concentration of droppings next to the other rabbit rather than randomly throughout the apparatus suggests that the rabbits were using the droppings as territory marking. An alternative possibility is that the social contact cage was the most suitable place to leave droppings. It may be that leaving droppings in the food cage was avoided to prevent food becoming tainted and that the platform cage was not used as this was the area where the rabbits tended to rest. Few visits were made to the empty cage and there would be no reason for entering this cage other than to use it as a latrine as there was no reward. If the only reason for entering the social contact cage was for territorial reasons, it might be expected that the rabbits would spend all or most of the time confronting this rabbit, as wild rabbits will aggressively defend their territory against intruders (e.g. Mykytowycz, 1958; Bell, 1983). The rabbits did spend significantly more time near to the rabbit in the social contact cage (where visual contact was possible) than away from it (out of visual contact), however, casual observations during video analysis found no aggressive behaviour between rabbits. The fact that the rabbits were paying the cost to enter the social contact cage, yet did not spend all of their time near to the rabbits where visual contact was possible indicates that as well as visual contact being of importance to the rabbits, being in close proximity to a conspecific, but not necessarily in direct visual contact, was also important.

It has been found that the presence or absence of cues can affect how animals work for certain resources (e.g. Warburton and Mason, in press) and it was found in Chapter 4 that olfactory appeared to be playing an important part in how the rabbits behaved in the experimental apparatus, and in Chapter 5 that whether or not rabbits were housed in olfactory contact with their cage-mate affected how hard they would work for social contact. It has been suggested (Warburton and Mason, in press) that the presence of cues from a resource might affect the validity of the application of the results to an applied situation. However, as with the experiments in Chapters 4 and 5 the apparatus in the current experiment was designed so that the rabbits could not see the other rabbit until they had paid the cost. This was partly to avoid the behaviour of the non-test rabbit affecting the behaviour of the test rabbit and partly to make the situation as applicable to the laboratory industry as possible, as within laboratories rabbits are often housed in olfactory and auditory contact but not visual contact.

The rabbits paid the same maximum price for the platform as they did for food and social contact, although the total expenditure per day for the platform was lower.

Although the rabbits made fewer visits to the platform cage, the mean duration of visits was longer than for visits to the food and social contact cages. The total duration decreased as the weights on the push-doors increased, therefore access to the platform was not defended as strongly as food or social contact. The platform in a laboratory cage provides rabbits with additional floor space on a raised area and a darkened semi-enclosed area underneath. However, when the position of the rabbits within the platform cage was investigated it was found that rather than utilise these two aspects of the platform, the rabbits spent the vast majority of the time resting in front of the platform rather than on or under it. When rabbits are observed in a laboratory cage with a platform fitted, they may be perceived as not 'using' the platform if they spend a large proportion of their time lying in front of it and not on or under it, and platforms may be thought of as unnecessary. The fact that the rabbits were willing to pay high costs to access the platform cage and spent a relatively long time in the cage with each visit suggests that simply being near a platform is what is most important about the platform. Rabbits in the wild will run to for cover when they are startled or under threat. It has been found that in areas of thick vegetation rabbits spend much time on the surface and may choose to hide in the undergrowth to avoid predators, however, when no other cover is available the rabbits may spend much of their time underground, and if on the surface will run to their burrow for cover (Kolb, 1991, 1994). Since bolting for cover is the natural survival strategy of the rabbit and no other cover was available within the cage it is perhaps unsurprising that they should choose to rest in a position which provided them with quick access to safety. Casual observations of rabbits in laboratory cages with platforms have found that the rabbits spend a much higher proportion of their time lying on top of the platform than was seen in the experimental apparatus. However, when the rabbits were on top of the platform in these laboratory cages they were able to see into the adjacent cages through the transparent sliding door which enabled the cages to be interlinked. It is therefore possible that although the human perception is that the rabbits are using the raised area to lie on, it is actually the case that the rabbits are using the platform as a means of gaining social contact.

6.5 Implications for laboratory rabbit housing

The experiment found that social contact was as important to the rabbits as food. It was found that whilst the rabbits were willing to pay increasing costs to enter the social contact cage, they spent more than a third of their time not in direct visual contact with the rabbit, although they were in close olfactory contact. This supports the findings of Chapters 4 and 5, that olfactory contact plays an important role in communication between rabbits, which is the main sense of the wild rabbit (McBride, 1988). It is therefore suggested that singly caged rabbits should be able to have visual and tactile contact with conspecifics, as well as having a means of avoiding direct visual contact. A platform was as important to the rabbits as food and social contact, depending on the economic measure used. In the platform cage, the rabbits spent the majority of their time in front of the platform, rather than on or under it, suggesting that the feature of the platform that is most important to the rabbits is the fact that it provides a means of escape if the rabbit feels threatened. It is suggested that laboratory rabbit cages should be fitted with a platform to provide the rabbits with an area to hide in if they are startled.

Chapter 7 – Platform use by singly and socially caged female rabbits

Abstract

Rabbits in the wild use bolt-holes as their primary means of escape from predators. Laboratory rabbits housed in groups tend to be provided with artificial bolt-holes, which they can hide in and may be able to lie on. Caged rabbits may be provided with platforms, which provide a raised area of additional floor space and a darkened area underneath which the rabbits may use as a bolt-hole. In Chapter 6, the rabbits were found to work to gain access to a platform, yet spent the majority of their time in front of the platform. It was noted that rabbits in cages tend to spend more time on their platform than the rabbits in the experiment did and that may this be related to social factors, as social contact was possible whilst on the platform in the cages. The cage environment of socially and singly caged rabbits was manipulated to investigate how platform use was affected by social stimuli from conspecifics. Socially caged rabbits were housed in their pairs, next to their cage-mate (with and without contact) and next to an unfamiliar rabbit (with and without contact). In each cage manipulation the rabbits spent more time in front of the platform than on or under it. The rabbits moved onto the platform most often and spent the shortest bouts in front of the cage when housed with their cage-mate. Similar behaviour was seen when contact was possible with the unfamiliar rabbit. More time was spent on the platform when the rabbits were housed with their cage-mate than when they were housed without contact. When contact was possible, more time was spent facing the adjacent cage than orientated away from it. Singly caged rabbits were housed with and without a platform, both with and without contact with the rabbit in the adjacent cage. When housed without a platform the rabbits spent more time at the front of the cage than at the back, and the reverse was true when housed with a platform. The rabbits spent more time on the platform than in front of or under the platform, and spent more time on the platform in more frequent, longer bouts when contact was possible than when it was not. The rabbits spent longer facing the

adjacent cage than orientated away from it, both with and without contact and spent longer facing the adjacent cage when contact was possible than when not. The results indicate that platform use is affected by social stimuli from other rabbits, whether physical, visual or olfactory and that platform use may be related to territorial behaviour. It is recommended that laboratory rabbits housed in cages should be provided with a platform, both as a means of gaining social contact and to provide an area to hide in if startled.

7.1 Introduction

Wild rabbits live in a warren, which consists of many narrow and often long burrows. The warren may be fairly simple, with few branches or may be very complex and full of twists and turns. The burrow has several functions including protection from the elements, providing an area for raising the young and perhaps most importantly protection from predators. In addition to the main burrow entrances the rabbits will dig extra entrances/exits. These additional entrances are known as bolt-holes and as suggested by their name, rabbits run down them when they are startled or threatened. Kolb (1991, 1994) found that the use of the burrows as a means of escape from predators varies depending on the habitat the rabbits live within. If there is a lot of ground cover the rabbits may stay hidden on the surface rather than go underground and this may provide better protection, particularly from small predators such as stoats, which could easily enter the burrow. If there is no other cover, the bolt-holes will be used as a means of escape.

Group housed rabbits within laboratories are almost always provided with artificial bolt-holes (e.g. cardboard or plastic tubes, cardboard boxes), although this is to escape from the unwanted attention of other rabbits rather than from any other sort of threat (e.g. Batchelor, 1991; Wolfensohn and Lloyd, 1994). In cages, floor space is at a premium, and it is interesting that platforms were probably originally introduced into laboratory cages as a means of increasing floor space to comply with new regulations, rather than to improve the welfare of the rabbits. The widening acceptance that rabbits do appear to use their platforms is leading to them becoming increasingly common within laboratories. Platforms within cages provide a raised area as well as a darkened area underneath, which the rabbits may use as a bolt-hole to hide under if startled. This can also be used to enable the rabbits to avoid visual contact with other rabbits and the laboratory technicians. Hansen and Berthelsen (2000) carried out a study of the use of platforms by laboratory rabbits and found that rabbits housed without a platform were vigilant for longer after a disturbance than rabbits housed with a platform. Rabbits housed without a platform were also found to be more restless than those housed with a platform - the former failing to complete ongoing activities. Rabbits housed in cages without platforms tend to freeze when startled, assuming a hunched position, and this is much reduced when rabbits are provided with areas to hide in (e.g. Stauffacher, 1997).

In the previous chapter (Chapter 6), the rabbits worked to gain access to the platform, yet spent the majority of their time lying in front of it rather than on or under it, appearing to not be using the platform, i.e. the rabbits were motivated for the opportunity to be near to the platform. It was noted from casual observations, that rabbits in laboratory cages spent more time on the platform than the rabbits within the experimental apparatus did. It was suggested in Chapter 6 that the use of platforms in the cages might have been partly due to the fact that being on the platform allowed the rabbits to look down into the adjacent cages in the rack and therefore to gain visual contact with conspecifics. This was not possible in the experimental apparatus used in Chapter 6. The aim of this chapter was therefore to investigate platform use by socially and singly caged rabbits and to manipulate the cage environment to determine how this was affected by physical, visual and olfactory stimuli from conspecifics. Two experiments are described in this chapter. Firstly, pair housed rabbits were housed in cages with platforms with their cage-mate, in the cage next to their cage-mate (with and without visual and minimal tactile contact) and next to an unfamiliar rabbit (with and without visual and minimal tactile contact). This was to determine how social factors affect platform use. In a second experiment, singly housed rabbits were housed with and without a platform, both with and without visual and minimal tactile contact with the rabbit in the adjacent cage. This was to determine how the presence of a platform affected the use of different areas within the cage and to investigate whether the effects of social factors on platform use differed between rabbits that had previous experience of being socially housed and rabbits that had been singly housed. The overall percentage of time and mean duration of time spent on, in front of and under the platform were calculated, as was the number of times the rabbits moved onto the platform and their orientation on the platform with respect to the adjacent cage.

Part 1 - Socially caged rabbits

7.2 Methods

7.2.1 Animals, housing and husbandry

The experimental animals were ten female New Zealand White rabbits, which were approximately 8 months old. The rabbits had previously been used in the experiment described in Chapter 5, and were housed as described in section *5.2.1*. Following the experiment in Chapter 5, the rabbits were housed with the cage manipulations described in section *7.2.2*. The dominance status of the individuals in each pair were determined in Chapter 5 (see section *5.2.2*).

7.2.2 Cage manipulations

The cage environment was manipulated in several ways, with five days between each of the manipulations, during which time the rabbits were housed in with their cage-mate. The manipulations were carried out in the following order:

i. 'In with cage-mate' (With c-m)

The rabbits were housed in their usual double cage in full physical contact with their cage-mate.

ii. 'Next to cage-mate, no visual and minimal tactile contact' (C-m, no contact)

The rabbits were separated from their cage-mate by closing the sliding door between the interconnecting cages. Boards were placed between the cages preventing visual and tactile contact, therefore the rabbits had only olfactory and auditory contact with their cage-mate.

iii. 'Next to cage-mate, visual and minimal tactile contact possible' (C-m, contact)
The rabbits were separated from their cage-mate by closing the sliding door between the two cages. The rabbits were able to gain visual contact with their cage-mate through the sliding door by moving onto the platform and could have both visual and minimal tactile contact through the mesh panel.

iv. 'Next to unfamiliar rabbit, no visual and minimal tactile contact' (Un, no contact)
The rabbits were housed in separate racks of cages from their cage-mate and were housed next to an unfamiliar rabbit. Boards were placed between the cages preventing visual and tactile contact, therefore the rabbits had only olfactory and auditory contact with the unfamiliar rabbit in the adjacent cage.

v. 'Next to unfamiliar rabbit, visual and minimal tactile contact possible' (Un, contact)
The rabbits were housed in separate racks of cages from their cage-mate and were housed next to an unfamiliar rabbit. The rabbits were able to gain visual contact with the unfamiliar rabbit through the sliding door by moving onto the platform and could have both visual and minimal tactile contact through the mesh panel.

During part of the experiment in Chapter 5 the rabbits had been separated from their cage-mate on a daily basis for up to 23 hours and were therefore used to being separated.

7.2.3 Data collection

The behaviour of the rabbits in their cages was recorded on videotape using a time-lapse video recorder (Panasonic AG-6024), a quad unit (Panasonic WJ-410) and CCTV cameras (Panasonic WV-BP330/B) set up on tripods in front of the cages. Infra-red lights were used to record behaviours during the dark hours of the light cycle. The tripods and cameras were set up for 24 hours prior to recording behaviour to allow the rabbits to habituate to the sight of the cameras.

In order to allow the rabbits to settle, cage manipulations were carried out one hour before filming began. For each cage manipulation the rabbits were filmed for 24 hours in each side of the interconnecting cage to avoid any positional effect, for example, rabbits showing a preference for lying on one their sides, therefore facing a particular direction. The body of the cages and the trays underneath the cages were moved with the rabbits to avoid housing them in cages with the scent of other rabbits. The videotapes were observed using continuous recording to determine the measures shown in Table 7.1. The behaviour of both rabbits of a pair was analysed from the same observation period.

Measurement	**Description**
Position within the cage	Time spent on, in front of and under the platform; (total time (as a percentage) and mean duration of bouts)
Number of times on the platform	Total number of times the rabbit jumped on to the platform
Orientation on the platform	Percentage of time on the platform spent facing or turned away from the adjacent cage (only recorded when housed singly)

Table 7.1 Measurements recorded from video observations to investigate platform use

7.2.4 Data handling and analysis

For each of the measurements in Table 7.1 the mean of the two 24 hour periods was calculated. Histograms were plotted for each of the measurements taken, then the data were transformed to improve normality. The percentage of time spent on, in front of and under the platform and the percentage of the time on the platform spent facing the adjacent cage were transformed using arcsine transformation, as is conventional for improving normality of percentage data. The number of times the rabbits move onto the platform and the mean duration of bouts on, in front of and under the platform were subjected to log transformation. Any differences between cage manipulations and any effects of dominance on the measurements in Table 7.1 were investigated using General Linear Models, blocking by pair and dominance. Tukey tests were used to highlight where any significant differences lay. For each cage manipulation, paired t-tests were used to compare the time on the platform that was spent facing the adjacent cage and the time spent orientated away from the adjacent cage. Analysis was carried out using Minitab 12.

7.3 Results

7.3.1 Position within the cage

In each of the cage manipulations the rabbits spent the majority of their time in front of the platform. The mean percentage of time spent on, in front of and under the platform in each of the different cage manipulations are shown in Figure 7.1.

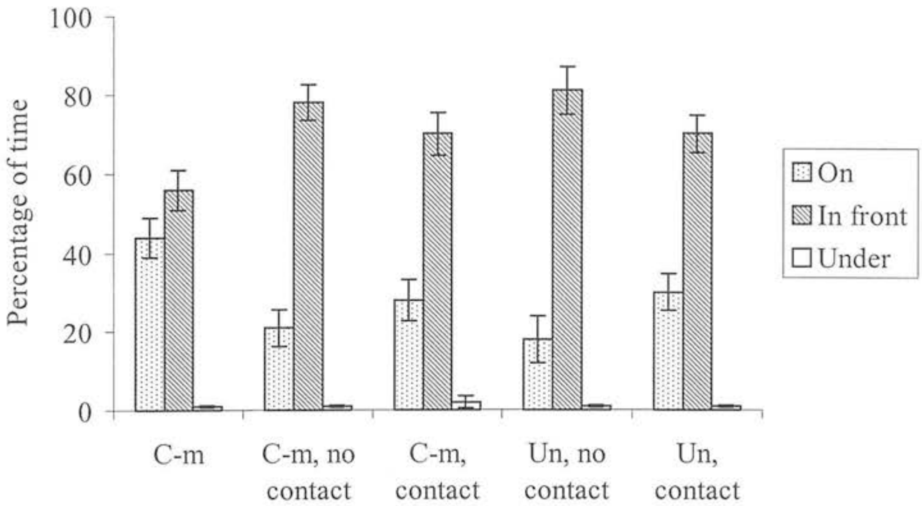

Figure 7.1 Mean (± se) percentage of time spent on, in front of and under the platform in each of the cage manipulations (c-m = cage-mate, un = unfamiliar rabbit)

The cage manipulations had a significant affect on the percentage of time the rabbits spent on the platform (GLM, $F_{(4,40)}=4.61$, $p<0.05$). When the rabbits were housed with their cage-mate they spent significantly more time on the platform than they did when housed without contact with either their cage-mate or an unfamiliar rabbit (both $p<0.05$). The percentage of time spent in front of the platform showed a corresponding difference between manipulations ($F_{(4,40)}=4.27$, $p<0.05$), with the rabbits spending less time in front of the platform when housed with their cage-mate than they did when housed with no contact with either their cage-mate or an unfamiliar rabbit (both $p<0.05$). The percentage of time spent under the platform did not differ significantly between the cage manipulations. The time spent on and in front of the platform differed between pairs of rabbits ($F_{(4,40)}=2.63$, $p<0.05$ and $F_{(4,40)}=2.84$, $p<0.05$ respectively), however, dominance did not have any effect.

7.3.2 Number of times on the platform

The mean number of times the rabbits moved onto the platform in each of the cage manipulations is shown in Figure 7.2.

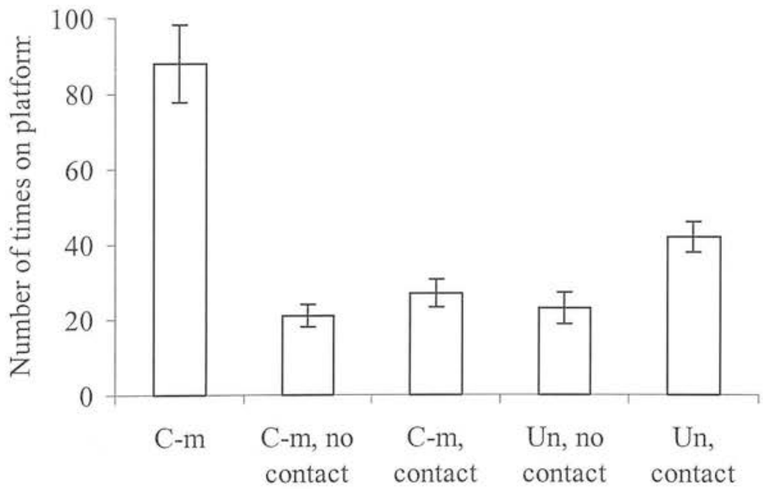

Figure 7.2 Mean (± se) number of times on platform (c-m = cage-mate, un = unfamiliar rabbit)

The mean number of times the rabbits moved onto the platform varied between the cage manipulations ($F_{(4,40)}=17.58$, $p<0.001$). The rabbits moved onto the platform significantly more often when housed with their cage-mate than in any of the other cage manipulations (with and without contact with their cage-mate and without contact with the unfamiliar rabbit, $p<0.001$; with contact with the unfamiliar rabbit, $p<0.05$). The rabbits moved on to the platform more often when housed with contact with the unfamiliar rabbit than they did when housed without contact, with either the unfamiliar rabbit or their cage-mate (both $p<0.05$). The number of times the rabbits moved onto the platform was not affected by dominance.

7.3.3 Mean duration of bouts

The mean duration of bouts of time spent on, in front of and under the platform are shown in Figure 7.3.

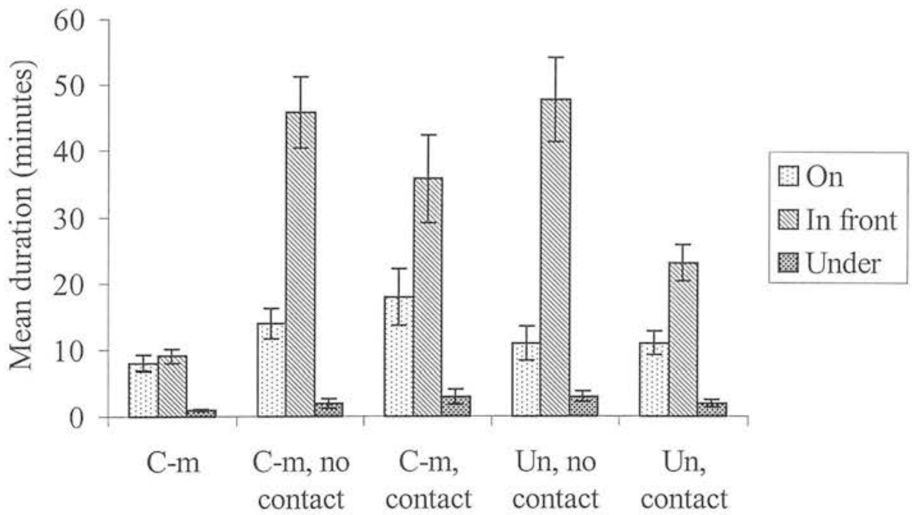

Figure 7.3 Means (± se) of the mean duration of bouts of time spent on, in front of and under the platform (c-m = cage-mate, un = unfamiliar rabbit)

The mean duration of bouts of time spent on the platform did not differ between cage manipulations. It was, however, affected by dominance ($F_{(1,40)}=5.64$, $p<0.05$), with dominant rabbits spending longer bouts on the platform than subordinate rabbits. The mean duration of bouts on the platform also differed between pairs of rabbits ($F_{(4,40)}=3.98$, $p<0.05$). The mean duration of bouts in front of the platform did, however, differ between the cage manipulations ($F_{(4,40)}=17.94$, $p<0.001$), with rabbits spending significantly shorter bouts in front of the platform when housed with their cage-mate than in any of the other cage manipulations (all $p<0.001$). The bouts in front of the platform were also shorter in when housed with contact with the unfamiliar rabbit than when housed without contact with either the unfamiliar rabbit or their cage-mate (both $p<0.05$). There was no difference between pairs of rabbits, nor between the dominant and subordinate rabbits. The mean duration of time spent under the platform did not differ between cage manipulations, nor was it affected by dominance. It did, however, differ between pairs of rabbits ($F_{(4,38)}=3.55$, $p<0.05$).

7.3.4 Orientation on the platform

The percentage of the time on the platform spent facing and orientated away from the adjacent cage is shown in Figure 7.4.

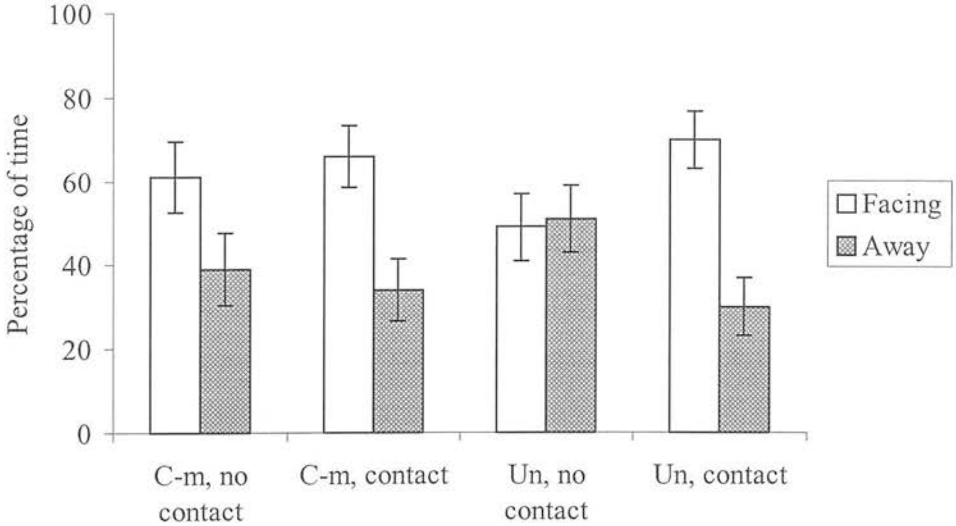

Figure 7.4 Mean (± se) percentage of time on the platform spent facing and orientated away from the adjacent cage (c-m = cage-mate, un = unfamiliar rabbit)

The percentage of the time on the platform that was spent facing the adjacent cage did not differ between the cage manipulations, nor did it differ between pairs of rabbits, or between dominant and subordinate rabbits. The amount of time spent facing the rabbit in the adjacent cage was significantly greater than the time spent orientated away from the adjacent cage when the rabbits were housed with contact, with both their cage-mate (Paired t-test, N=12, T=2.73, $p<0.05$) and an unfamiliar rabbit (N=12, T=3.40, $p<0.05$).

7.4 Discussion

Regardless of how the rabbits were housed the greatest proportion of their time was spent in front of the platform and the least amount of time was spent under the platform. This is similar to the findings of the experiment in Chapter 6, where the rabbits were found to be willing to work to gain access to the platform, yet spent the majority of their time lying in front of it. It was suggested in Chapter 6 that the aspect of the platform that was most important to the rabbits was that it provided a means of 'escape' if they felt under threat. The findings of the current study support this suggestion. The time spent on the platform was affected by the cage manipulation. The rabbits spent the longest amount of time on the platform when they were housed with their cage-mate (and could therefore have social contact at all times) and the least amount of time on the platform when no contact was possible, with either their cage-mate or the unfamiliar rabbit, indicating that the use of the platform is affected by social contact. From casual observations it was noted that both individuals of the pairs often spent time on the platforms together, either in separate sides of the double cage, or sharing one platform.

There was no difference in the time the rabbits spent on the platform when housed in visual contact with either their cage-mate or an unfamiliar rabbit, suggesting that social contact with a familiar or unfamiliar rabbit is of equal importance to the rabbits. This is consistent with the findings in Chapters 4 and 5 where rabbits were found to show no difference in how hard they would work for visual and minimal tactile contact with either a familiar or unfamiliar rabbit. As in all of the treatments in this chapter, when the rabbits were housed with visual and minimal tactile contact they spent more time in front of the platform than they did on it, indicating that when allowed visual contact they do not want to spend all of their time in direct visual contact with another rabbit. This is similar to the findings of Chapter 6, when after paying the cost of entering the social contact cage the rabbits spent almost a third of their time out of visual contact with the other rabbit. This is also similar to the findings of Chapters 4 and 5, when again the rabbits paid the cost of reaching the rabbit, yet did not spend all of the time allowed in visual contact. These findings therefore indicate the importance of providing the rabbits with the opportunity to avoid direct visual contact. The time spent facing the adjacent cage did not differ between manipulations, and therefore was not affected by whether or not the rabbits had visual contact with the rabbit in the adjacent cage. Perhaps facing the cage when no contact was present allowed the rabbits to receive stronger olfactory cues from the adjacent cage. The time spent facing the adjacent cage was, however, significantly greater than the time spent away from the adjacent cage when housed with contact with either their cage-mate or the unfamiliar rabbit, indicating that the rabbits were using the platform as a means of gaining visual contact.

The number of times the rabbits moved onto the platform and the mean duration of bouts on, in front of and under the platform can give an indication of how active the rabbits were within the cage. When housed with their cage-mate the rabbits moved onto the platform more times than in any of the other cage manipulations. The bouts of time spent in front of the platform were significantly shorter when the rabbits were housed with their cage-mates than in any of the other cage manipulations. This and the elevated number of times the rabbits moved onto the platform indicate that the rabbits were more active when housed with their cage-mate. Casual observations of the rabbits when housed with their cage-mates found that these short bouts of time on the platform tended to be as a result of interaction between the cage-mates, particularly at certain times of the day such as just after the lights came on in the morning and late afternoon. In this experimental housing, moving onto the platform was the rabbits' only means of moving between adjacent cages, which was usually their response to aggression interactions, therefore the number of times the rabbits moved onto their platforms is artificially high. The rabbits moved onto the platform more often when they had contact with the unfamiliar rabbit than they did when contact was not possible (with either the unfamiliar rabbit or their cage-mate) and the mean duration of time spent in front of the platform was significantly lower than when they were housed without contact. This suggests that moving onto the platform to see the unfamiliar rabbit may have been partly for territorial reasons, making frequent checks on the unfamiliar rabbit in such close proximity to their cage. The rabbits in the experiment in Chapter 6 showed a similar response of making frequent, short visits to the social contact cage, even as the cost of entering the cage increased. The finding that the dominant rabbits spent longer bouts on the platform that the subordinate rabbits also supports the suggestion that platform use may be related to territorial behaviour.

The findings of the cage observations of these rabbits indicate that physical social factors seem to have most influence on platform use and that the time spent on the platform is affected by both olfactory and visual cues from rabbits in adjacent cages. This could therefore explain why the rabbits in the experiment in Chapter 6 spent so little of the time in the platform cage actually on the platform, as in that experiment there were no social cues from other rabbits which could influence the use of the platform and using the platform did not allow the rabbits to gain social contact.

Part 2 - Singly caged rabbits

7.5 Methods

7.5.1 Animals, housing and husbandry

The experimental animals were eleven female New Zealand White rabbits, which were approximately 8 months old. These rabbits had previously been used for the experiment reported in Chapter 6, and were housed as described in section *7.2.1*. Throughout the experiment in Chapter 6 the rabbits were able to gain access to a platform and visual and minimal tactile contact with a conspecific in the experimental apparatus, although they did not have access to either of these resources in the cages prior to the experiment. Following the completion of the experiment in Chapter 6, the rabbits were housed with the cage manipulations described in section *7.5.2*.

7.5.2 Cage manipulations

i. 'No platform, no visual and minimal tactile contact' (No platform, no contact)
The platforms were removed from the cages. Boards were placed between adjacent cages preventing visual and tactile contact, therefore the rabbits had only olfactory contact with the unfamiliar rabbit in the adjacent cage.

ii. 'No platform, visual and minimal tactile contact possible' (No platform, contact)
The platforms were removed from the cages. The rabbits were able to gain visual and minimal tactile contact with the unfamiliar rabbit in the adjacent cage, if they reared up onto their hind legs.

iii. 'Platform, no visual and minimal tactile contact' (Platform, no contact)
The platforms were fitted into the cages. Boards were placed between adjacent cages preventing visual and tactile contact, therefore the rabbits had only olfactory contact with the unfamiliar rabbit in the adjacent cage.

iv. 'Platform, visual and minimal tactile contact possible' (Platform, contact)
The platforms were fitted into the cages. The rabbits were able to gain visual and minimal tactile contact with the unfamiliar rabbit in the adjacent cage by moving onto the platform.

7.5.3 Data collection

Data was collected as in section *7.2.3*, with the measurements in Table 7.2 being recorded.

7.5.4 Data handling and analysis

For each of the measurements in Table 7.2 the mean of the two 24 hour periods was calculated. For each of the four cage manipulations comparisons were made between the time spent in the front half and the back half of the cage using paired t-tests. This was to investigate whether the platform influenced the preferred position within the cage. Paired t-tests were also used to compare the time spent on, in front of and under the platform and the mean duration of bouts in each position within the cage when rabbits did and did not have contact with the rabbit in the adjacent cage, to investigate whether visual contact affected platform use. The number of times the rabbits moved onto the platform was also analysed using paired t-tests to investigate the effects of visual contact. Comparisons were made between the time spent facing and the time spent orientated away from the adjacent cage, both when the rabbits had contact and did not have contact. The time spent facing the adjacent cage was compared between when the rabbits did and did not have contact. Again, these comparisons were made using paired t-tests. Analysis was carried out using Minitab 12.

Measurement	Description
Position within the cage	Overall percentage of time spent on, in front of and under the platform; overall percentage of time spent in the front and back halves of the cage (when platform present, time in back half = time on and under platform); mean duration of bouts calculated
Number of times on the platform	Total number of times the rabbit jumped on to the platform
Orientation on the platform	Percentage of time on the platform spent facing or turned away from the adjacent cage

Table 7.2 Measurements recorded from video observations to investigate platform use

7.6 Results

7.6.1 Position within the cage

The percentage of time the rabbits spent at the front and back of the cage, both with and without a platform, and both with and without contact with the rabbit in the adjacent cage are shown in Figure 7.5.

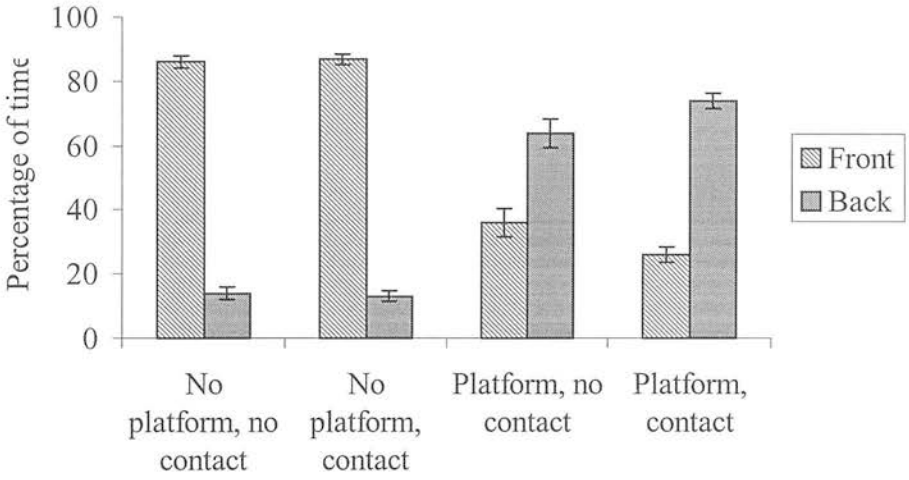

Figure 7.5 Mean (± se) percentage of time spent at the front and the back of the cage, with and without a platform at the back of the cage

When housed without a platform the rabbits spent significantly more time at the front of the cage than at the back, both with and without contact with rabbits in the adjacent cage (Paired t-test, N=11, T=23.11, $p<0.001$ and N=11, T=18.17, $p<0.001$ respectively). However, when housed with a platform the rabbits spent significantly more time at the back of the cage than at the front, both with and without contact (N=11, T=10.19, $p<0.001$ and N=11, T=3.14, $p<0.05$ respectively).

When housed with a platform, the rabbits spent significantly more time on the platform when contact was possible with the rabbit in the adjacent cage (see Figure 7.6; N=11, T=3.08, $p<0.05$). The rabbits correspondingly spent more time in front of the platform when no contact was possible (N=11, T=3.16, $p<0.05$). No difference was found in the time spent under the platform.

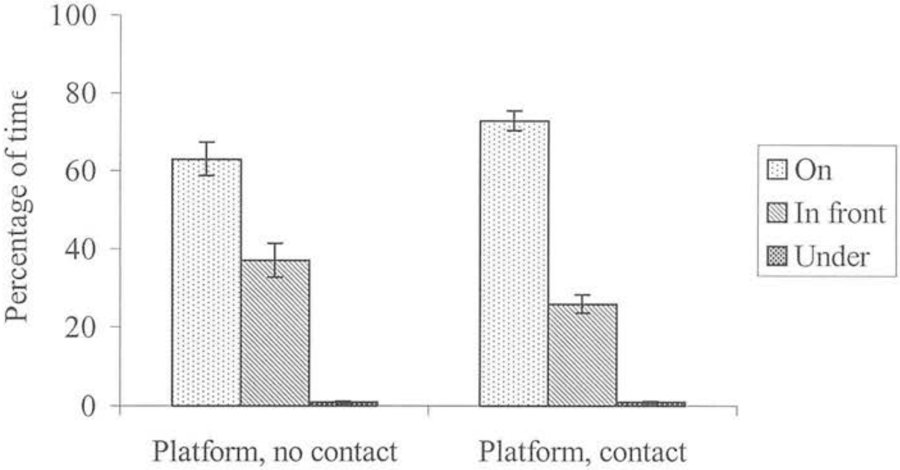

Figure 7.6 Mean (± se) amount of time spent on, in front of and under the platform, both with and without contact

7.6.2 Number of times on platform

The number of times the rabbits moved onto the platform when no contact was possible and when contact was possible are shown in Figure 7.7.

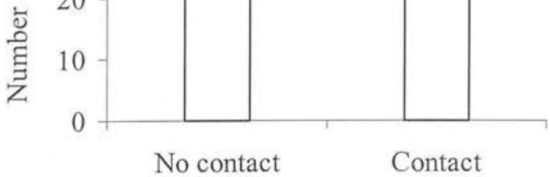

Figure 7.7 Mean (± se) number of times the rabbits moved on to the platform both with and without contact

The rabbits moved on to the platform significantly more times when contact with the rabbit in the adjacent cage was possible compared to when contact was not possible (Paired t-test, N=11, T=6.60, $p<0.001$).

7.6.3 Mean duration of bouts

The mean duration of the time the rabbits spent on, in front of and under the platform are shown in Figure 7.8. The mean duration of bouts on the platform was significantly greater when the rabbits were housed with contact than when housed without contact (Paired t-test, N=11, T=4.60, $p<0.05$). The mean duration of time spent in front of the platform was, however, significantly greater when the rabbits were housed without contact than when housed with contact (N=11, T=5.81, $p<0.001$). The mean duration of time spent under the platform did not differ between cage manipulations.

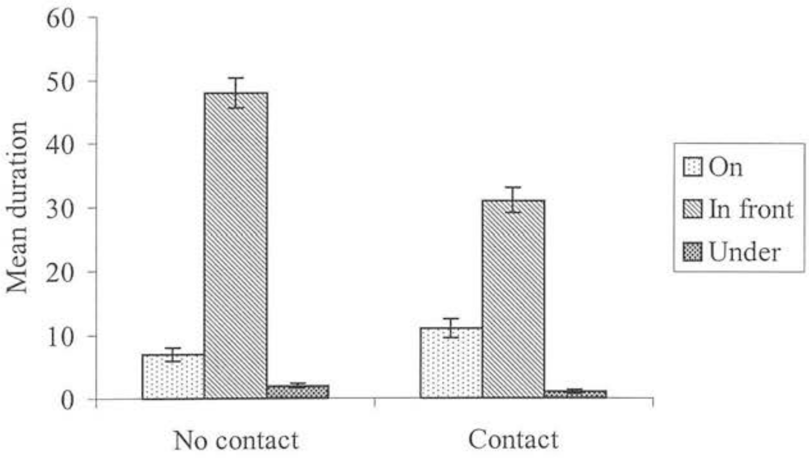

Figure 7.8 Means (± se) of the mean duration of time spent on, in front of and under the platform

7.6.4 Orientation on the platform

The percentage of the time on the platform spent facing and orientated away from the adjacent cage is show in Figure 7.9.

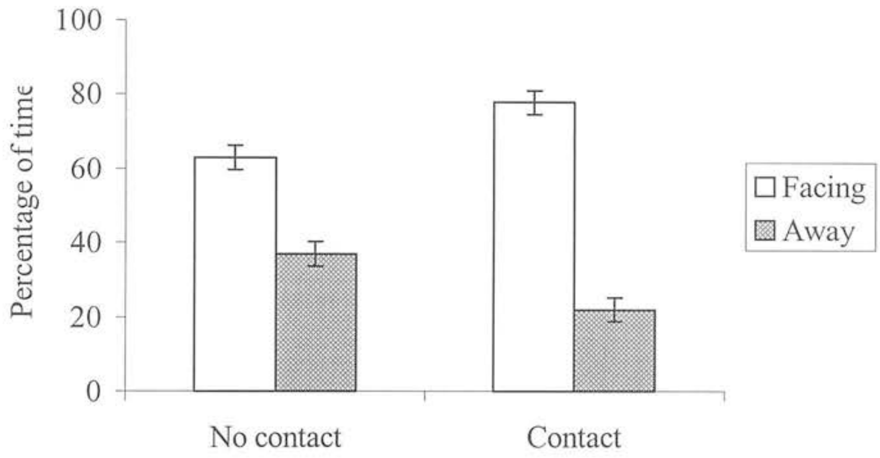

Figure 7.9 Mean (± se) percentage of time on the platform spent facing and orientated away from the adjacent cage

The rabbits spent significantly more time facing the adjacent cage than they did orientated away from it, when housed both with and without contact (Paired t-test, N=11, T=8.86, $p<0.001$ and N=11, T=4.07, $p<0.05$ respectively). The rabbits spent more time facing the adjacent cage when housed with contact than they did when they did not have contact (N=11, T=3.87, $p<0.05$).

7.7 Discussion

When housed with no platform the rabbits chose to spend significantly more of their time at the front of the cage rather than at the back of the cage, indicating that this was the preferred position. However, when provided with a platform they choose to spend significantly more time at the back of the cage (mostly on the platform rather than under it) than at the front suggesting that the preference for the platform was stronger than the preference for the front of the cage. This was also found in Chapter 2, when rabbits were filmed in cages within the pharmaceutical industry. Hansen and Berthelsen (2000) similarly found rabbits in cages without platforms spent the highest frequency of scan samples in the front and middle of the cage and those with platforms spent the highest frequency of scan samples at the back of the cage.

Unlike the socially housed rabbits, which spent most time in front of the platform, the singly housed rabbits spent most time on the platform. These rabbits were the same rabbits that had been used in the experiment in Chapter 6 and in the experimental apparatus in that chapter were found to spend little time on the platform, indicating that their time on the platform was strongly influenced by the presence of social cues. It is possible that the singly caged rabbits spent more time on the platform than the socially caged rabbits due to the fact that as these rabbits had always been housed singly, social contact was more attractive because it was novel to them, or perhaps more threatening, suggesting that they were motivated to investigate the threat and defend their territory as rabbits do in the wild (e.g. Bell, 1983). Similarly to the socially housed rabbits, the singly housed rabbits spent more time on the platform when contact was possible than they did when contact was not possible, again indicating that platform use is affected by social factors and that social contact is something that singly housed rabbits want to have. Of the time spent on the platform, the rabbits spent significantly more time facing the adjacent cage when housed with contact than when housed without contact. When housed both with and without contact the amount of time spent facing the adjacent cage was significantly longer than the amount of time spent orientated away from it. This was also seen with the socially housed rabbits and it was suggested that facing the adjacent cage perhaps allowed the rabbits to receive stronger olfactory cues than when they were orientated away from the cage.

The singly housed rabbits moved onto the platform more frequently when it was possible to gain contact with the rabbit in the adjacent cage, which was also seen with the socially housed rabbits. The mean duration of bouts on the platform was longer when contact was possible compared to when it was not and the mean duration of bouts in front of the platform were longer when contact was not possible. Again, the frequent, short bouts on the platform are consistent with the short, frequent visits to the social contact cage in the experiment in Chapter 6, again indicating there was an aspect of territory inspection in their behaviour.

As with the findings of observations of the socially caged rabbit, the observations of the singly caged rabbits indicate that the time spent on the platform is affected by both olfactory and visual cues from rabbits in adjacent cages.

7.8 General discussion

The rabbits that had been housed socially prior to the cage manipulations spent most of their time in front of the platform rather than on or under it, whereas the rabbits that had always been housed singly spent more time on the platform. Both groups moved onto the platform more frequently when they were able to gain social contact than when they were not, and spent more time on the platform when contact was possible. Both made frequent, short duration visits (approximately 10 minutes long) to the platform when they were able to gain contact with an unfamiliar rabbit. Both groups spent more time facing the unfamiliar rabbit when contact was possible than they did orientated away from it. The findings suggest that both the singly and socially housed rabbits may have been behaving in a territorial way towards the unfamiliar rabbit in the adjacent cage, choosing to have frequent, short bouts of visual contact. Rabbits in the wild are territorial and patrol their territories regularly, scent marking using anal and chin glands and depositing faeces to inform intruders that the territory is occupied (Mykytowycz, 1962, 1968; Thompson and Worden, 1956). It is possible that having rabbits in such close proximity to each others territories may cause the rabbits to feel threatened, motivating them to continually 'patrol' their environment.

The main difference between the two groups of rabbits was in the amount of time spent on the platform, with the singly housed rabbits spending approximately twice as much time on the platform compared to the socially housed rabbits. Although the singly caged rabbits were the same rabbits that had been used in the experiment in Chapter 6, it was the socially caged rabbits that showed behaviour more like that seen in Chapter 6. It was mentioned in the discussion of Chapter 6 that in the experiment the rabbits spent very little time on the platform and the majority of time in front of it, and that this was unlike casual observations made of rabbits in their laboratory cages. It was suggested that this might have been due to the fact that no social cues (either visual or olfactory) were available when the rabbits were on the platform, or even in the platform cage and therefore the use of the platform was not confounded with motivation for another resource. With the suggestion that the platforms in cages were used for social purposes, it was also suggested that in the absence of social contact it was not the raised area of the platform that was important aspect, but the area underneath it which provided the rabbits with the darkened bolt-hole. Lying in front of the cage meant that from this position the rabbits could move into the bolt-hole most quickly. The use of bolt-holes or the cover of vegetation is naturally the primary means of escape for a rabbit (Thompson and Worden, 1956; Kolb, 1991, 1994) and casual observations of laboratory rabbits with access to platform have found that they respond in such a way when startled. It has been suggested by Stauffacher (1997) that the platform should be shorter than the length of the cage and that the entrance to the bolt-hole area should be at the side, as this takes into account a further aspect of normal rabbit behaviour. It has been found that even when rabbits are able to access a bolt-hole from in front of the platform, rabbits will often use this side entrance when startled.

Despite the fact that the wild rabbit spends much of its time underground and that the main escape response is to hide in burrows and bolt-holes, both the socially and singly caged rabbits were found to spend very little time underneath the platform. This was also found by Batchelor (1991), Whary *et al.* (1993) and Hansen and Berthelsen (2000). Brooks *et al.* (1993) allowed pairs of rabbits to have access to two cages via a PVC pipe and similarly found that the use of this by the rabbits as a hiding area was infrequent. It is therefore likely that the rabbits have learnt that there are few threatening stimuli in the environment they are in. It has, however, been found that rabbits housed without platforms are more restless than rabbits provided with an area to hide and are more easily affected by the environment (e.g. Hansen and Berthelsen, 2000). Perhaps merely the presence of the platform is sufficient to reduce the restlessness of the rabbits, rather than actually using it. Hansen and Berthelsen (2000) found that although none of the rabbits spent much time under the platform, females spent more time under the platform than males. This is also seen in wild rabbits (Kolb, 1991, 1994) with females spending longer in the burrows than males. Hansen and Berthelsen also found that the female rabbits were more timid than the males (both in the cage and in open-field tests) and also gnawed at the environment more in the cages without platforms. They suggested that perhaps female rabbits in particular benefited from the presence of the platform.

The behaviour of the socially housed rabbits appears to agree more closely with the suggestion that the opportunity to hide underneath the bolt-hole if startled was the most important aspect of the cage and that the platform was used to gain visual contact, compared with that of the singly caged rabbits. The socially caged rabbits spent more time in front of the platform than on it and increased their use of the platform when visual and minimal tactile contact was possible. The singly caged rabbits spent more time on the platform than in front of it, even when visual contact was not possible, however it is possible that social contact is more novel to rabbits that have always been singly housed and therefore visual and olfactory cues have more effect. The findings suggest that for rabbits which have previous experience of being socially housed, when in a cage with both a bolt-hole and a means of gaining social contact that the motivation for the two resources are fairly similar, with much time being spent next to the bolt-hole but time on the platform increasing when visual and minimal tactile contact are available as opposed to just olfactory contact. This is consistent with the economic measure of the 'maximum price paid' used in Chapter 6, which ranked gaining social contact and gaining access to a platform (and then spending the majority of time in front of it) as being of equal importance. However, the behaviour of the singly caged rabbits suggests that rabbits that have been singly caged are more strongly motivated for social contact than for a bolt-hole, as much time was spent on the platform even when only olfactory contact was available. This is consistent with the economic measure of 'total expenditure' used in Chapter 6, which ranked social contact as being more important than gaining access to a platform. It is possible, however, that as singly caged rabbits become more accustomed to being able to gain visual contact they may behave more like the socially caged rabbits and spend more of their time in front of the platform.

Platform use therefore appears to increase when social contact is available through its use, with the effects varying depending on the environmental cues available and the previous experience of the rabbits. This variation in resource use and apparent motivation for resources depending on the environmental cues and previous experience is therefore a factor which must be taken into account in the design and analysis of preference and motivational studies. Other authors (e.g. Warburton and Mason, in press) have also discussed the importance of environmental cues.

7.9 Implications for the housing of laboratory rabbits

The findings suggest that even if rabbits in cages appear not to be using their platform, i.e. are lying in front of the platform, the rabbits are likely to perceive this position within the cage differently to humans. Although the platform may be used as a means of gaining social contact, it appears that being in close proximity to a platform and therefore a means of 'escape' if startled is also important to the rabbits. It is therefore recommended that laboratory rabbits should be provided with a platform regardless of their previous experience of housing types, to provide both a bolt-hole and a means of gaining visual (if possible) and olfactory stimuli from conspecifics.

Chapter 8 - General discussion

8.1 Current situation within the laboratory industry

The survey of the pharmaceutical industry at the onset of the research and subsequent discussions with the Pharmaceutical Housing and Husbandry Steering Committee (PHHSC), laboratory technicians and laboratory rabbit breeders meant that up-to-date information was gathered to determine which aspects of laboratory rabbit housing and husbandry were of most concern. This information, along with observations of rabbits in different types of housing, highlighted the main issues. The primary concern was the rabbits' social environment and second most was the importance of the provision of a platform within cages. The questionnaire found that where possible, female rabbits were housed socially, although male rabbits are always housed singly due to problems with aggressive behaviour. Female rabbits may be housed in groups in cages or in floor pens, and whilst this is often a viable method of housing it was reported as not being guaranteed to be successful, as aggressive behaviour may erupt when the group appears to be stable. It was found from the questionnaire that within the previous year, 46% of female laboratory rabbits were housed singly, 12% were housed socially in cages and 42% were housed socially in floor pens. Although the provision of social contact was said by the industry to be the area of most concern it is often the case that certain experimental needs take greater priority, often preventing social housing from being used. Examples of such experimental needs, as reported in the questionnaires, included the ability to monitor food and water intake and the need for topical drug administration. Under these circumstances rabbits have to be housed singly. From the questionnaires it was found that of the six companies that singly caged rabbits, four of them used cages that allowed the rabbits to have visual and minimal tactile contact with rabbits in adjacent cages.

The questionnaire also found that of the rabbits used in the previous year, 91% were New Zealand White (NZW) and that 69% of the total number of rabbits used were female. The investigations into housing of laboratory rabbits therefore focused on female NZW rabbits to ensure that the findings of the research were applicable to as large a proportion of the rabbits used within the industry as possible.

8.2 Social contact

Research has previously been carried out to assess whether social housing of female rabbits can be successful and whether rabbits show a preference for social contact. It has been found that social housing is often a viable method to use (e.g. Heath and Stott, 1990; Batchelor, 1991; Stauffacher, 1992) and that rabbits choose to spend time with other rabbits when given the opportunity to do so (e.g. Huls *et al.*, 1991; Brooks *et al.*, 1993; Held *et al.*, 1995). However, when experimental constraints prevent rabbits from being housed socially (for example, high turnover of rabbits in tissue harvesting), the use of some designs of single caging allows rabbits to have visual and minimal tactile contact through a mesh panel between cages. This only allows the rabbits to have restricted social contact and contact which they are unable to have complete control over. An individual is only able to gain visual contact if the adjacent rabbit is either on or in front of its platform and an individual is only able to avoid being in visual contact with a rabbit on the platform in the adjacent cage by moving under the platform. Although rabbits have been found to show a preference for full social contact, it was not known whether in the absence of full physical contact, such limited contact was of value to rabbits.

In this thesis, the importance of social contact was assessed using three different approaches, namely short term tests of motivation, longer term tests of motivation and cage observations (both within the laboratory industry and of the experimental rabbits used in the motivation tests). The findings of each of the approaches used indicated that visual and minimal tactile contact was something that rabbits wanted and was something that they were willing to work for. When rabbits were given the opportunity to access more than one resource at a time and had control over the duration of time spent with each resource (Chapter 6), the rabbits were found to be as motivated for visual and minimal tactile contact as they were for food, in terms of both the maximum price paid for the resources and the total expenditure per day. Rabbits also worked to gain visual and minimal tactile contact when the bouts of contact were pre-determined and restricted to periods of only one minute. Singly caged rabbits were willing to push through weights of up to two thirds of their body weight to gain short periods of visual and minimal tactile contact with a conspecific, whether that conspecific was familiar or unfamiliar to them (Chapter 4). Rabbits that had been pair housed prior to testing and were re-familiarised with their cage-mate during short periods of physical contact each day (Chapter 5) were less motivated to gain short bouts of restricted contact compared to singly housed rabbits (Chapter 4). These socially housed rabbits also showed no difference in how hard they were willing to work for a familiar rabbit (their cage-mate) or an unfamiliar rabbit. The lack of discrimination between contact with an unfamiliar and familiar rabbit was also seen during the observations of the experimental rabbits in their cages (Chapter 7). When the previously socially housed rabbits were able to gain visual contact by moving onto the platform they showed no difference in the time spent on the platform, nor in the time spent facing the adjacent cage when the rabbit in the adjacent cage was their cage-mate or an unfamiliar rabbit. It appears that visual and minimal tactile contact is important to rabbits, regardless of whether the rabbit is familiar to them and regardless of whether the bout length is unlimited or restricted to one minute.

The importance of visual and olfactory contact was again highlighted during the physical contact test with the socially caged rabbits (Chapter 5). During this test the rabbits did not push through significantly heavier weights for physical contact with their cage-mate compared to those pushed through for visual and minimal tactile contact. On several occasions, both dominant and subordinate rabbits pushed through the weighted push-door, yet did not push through the second unweighted door to gain physical contact with their cage-mate. This was however only seen in a small percentage (11%) of the trials. The social contact available when rabbits were in visual contact was clearly of value to some of these rabbits without having to be in actual physical contact. Studies carried out to determine whether rabbits showed a preference for being in physical contact with a conspecific or alone have found that rabbits show a preference for physical contact and spend almost 90% of their time together (e.g. Huls *et al.*, 1991; Brooks *et al.*, 1993).

In each of the approaches used, as well as finding that the rabbits were motivated to gain social contact, it was also found that the rabbits did not spend all of the available time directly in visual contact with the conspecifics. This was perhaps most notable in the experiments in Chapters 4 and 5 where the rabbits were only allowed short periods of contact. Although they moved opposite the unfamiliar or familiar rabbit almost immediately after pushing through the push-door, they did tend to move away from the rabbit and spend some of the time allowed in close proximity to the rabbit but not in direct visual contact. When the rabbits were allowed to determine the duration of the bouts themselves (Chapter 6), they again chose to spend time in close proximity to the other rabbit but not necessarily in direct visual contact. This was also seen in the cage observations (Chapter 7), with rabbits that were able to gain visual contact whilst on their platform spending much of their time in front of the platform, particularly the rabbits that had previously been socially caged. In addition, when the rabbits were on the platform not all of their time was spent facing the adjacent cage. Thus, although rabbits are motivated to gain social contact, they also choose to spend time out of direct contact.

At the end of Chapter 3, it was stated that younger rabbits, of approximately 13-15 weeks of age, would be used for future experiments to ensure that the findings of the experiments were as applicable to the industry situation as possible. It was thought that younger rabbits may be more active during the behavioural tests than the rabbits used in the experiments reported in Chapter 3 (one to three years of age). From casual observations during the behavioural tests, this did appear to be the case. The experiments reported in Chapters 4, 5 and 6 were each carried out over a period of several months. There were no obvious differences in the behaviour or activity levels of the rabbits at the end of the experiments compared to that at the start of the experiments. This indicates that using younger rabbits in the experiments in Chapters 4, 5 and 6 was a valid amendment to make to the experiment reported in Chapter 3.

It would be possible to use the methods developed in this thesis to assess how motivated rabbits were to have the opportunity to avoid social contact, by housing rabbits in direct contact which they are unable to avoid and then allowing them the opportunity to avoid direct contact, for example, behind a screen. As well as laboratory rabbits, this would also be applicable to the farmed rabbit situation, where the rabbits (often New Zealand White) may be housed in wire mesh cages, in visual contact with rabbits all around them and no means of avoiding it. Although the rabbits have been found to be motivated to gain visual and minimal tactile contact, it has, however, not been determined which aspect of such contact is most rewarding (i.e. visual contact alone or both visual and tactile contact). Further investigations using the methods in the current study could allow rabbits only visual contact without allowing tactile contact to determine this. Investigations of the social environment of laboratory rabbits are likely to be applicable to the pet rabbit situation, where rabbits are often housed singly and perhaps with no visual or olfactory cues from conspecifics.

8.3 Platforms

Rabbits were found to work to gain access to a platform, however, once the cost was paid they spent the majority of time lying in front of the platform rather than on or under it (Chapter 6). It was noted that the rabbits spent less time on the platform during the experiment than in casual observations of rabbits in cages and it was thought that this may be due to the absence of social cues. Observations of rabbits were therefore made in cages with manipulations to the social environment to investigate the influence of social cues on platform use. It was found that both visual and olfactory cues influenced the use of the platform. The observations of previously socially caged rabbits found that they showed similar behaviour to that seen in Chapter 6 in the experimental apparatus, spending much of their time in front of the platform and little time on it. In contrast, the rabbits that had always been singly caged spent more time on the platform than in front of or under it. This was seen regardless of whether the rabbits could gain visual contact whilst on the platform. It is thought that the large proportion of time spent in front of the platform in the experimental apparatus and as shown by the socially caged rabbits was due to the fact that this position allows the rabbits to hide under the bolt-hole more quickly if startled. The previously socially housed rabbits appeared to perceive the bolt-hole as being important, however, the singly caged rabbits appeared to find social contact more important than the bolt-hole. It was thought that the visual and olfactory cues gained from being on top of the platform were perhaps more novel to the singly caged rabbits, or were perhaps perceived as more threatening, as the rabbits may not have become used to being housed with social contact. It is therefore possible that over time as the rabbits would become more familiarised with social cues from rabbits in adjacent cages they may spend more time in front of the platform than on it, as seen by the socially housed rabbits. The code of practice for the welfare of laboratory animals (Home Office, 1989) suggests that the platform can be included in the calculation of the floor area of animal cages where there is adequate height for the animal above the platform. This may perhaps not be appropriate, as rabbits seem to prefer to lie in front of the platform rather than on it. If the time spent on the platform is influenced by the presence of social stimuli, in particular visual stimuli, rabbits housed without such stimuli may be less likely to use the platform and therefore do not benefit from this area of floor space.

It was found during cage observations of the rabbits in the laboratory industry (Chapter 2) that the singly caged rabbits spent most of their time on the floor of the cage rather than on the platform. These cages did not allow visual contact between rabbits in adjacent cages and the rabbits had been housed in these cages for some time and therefore any olfactory cues received would not be novel to them. This supports the suggestion above that the singly caged experimental rabbits in Chapter 7 were using the platform due to the novelty of the cues received. When the socially housed experimental rabbits were housed in with their cage-mate they spent little time under the platform (Chapter 7). This was also seen during all of the cage manipulations with the socially and singly caged rabbits (Chapter 7) and in the experimental apparatus (Chapter 6). It was found, however, that the socially caged rabbits observed in the industry (Chapter 2) spent a substantial amount of time under the platform in the bolt-hole, despite being in stable groups. The reason for this is unclear, although this could be due to the fact that the rabbits were housed in groups of three rabbits in the industry whereas the experimental rabbits were in pairs. The larger groups may have increased territorial behaviour, resulting in the rabbits using the bolt-holes to avoid aggressive interactions. It is also possible that the socially caged rabbits in the industry were in a more unpredictable environment than the experimental rabbits. These rabbits were housed in a large room with other racks of cages and several floor pens of rabbits in the room, whereas the experimental rabbits were in a relatively small room and were not disturbed during cage observations. It is therefore possible that the industry housed rabbits were exposed to more noise from other rabbits and were disturbed more often by laboratory technicians entering the room, causing them to seek the safety of the bolt-hole under the platform.

The suggestion that the main use of the platform is as a bolt-hole is supported by the findings of a study by Hansen and Berthelsen (2000). It was found that rabbits in cages with no platforms were more restless than those in cages with platforms and that the former were more easily affected by the environment. This was however not apparent from the observations of rabbits housed with and without platforms (Chapter 7), however, the focus of these observations was on where within the cage the rabbits spent their time, rather than to record their full behavioural repertoire and time budget. Rabbits housed without a platform freeze when startled, assuming a hunched posture (Stauffacher, 1997) and remain vigilant for longer after a disturbance than rabbits housed with a platform (Hansen and Berthelsen, 2000). Female rabbits housed without a platform have been found to be more timid than those housed with a platform and it has been suggested that female rabbits may be more affected by the cage environment and therefore may have more difficulties in coping with the environment than male rabbits (Hansen and Berthelsen, 2000). Laboratory rats have been found to show a strong preference for cages with shelters (Townsend, 1997) and the provision of shelters has similarly been found to affect behaviour, with rats housed with shelters being less fearful and showing more exploratory behaviour.

The methods used to assess the importance of a cage platform could also be adapted to investigate the importance of other features of a laboratory cage such as environmental enrichment, as well as investigating potentially aversive aspects of the environment, such as ultrasound from laboratory equipment.

8.4 Dominance

Wild rabbits can be extremely territorial and the lowest ranking rabbits live on the edges of territories and are known as satellite rabbits (e.g. Bell, 1983). Satellites can be both male and female and are driven away from the main warren by the territory holders. Dominance status is often thought to be an issue in group housing of rabbits and concern has been expressed for the welfare of subordinate rabbits in a group (e.g. Batchelor, 1991). Attempts to 'drive away' subordinate rabbits (as in the wild) clearly do not succeed in the limited space available to captive rabbits, perhaps leading to persistent aggression. The inability to escape aggression is thought to adversely affect the subordinate rabbits. Although dominance did not significantly affect the maximum weights pushed through by the socially housed rabbits to access limited social contact compared to the subordinate rabbits (Chapter 5), it did affect other aspects of their behaviour, both in the experimental apparatus and in their cages. The subordinate rabbits were found to move through the runway to reach the rabbit in the pen at the end of the runway more quickly than the dominant rabbits, although they were more hesitant to first approach the push-door than the dominant rabbits. The subordinates also spent more of the time allowed in visual contact with the conspecific. It is possible that the subordinate rabbits pushed through the weighted push-door to gain the security of being in close proximity to a conspecific, whereas the dominant rabbits may have pushed through the weighted push-door partly for territorial reasons. During the cage observations (Chapter 7) the dominant rabbits were found to spend longer bouts on the platform compared to subordinate rabbits, and again this may be for territorial reasons, assessing the threat imposed by the rabbits in close proximity. It may also be that subordinate rabbits cut short their platform visits in response to the approach of, or threats by, the dominant rabbit.

8.5 Territorial behaviour

When the rabbits were allowed to determine the duration of bouts of social contact themselves (in the experiment in Chapter 6) the mean duration of bouts was relatively consistent throughout the experiment as the weights on the push-doors increased. The rabbits tended to make relatively short, frequent visits to the social contact cage, similar to the short frequent visits to the platform shown in the cage observations (Chapter 7). It was thought that this may be partly for territory inspection, as the lack of re-scheduling of behaviour as the cost was increased suggests that it was the frequency of the visits that was more important than the duration of visits. Interestingly, both the test rabbits and the rabbits in the pen behind the mesh panel were found to use the corner nearest the mesh panel as their latrine area. Rabbits in the wild will use latrines or dunghills to mark their territory and these tend to be situated in the warren area (e.g. Thompson and Worden, 1956; Mykytowycz, 1968). This was similarly seen in the experiment reported in Chapter 5, when the previously socially caged rabbits were first housed next to an unfamiliar rabbit (with only olfactory and auditory contact). Many of the rabbits began to use the top of the platform in the corner nearest to the adjacent cage as the latrine area. This strongly suggests a territorial response underlying the motivation of rabbits to access limited social contact, at least where the rabbits are unfamiliar to each other.

The territorial behaviour shown by the rabbits throughout the experiments is perhaps not surprising, as domestic rabbits have been found to have a similar behavioural repertoire to wild rabbits in terms of feeding and resting patterns, social, reproductive and maintenance behaviour and responses to predators. Group housed rabbits in the laboratory are often found to show aggressive, territorial behaviour and it seems that perhaps the presence of visual and olfactory cues is enough to stimulate territorial behaviour in caged rabbits.

8.6 Importance of olfaction

Olfactory cues were found to be important throughout the experiments. During the short term tests with singly caged rabbits (Chapter 4), several of the rabbits did not push through the push-door during the control when there was no rabbit at the end of the runway. The rabbits appear to have been receiving olfactory (and perhaps also auditory cues) from the rabbit at the end of the runway during the unfamiliar and familiar rabbit tests, as this was seen even in the first control trial before the rabbits had any opportunity to learn that social contact was no longer available. These cues therefore appeared to be stimulating the rabbits to push through the push-door.

Olfactory cues available in the home cage were also found to have an important influence on the motivation for limited social contact during the behavioural tests. When the previously pair housed rabbits (Chapter 5) were housed with olfactory contact with their cage-mate and were allowed up to one hour of physical contact each day, their motivation for social contact in the test environment was reduced, compared to when the rabbits were allowed the short period of physical contact but were housed without olfactory contact with their cage-mate.

Both the singly and socially caged rabbits were found to spend time on the platform facing the adjacent cage when visual contact was not possible. It may be that olfactory cues from adjacent cages (and perhaps cages in the above tier) are stronger whilst on the platform. Although the presence of cues can affect the motivation of animals for resources (e.g. Warburton and Mason, in press), the presence of cues in the current study was an important feature, as laboratory rabbits are always housed in at least olfactory (and auditory contact) with conspecifics and perhaps visual or physical contact.

8.7 Recommendations for housing of female laboratory rabbits

8.7.1 Social contact

The findings of the different approaches of behavioural assessment all suggest that social contact is important to rabbits. It has been found that singly caged rabbits will work for short periods of visual and minimal tactile contact, therefore it is recommended that cage designs should take this into account, allowing singly caged rabbits to gain such contact through mesh panels. Socially caged rabbits have also been found to push through weighted push-doors for visual and minimal tactile contact. They were found to be more motivated for visual and tactile contact when housed without olfactory contact with their cage-mate. This suggests that if a socially housed rabbit has to be removed from its social group and housed singly it should be able to have such contact with a conspecific, regardless of whether that rabbit is from its social group or is unfamiliar. If the rabbit is unable to be housed next to a familiar rabbit, placing bedding from the group pen in the tray under the cage may provide them with olfactory cues from familiar rabbits and may therefore reduce their motivation for visual and tactile contact.

Both singly and socially caged rabbits were found to choose to spend time in close proximity to other rabbits, but not always in direct visual contact. It is therefore suggested that rabbits should be allowed the opportunity to avoid contact if they wish to. All of the rabbits spent very little of their time under the platform in the bolt-hole, suggesting that this is not a favoured place to be. It would therefore be suggested that partially solid partitions between cages would be a more suitable way of allowing the rabbits to avoid direct contact.

The fact that rabbits previously kept in pairs remained 'familiar' when allowed up to one hour of contact a day, and that this experience reduced their demand for social contact (compare Chapters 4 and 5) might suggest a compromise in rabbit housing. If rabbits were to be singly housed, allowing them to have a short period of contact each day with rabbits they were familiar with (e.g. litter mates or members of a group they were reared with at the suppliers), whilst being singly housed with visual and minimal tactile contact for the rest of the day would reduce their motivation for further social contact. This is however, how the results would be interpreted without taking practical considerations of the laboratory situation into account. Whilst this may benefit the rabbits in terms of partly satisfying their social needs and thus improving their welfare, it is unlikely to be practical due to experimental reasons, time constraints and potential problems of aggressive behaviour.

8.7.2 Platforms

The experiments and cage observations have also found that a platform is an important resource for rabbits. The most important aspect of the platform appears to be the ability for rabbits to hide underneath the platform in the bolt-hole when startled. It is therefore recommended that cages should be fitted with a platform. The use of the platform appears to be strongly influenced by social cues, in particular visual cues, therefore caution should be taken when including the platform area in the calculation of floor area, as in the absence of visual contact the use of the platform as an area to lie on is often reduced.

Chapter 9 - References

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Appendix 1

Questionnaire On The Housing And Husbandry Of Laboratory Rabbits

RABBITS

Which breed/strain of rabbits does your organisation use and why? ....

....

....

How many rabbits are used each year? How many of each breed? ....

....

....

What percentage of rabbits used within the last year were: Male .... Female ....

How many rabbits are typically held on the premises at any one time? ....

Are rabbits: Bred on the premises Bought from a supplier Both

If both, within the last year, what percentage were: Bred .... Bought ....

Which supplier is used? ....

Do you know what housing systems the supplier uses? YES / NO

Are you able to specify to the supplier what housing system you want the rabbits to be raised in? YES / NO

HOUSING

Within the last year, how many rabbits were housed in the following systems?

	Male	Female
Singly in cages		
In groups in cages (social cages)		
In groups in floor pens		

For what reasons are rabbits singly housed? ....

....

....

If any rabbits are housed in cages (either singly or socially) please answer the following section, otherwise please move to 'Floor Pens' on page 4.

Cages

What materials are cages made of?

Shell (metal, plastic *etc.*) ....

Flooring (solid, grid, dimple *etc.*) ....

Who is the cage manufacturer and/or what is the brand name?

....

What are the dimensions of the single cages (in cm)?

Length..................... Depth...................... Height.......................

Is *visual* contact possible between singly caged rabbits and rabbits in:

-adjacent pens YES / NO

-opposite pens YES / NO - if yes, how far apart are these rabbits (in metres)? ....

Is *physical* contact possible between rabbits in adjacent single cages? YES / NO

What are the dimensions of the social cages (in cm)?

Length.................... Depth..................... Height......................

How many rabbits are kept in these social groups? ....

Which of the following are caged rabbits fed?

Pellets Hay Fruit/Veg Other (please specify)

....

Are rabbits fed *ad lib*. in: Single cages YES / NO Social cages YES / NO

What is the light intensity in the rooms (in lux)? ....

What light/dark cycle is used? ....

Is there a dawn/dusk facility? YES / NO

Do rabbits have a hide/bolt hole in: Single cages YES / NO Social cages YES / NO

Are rabbits in cages provided with environmental enrichment? YES / NO

If yes, in what form ? ....

....

....

Is enrichment continued throughout the experiments? YES / NO / SOMETIMES

If only single cages are currently being used, have social housing systems been tried previously? YES / NO

If yes, what advantages and disadvantages were found? ....

....

....

....

If any rabbits are kept in floor pens please answer the following section, otherwise please move to 'Behaviour' on page 5.

Floor pens

What materials are the pen walls made of (plastic, wood *etc.*) ....

....

What are the dimensions of the pens (in cm) and the group sizes? ....

....

Which of the following are penned rabbits fed?

Pellets Hay Fruit/Veg Other (please specify)

....

Are penned rabbits fed *ad lib.*? YES / NO

What is the light intensity in the rooms (in lux)? ....

What light/dark cycle is used? ....

Is there a dawn/dusk facility? YES / NO

Do rabbits in pens have hides/bolt holes? YES / NO

Are rabbits provided with environmental enrichment? YES / NO

If yes, in what form?..

....

....

Is enrichment continued throughout the experiments? YES / NO / SOMETIMES

Are new rabbits ever introduced to an already existing group? YES / NO

If yes, are any precautions taken to reduce aggression during introduction and what are these? ....

....

....

BEHAVIOUR

Has behaviour been observed during the night as well as during the day? YES / NO

What percentage of rabbits in cages perform the following behaviours?

Hair chewing/plucking .... Biting - wire, food/water hoppers ....

Circling .... Kicking walls ....

Weaving .... Digging ....

Nose-sliding ....

Other (please specify) ....

....

At what times of day do you think abnormal behaviours are most frequent? ....

....

Have steps been taken to reduce the incidence of abnormal behaviours? YES / NO

If yes, in what way? ....

....

....

INJURY AND DISEASE

Within the last year what percentage of rabbits in each housing system suffered from the following? If a system is not used please leave the column blank.

	Single cages	Social cages	Floor Pens
Sore hocks			
Malocclusion			
Broken legs			
Broken backs			
Hairballs			
Bites and scratches			

Are any diseases more prevalent in one housing system than another? YES / NO

If yes, please specify ....

....

....

EXPERIMENTS

Broadly, what types of experiments are rabbits used in? ....

....

What is the average duration of experiments (in days)? ....

If there is large variation, please indicate the range ....

What percentage of experiments in the last year required rabbits to be singly housed?

....

What is the average duration of such experiments (in days)? ....

Are there any other constraints of experiments which could affect design of rabbit housing implemented in the future? ....

....

....

Any additional comments ....

....

....

....

....

What is your position within the company? ....

Thank you very much for taking the time to fill in this questionnaire.

Appendix 2 – Papers

The following papers have been published from the research:

Seaman, S., van Driel, K., Waran, N and Appleby, M., 2000. What do rabbits want? Measuring motivation in laboratory rabbits. In *Proceedings of the 34th International Congress of the ISAE, Brazil,* (A. Ramos, L.C. Pinheiro Machado and M.J. Hötzel, eds), Federal University of Santa Catarina, p. 195.

Seaman, S.C., Waran, N.K. and Appleby, M.C., 2001. Motivation of laboratory rabbits for social contact. In *Proceedings of the International Congress of the ISAE,* (J.P. Garner, J.A. Mench and S.P. Heekin, eds), Center for Animal Welfare at UCDavis, p. 88.

Presentations of the research have also been given at the following meetings:

Laboratory Animal Science Association, 1999

UFAW/RSPCA Rabbit Behaviour and Welfare Group, 1999

UFAW Vacation Scholarship Meeting, 2002

Scottish Conference on Animal Behaviour, 2000 and 2002

Companion Animal Behaviour Therapy Study Group, 2002