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# INFRA-RED VISION IN FERRETS (*Mustela furo*)

A thesis

submitted in partial fulfilment

of the requirements for the Degree

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## **Abstract**

Ferrets are labelled Unwanted Organisms under the Biosecurity Act (1993) due to their predation on New Zealand's native protected species and their status as potential vectors of Bovine Tuberculosis. There was suspicion that ferrets could detect the infrared light-emitting equipment used to monitor predator and prey behaviour. A two-alternative forced-choice operant procedure was used to test whether five pigmented male ferrets could detect infrared (870 and 920 nm) light.

First, the ferrets were taught to press a lever under a lit visible (white) light emitting diode (LED) for food rewards. After up to 101 40-minute sessions, each ferret could lever press under the lit-light at or above the pass criteria of 75% responses over four consecutive (or five out of six) sessions.

The same ferrets were then tested for stimulus generalisation over different stimulus properties by changing the wavelength/colour and intensity of the lit-light. The overall mean accuracy of each ferret's response to each coloured light varied between 92% and 84%. When a red light was systematically dimmed to halve the intensity nine times, all five ferrets still met the set pass criteria with overall accuracies of between 88% and 78%. This indicated that changing the properties of the light stimuli would not disrupt the ferrets' abilities to perform the learned task. This test was a necessary prerequisite before changing the light stimuli to potentially invisible wavelengths in the infrared spectrum.

The light stimulus was changed to a single infrared (870) nm LED. Two of five ferrets showed strong evidence (response accuracies of  $77\% \pm 4$  and  $72\% \pm 2$ ) and one ferret showed weak evidence ( $60\% \pm 3$ ) that they could see the light at this wavelength. Extraneous cues such as ultrasound emitted at the onset of a stimulus light or a predictable schedule of reinforcement were eliminated as potential response cues. These tests helped to prove that the ferrets were using only the light stimulus to discriminate which stimulus was lit. It may be possible that at least some feral ferrets can detect the light produced from infrared monitoring equipment that emits light wavelengths at or below 870 nm. This has significant implications for conservation because infrared equipment is used by

## *Abstract*

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conservation agencies in New Zealand and overseas to monitor predator and prey behaviour in the wild. If the infrared lighting is detected by the subject being observed, then it may potentially influence the behaviour of the animal, or attract a predator towards threatened native species.

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# **Chapter 1**

## **Literature Review**

### **General Introduction**

#### **New Zealand's Historical Background**

Animals and plants endemic to New Zealand evolved independently from the rest of the world, virtually without herbivorous and predatory mammals (King, 2005). Over time many bird species colonised New Zealand (most from Australia). The longer each species was resident, the more likely it was to become flightless and ground-dwelling, and to lose its predator avoidance instincts. Together, these factors made New Zealand's birds particularly vulnerable to future mammalian introductions (Wilson, 2004).

New Zealand was subject to two main waves of introduced mammals. First, Polynesians arrived (1250 to 1300 AD) bringing the kiore (Pacific rat, *Rattus exulans*) and kuri (Polynesian dog, *Canis familiaris*). Then, after Cook's first visit in 1769, Europeans began to settle in increasing numbers, thus introducing a total of 54 mammal species, 32 of which established resident populations. At least 14 of these species became common and widespread, taking over the country at speed, and have since caused severe and irreversible damage to New Zealand's existing biota. Among these introduced pests, possums (*Trichosurus vulpecula*), goats (*Capra hircus* L.) and deer (*Cervidae* spp.) have modified native vegetation; ferrets (*Mustela furo*), stoats (*Mustela erminea*), cats (*Felis catus*) and human hunters have caused extinctions among birds; and rabbits (*Oryctolagus cuniculus*) have contributed to pasture destruction and soil erosion (King, 2005).

### **Introduction of Ferrets (*Mustela furo*) to New Zealand**

The first ferrets were brought to New Zealand in 1879 as a biological control against wild rabbits (Clapperton and Byrom, 2005). Within 25 years of introduction, wild rabbits had bred uncontrollably, destroying grassland and leaving thousands of farmers at economic loss. Rabbits did not reach such huge numbers in the Northern Hemisphere (their original home) because (it was assumed) their populations were controlled by their natural predators – polecats, stoats, foxes, hawks and owls. Therefore, farmers demanded the New Zealand government bring in the natural predators of the rabbit, starting with the ferret (King, 1984), a domesticated version of the wild polecat (King and Moors, 1979). Farmers saw this as a cheap and simple biological control, and the last possible chance to save the future of pastoral farming in New Zealand (King, 1984). After their initial release, ferrets swiftly spread over the country, and now New Zealand has the highest population of feral ferrets in the world (de Lisle et al., 1993).

In 1985, farming of ferrets for their skins ('fitch farms') was made legal, and by 1986 over 100 registered farms had together exported 80,000 skins. This industry crashed a few years later, but the popularity of owning ferrets as pets grew. The Department of Conservation (DOC) published a discussion document to gauge the extent of public concern about ferrets as a threat to conservation (Department of Conservation, 1999).

### **Impact of Ferrets on New Zealand's Fauna**

Ferrets pose a threat to New Zealand's conservation values because they kill threatened native animals. They have been reported to prey on the kiwi (*Apteryx spp.*) (Miller and Pierce, 1995), weka (*Gallirallus australis*) (Graeme, 1996), and have contributed to the extinction of kakapo (*Strigops habroptilus*) from mainland New Zealand (Department of Conservation, 1999). The ferret's predation on ground-nesting birds is severe, particularly water birds including the brown teal (*Anas aucklandica*) (Pierce, 1996), black stilt (*Himantopus novaezelandiae*) (Pierce, 1986) and yellow eyed penguin (*Megadyptes antipodes*) (Darby and Seddon, 1990).

## Chapter 1: Literature Review

It can be difficult to separate the threat of ferret predation from that of other mammalian predators (Murphy, 1996), but ferrets have been observed numerous times on video removing eggs or chicks from native bird nests. Sanders and Maloney (2002) conducted a five year project monitoring predation of several species of native ground nesting birds in the Upper Waitaki Basin. They found that ferrets were responsible for 18% of the mortality at nests, third only to cats (43%) and hedgehogs (20%). Birds are not the only species at risk. In open country, ferrets also feed on native frogs (*Leiopelma* sp.), kauri snails (*Paryphanta* sp.), skinks (*Scincidae.*), hedgehogs (*Erinaceus europaeus occidentalis*), rodents (*Rattus* sp. and *Mus musculus*), brushtail possums (*Trichosurus vulpecula*), eels (*Anguilla* sp.), freshwater crayfish (*Paranephrops* sp.), and of course rabbits (*Oryctolagus cuniculus*) (King, 2005; Murphy, 1996).

Ferrets are potential vectors of TB (bovine tuberculosis), a disease that can be transmitted to New Zealand's cattle and deer herds (Ragg and Walker, 1996). Lugton et al. (1997b) conducted a large scale study to investigate the true prevalence of TB infection in wild ferrets in selected areas of the North and South Island. Seventy out of 228 ferrets examined were infected, with especially high prevalence in the Wairarapa. The disease is suspected to be transmitted by intra-species fighting, cannibalism and ingestion of infected possum carrion or domestic animal carcasses discarded in offal pits (de Lisle et al., 1993; Livingston, 1996; Lugton et al., 1997a; 1997b).

In 2003 ferret farming and keeping ferrets as pets were banned, and it became illegal to breed, buy or sell ferrets without a license (Clapperton and Byrom, 2005). Ferrets were declared an 'Unwanted Organism' under the Biosecurity Act 1993 due to their predation on native species and their potential to spread TB. Attempts were made to eliminate ferrets from New Zealand altogether, but their numbers and distribution were already too high. Today feral ferrets are widely distributed throughout the mainland of New Zealand, but they are not present on any off-shore islands and it is illegal to take them there (Department of Conservation, 1999).

### **Ferret Biology and Behaviour**

Ferrets belong to the family Mustelidae, which includes 26 genera and 67 species. New Zealand has three mustelids (all introduced); the ferret, stoat and weasel (*M. nivalis vulgaris*). They all have long bodies, short legs, a sharp pointed face and small round ears (King, 2005). Ferrets are the largest of the three mustelids, and males are much larger than females reaching an average of 1.3 kg (Clapperton and Byrom, 2005: Table 57).

The ferret pelage usually comprises a layer of dark brown guard hairs over creamy yellow under-fur, with a dark mask across the eyes. The ferret is a seasonally breeding carnivore with a polygamous mating system (Woodley and Baum, 2003). Ferrets mate in spring, gestate for about 42 days and produce 4-8 young per litter. If food is plentiful, ferrets may breed more than once in a season, but this is rare (Clapperton and Byrom, 2005). At 30 days old, the eyes of young ferrets begin to open and they are already mobile and eating meat brought back to the den by their mother. At three months old they leave their den and disperse about five kilometres from their natal home range (Byrom, 2002).

The ferret's main sense is olfactory (Apfelbach, 1986); although tactile, visual and auditory cues are also important. Ferrets have scent glands in the skin, producing odours that are sexually and individually distinct. Olfaction facilitates mate recognition and sexual partner preference (Woodley and Baum, 2003). Apfelbach (1986) investigated olfactory sign stimulus for prey selection in ferrets, and found that there is a sensitive phase at two to three months of age when ferrets learn to distinguish prey from non-prey smells. In adult ferrets, prey-searching behaviour could be induced only by the presence of a familiar prey odour – they did not respond to odours from unknown potential prey. This experiment shows that olfaction also plays an important role in prey recognition.

Auditory cues are important for ferrets hunting in the wild (Moore, 1982). The ferret's threshold for pure tone detection was determined by Kelly et al. (1986) using a standard behavioural experiment. Ferrets responded to frequencies ranging from 36 Hz to 44 kHz at 60 dB; the sharpest tuning was found in the region of 8-12 kHz. This hearing range is comparable with that of the least weasel

(*Mustela nivalis nivalis*). Both species have similar sensitivity at the low and mid ranges, but the least weasel has a higher upper limit of hearing, reaching up to 60.5 kHz (Heffner and Heffner, 1985). These upper frequencies are much higher than those that humans can hear (20 kHz), and may have an adaptive advantage since these high frequencies are also produced by mice (Nyby and Whitney, 1978) and mice are prey for all small mustelids (King and Powell, 2006).

Ferrets are mostly nocturnal, and are seldom active during daylight (Alterio and Moller, 1997). They walk with their body level to the ground but run with their back arched, occasionally squatting up on their hindquarters to get elevation (Clapperton and Byrom, 2005). Within pastoral habitats they concentrate their movements along boundary lines such as fences, stock tracks, and edges of ploughed fields (Baker, 1989). Ferrets are widely distributed throughout the North and South Island, and are locally numerous wherever rabbits are found (i.e. farmland, grassland, tussock, scrubland, dune-lands, swamps, river valleys, forests, and around forest lakes and rivers). Ferrets are found both above and below ground, often nesting in rabbit holes.

## **Ferret Management**

### **Current Control Efforts**

Ferret management is carried out by different agencies in New Zealand for different reasons. The Department of Conservation (DOC) removes ferrets from protected areas to prevent predation on endemic animals. The regional councils and the Animal Health Board remove ferrets from farmland, to enable routine monitoring of the present extent of TB and to prevent further spread of it by removing TB vectors. Ferrets are difficult pests to control in New Zealand where rabbits (their main prey) are abundant, because they can produce many young which disperse widely and have few natural enemies other than humans. Conventional techniques for ferret management include trapping, poisoning,

habitat manipulation and predator/prey monitoring. These techniques all have advantages and disadvantages, discussed below.

The main control method implemented by DOC is kill-trapping. This activity has not always met the ethics requirements of The Animal Welfare Act 1999, which prohibits traps that cause unacceptable pain or distress. Fenn (Mk4 and Mk6) traps, Victor Professional Snapbacks and Waddington Back Crackers, are among the traps that failed to meet the National Animal Welfare Advisory Committee (NAWAC) requirements for kill-trap performance because they did not render ferrets unconscious within three minutes and could be a threat to non-target animals (Poutu and Warburton, 2005; Warburton et al., 2002). A recent development is the DOC 250 kill-trap reported by Poutu & Warburton (2005) to be the world's first kill-trap that targets and kills ferrets, stoats, rats and hedgehogs in a relatively humane manner. The trap is placed inside a tunnel-like trap-box to ensure that birds, children or pets cannot get access to it. All ferrets tested with the DOC 250 were unconscious within 30 seconds, because all received head strikes, although the heart still took up to 7:09 minutes to stop beating.

Other than ethical issues, the main disadvantage of trapping ferrets is that setting and maintaining traps is labour intensive. With limited departmental budgets, only a small proportion of the total area occupied by ferrets can be treated (Parkes and Murphy, 2004). Additionally, many ferrets avoid traps or are inherently wary (trap-shy), and may be less trappable when abundant natural foods are available. Furthermore, ferrets have a high rate of reinvasion due to their large (on average 126 ha male and 88 ha female) home ranges (Moller et al., 1996). Trapping also requires meat baits that have a limited life (especially over summer) and may not be accepted by ferrets, even when fresh. Alternative, less perishable means of drawing the ferret to the trap are still being developed including auditory and olfactory lures (Clapperton, 1996).

Poison baits (e.g. poisoned eggs containing generic poison brands such as 1080, cholecalciferol or diphacinone) can be effective at killing some ferrets, but many are bait-shy, especially since ferrets are subject to prey imprinting during their first three months of life and are not likely to try foods with unfamiliar

smells after this age (Apfelbach, 1978). Poison baits are also expensive, labour-intensive to prepare and dispense (Brown, 2004) and can be a threat to non-target animals such as dogs, native birds, livestock and even humans (DOC website, June 2006).

Habitat manipulations have been trialled for predator control including vegetation buffers and pest-proof fencing. Vegetation buffers (used to surround and protect potential prey) have proved to be an ineffective means of excluding predators (Alterio and Moller, 1998). This method can even have the opposite effect, attracting predators, like ferrets, known to use pathways through grass and concentrate their movements along boundary lines (Baker, 1989). Quality predator-proof fencing can be successful at excluding ferrets and preventing reinvasion. However, fencing with electric components can be prone to technical failure (Clapperton and Day, 2001) and all pest-proof fencing is expensive to set up and maintain, so only a small area can be managed in this way (Parkes and Murphy, 2004).

Biological controls (such as the use of canine distemper disease or other possible agents) have been disregarded as viable control techniques for any mustelids due to welfare issues, lack of public acceptability and the risk to non-target animals (Parkes and Murphy, 2004). Controlling primary prey (rabbits) can reduce ferret numbers if the operation is extensive (at least 4000 ha) and successful (a rabbit kill rate of at least 95%) (Clapperton and Byrom, 2005). But that is as difficult as controlling the ferret itself, and the risk of prey switching after rabbits are suddenly removed can have devastating consequences for native birds (the prey that should ultimately be protected) (Norbury and McGlinchy, 1996).

Shooting is another impractical option for ferret control. It is not only labour-intensive and time-consuming, but also ineffective; it cannot meet the primary requirement of an effective control method, that all members of the target population can be put at risk (Parkes, 1993).

Predator/prey monitoring is an important and frequently used technique exercised by DOC and other organisations concerned with protecting native

animals. Although it does not usually result in the immediate control of predators, the information gained from monitoring can be used to learn more about predator behaviour in order to prevent nest mortality in the future. Once predator behaviour has been observed and understood, then new, more cost effective and sustainable approaches to controlling them can be developed (Department of Conservation, 2000).

Predation is seldom witnessed despite its high frequency, since predators hunt mostly at night or in thick vegetation, out of the range of human observation (Major and Gowing, 1994). Traditionally, predator monitoring and identification consisted of anecdotal sightings, examining prey remains and matching them with predator stomach contents, collecting fur or feather samples (Major, 1991b), and identifying predator sign such as tooth patterns (Lyver, 2000), tracks and scats (Culter and Swann, 1999; Laurance and Grant, 1994). However, these methods can be too invasive, potentially disturbing nest sites, and are not especially reliable for correctly identifying mustelids because their predation can be 'clean' with little to no signs left at the nest (Major, 1991a).

Unobtrusive monitoring using a camera enables the study of animals that are difficult to monitor through the traditional methods of observation and capture. Camera monitoring produces images that are permanent and can identify visiting predators and determine their relative importance and temporal patterns (Picman and Schriml, 1994). Remote photography is one such technique, and its use has surged since the development of infrared triggered camera systems (Culter and Swann, 1999).

### ***Monitoring with Infrared Equipment***

Infrared (IR) video surveillance has been widely used in the observation of predator/prey behaviour in New Zealand (Brown et al., 1998; Innes et al., 1994; Sanders and Maloney, 2002) and overseas (Laurance and Grant, 1994; Savidge and Seibert, 1988). The advantage of using IR light is that it can identify and capture the behaviour of predators as well as the rate, timing and sequence of predation events in complete darkness (Innes et al., 1994). This is important for

monitoring most of New Zealand's pest mammals (including ferrets) because many are nocturnal. IR video surveillance is also less invasive and disruptive than traditional methods, and since the 1990s has become the most frequently used type of surveillance monitoring equipment (Culter and Swann, 1999).

IR video surveillance can be done either with large floodlights consisting of clusters of IR light emitting diodes (LEDs) (Innes et al., 1994; Sanders and Maloney, 2002), or with a large standard white bulb covered by filters that cut out all light wavelengths except IR (S. Cockburn, pers. Comm., September 2006<sup>1</sup>). Active IR sensors are also available in the form of a snapshot camera triggered by an approaching predator breaking a beam (Carthew and Slater, 1991; Hernandez et al., 1997; Laurance and Grant, 1994; Savidge and Seibert, 1988), or traps may be set with pairs of active IR light gates that allow size classification of the animal before the trap is triggered (Prout, 2003). Both passive and active methods produce a field of IR light commonly reported to contain frequencies between 830 and 880 nm (Carthew and Slater, 1991; Innes et al., 1994). However, filters and IR LEDs are known to degrade over time, and then they emit light that contains visible wavelengths (S. Cockburn, pers. comm., September 2006), risking detection by both predators and protected species.

If IR lighting becomes detectable by the subject being observed, it may potentially influence the behaviour of interest. For example, the subject may show curiosity towards video equipment, or be wary of entering areas that have been disturbed by humans or flooded with IR light. Some of the IR lights used to illuminate nests emit a small amount of visible light, visible by humans at close range in the dark (Sanders and Maloney, 2002) and possibly also visible to the predators (Brown et al., 1998; S. Cockburn, pers. comm., September 2006). An animal that can detect monitoring equipment or night-time lighting may behave differently compared with when it is undisturbed. However, there is little

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<sup>1</sup>Stuart Cockburn is the head of the Science Electronics Section at the Department of Conservation, Wellington, New Zealand. He designs electronic equipment for research and monitoring purposes, and supplies and maintains such equipment (including infrared lights) for Departmental use.

information on how monitoring with IR lighting affects predator behaviour, if at all.

Brown et al. (1998) used time-lapse video recording with night time IR illumination (a cluster of 15 LEDs, laser diodes and collimated lasers) to identify predators at tomtit (*Petroica macrocephala*) and New Zealand robin (*Petroica australis*) nests in the central North Island. They observed no evidence that the predators reacted strongly to the cameras or lights, or that filming altered predation rates. However, simply failing to observe any reaction is not evidence of lack of perception. A predator that does not react to an IR light could either (1) not perceive it or (2) perceive it but choose to ignore it. It is essential, but very difficult, to distinguish between these two possible explanations. Brown et al. (1998) noted that they themselves could detect a faint red glow from the collimated laser and LED cluster, suggesting that the predators may also have been able to detect the IR light sources used during filming even though they did not respond.

Prout (2003) conducted a thesis on a new automatic bait delivery system called the Scentinel ®. The second and third prototypes used a pair of IR light-gates set 10 cm apart in the entrance tunnel, to measure the length of animals that entered the tunnel, and so exclude mice from accessing the bait. There were significantly fewer visits by rats (*Rattus rattus*) in tunnels with IR light-gates compared with tunnels set in the same positions with no IR light-gates, leading to speculation that the rats may have been able to see the IR beams inside the tunnel and avoided entering.

Further anecdotal evidence was reported by S. Cockburn, (pers. comm., September 2006). When flood-lights with IR filters were positioned above monitored nesting sites, possums and rats were reported to wait on the edge of the IR light field and move quickly in and out of the lit area, suggesting they could detect it to some degree.

Undetectable monitoring is vital in the control of pest and unwanted species. Daily human disturbance or visitation has been reported to increase nest predation (Major, 1991a) and predators may be either attracted or repelled by an

observation device set to monitor the nest (Innes et al., 1994). This fact has implications for bird protection. Since infra-red light is also used to monitor the nests of native birds, it is vital to be sure that the light is not detected by any of their major predators. Animals that can detect the IR light can also potentially alter their behaviour around the monitoring equipment. A detectable IR light could even attract predators that learn to associate IR illumination with nesting areas (W. Temple, pers. comm.<sup>2</sup>). Several researchers have become alarmed enough to call for research exploring whether either birds or predators are disturbed by, or attracted to, the surveillance equipment used (Culter and Swann, 1999; Innes et al., 1994; Prout, 2003; Winder, 2003). That is the aim of this study.

## **Wavelength Sensitivity**

### **The Electromagnetic Spectrum**

The electromagnetic spectrum divides up electromagnetic radiation into arbitrary categories based on wavelength. From shortest to longest wavelengths, the spectrum is as follows: gamma radiation, ultraviolet radiation, visible light, near-infrared light, far-infrared heat, micro waves and radio waves.

### ***The ‘Visible’ Spectrum***

When the earth receives radiation from the sun, only the narrow band of wavelengths producing visible light is detected by the eyes of humans, and is termed the ‘visible spectrum’. The components of light are described in terms of their wavelengths, the distance between successive troughs or crests in the light wave, measured in nanometers (nm). There is no agreement on the exact limits of spectral wavelengths visible to humans. Many publications state that humans are sensitive to light within the range from 400 nm (violet) to 700 nm (red) (Ali and

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<sup>2</sup> William Temple is an Associate Professor at the University of Waikato, Hamilton, New Zealand. His expertise is in experimental and applied analysis of behaviour, both animal and human.

Klyne, 1985; Jacobs, 1981; Jacobs, 1983; Lomas et al., 1998). However, some cite a broader range from about 380 nm to 750 nm (Boynton, 1979; Kaiser and Boynton, 1996). Any frequency below the lower frequency is called ultraviolet, and anything above the upper frequency is infrared, both invisible to humans.

There are several problems with these definitions. (1) They relate to human perceptions only, but the terms are still applied to other species, even though what is infrared or ultraviolet to humans may be clearly visible to other animals. (2) Even when applied only to humans, the discrepancy in the published estimates of the limits of the spectral range creates confusion as to exactly when violet light becomes ultraviolet and red light becomes infrared. (3) Perception of colour is variable because it depends on the brightness of the light and background luminance (Jacobs, 1993).

Spectral sensitivity is variable between species and individuals. There is evidence of genetic variation in human vision (Chowers et al., 2004) and variation between other species in visual abilities. Many animals are sensitive to parts of the same spectral range as humans (primates, fish, amphibians, birds, reptiles and many insects can all see some form of 'visible light' (Autrum, 1968)). We are still discovering animals that are sensitive to light beyond the range of frequencies we can detect.

For example, diurnal birds have a much larger spectral sensitivity range than humans. Both hummingbirds (*Stellula calliope*) and pigeons (*Columba livia*) can achieve good spectral discrimination among wavelengths in the near ultraviolet (Emmerton and Delius, 1980; Jacobs, 1983). The turtle (*Pseudemys scripta elegans*), brown trout (*Salmo trutta*) and several common species of rodents can all detect wavelengths within the ultraviolet region (Arnold and Neumeyer, 1987; Autrum, 1968; Bowmaker and Kunz, 1987). Electrophysiological studies on the compound eye of a stalked eye fly (*Cyrtodiopsis dalmanni*) have demonstrated reactions to stimuli above 700 nm, commonly reported to be IR (Burkhardt, 1972). The desert ant's (*Cataglyphis*) spectral sensitivity extends into the long wavelength range of the spectrum, thought to be an adaptation to an environment reflecting the yellow and red part of the spectrum only (cited in Burkhardt, 1973). Vanstone (2006) used an operant

procedure to show that possums (a pest species in New Zealand) can detect a high intensity IR light peaking in the near-infrared at 870 nm. For these animals the terms infrared and ultraviolet, defined with reference to humans, are no longer appropriate, but are still used here for consistency with the literature.

### **Adaptive Advantage of Variations in Spectral Sensitivity**

Spectral differences between species provide a unique source of information about objects in the environment and support those colour or light differences an animal is required to make in order to survive and thrive (Jacobs, 1983). Correlations can be made between the photic environment an animal lives in, and the ability of its eyes to absorb photons in that particular range. For instance, many birds are able to detect ultraviolet light, and the petals and berries of many bird-pollinated plants contain substances that reflect ultraviolet (Burkhardt, 1982; Burkhardt and Finger, 1991). The paired far-IR (heat) detection organs ('pits') on the nose of the pit viper snake (Crotalinae.) enable it to distinguish a small animal from a relatively cooler background, and strike with 100% accuracy in total darkness (Campbell et al., 2002).

The ability to detect IR light could have several adaptive advantages for nocturnal animals such as ferrets. No animal can see (with its eyes) in complete darkness; all visual perception requires some source of illumination to irradiate the objects that are seen (Kaiser and Boynton, 1996). At night the light is provided primarily by star- and moon-light, rich in the longer light wavelengths (including IR) (McFarland and Munz, 1979). Additionally, animals living under the canopy of a forest receive mostly green light peaking around 520 nm and reaching into the long wavelength range above 700 nm (Burkhardt, 1983). If nocturnal animals (including the ferret) could detect near-IR light, it could enhance their ability to navigate and to detect prey, predators and mates. Sensitivity to the red end of the spectrum might also act as a sexual stimulant when the vulva is reddened during oestrus, as an alarm signal when blood is drawn by a predator, or as an appetite stimulus when blood is drawn from prey (Lomas et al., 1998).

## **Methods for Studying Animal Vision**

### **Physiological Studies**

Physiological studies of animal vision involve learning about the anatomy and function of the eye. Information on an animal's visual capabilities can be obtained from knowledge of the size, shape and position of the eye. For example, nocturnal animals generally have large round eyes to make the most of the available light (Muntz, 1974) while diurnal animals have smaller eyes. Predators typically have forward directed eyes to allow for binocular vision, better depth perception and judgment of distance to strike, whereas prey animals have laterally directed eyes to allow panoramic vision for detection of predators (Saslow, 2002).

Examination of the visual pigments (e.g. how many pigment types, what ratio) is also important since these act as the initial filter for light wavelengths (frequency), intensity and image motion (Lomas et al., 1998). However, there are several problems with relying on only physiological experiments: (1) they are often performed with subjects under the influence of anaesthetics or other drugs; (2) they deal with small isolated parts of the visual system, and the results cannot be extrapolated with any certainty to the whole animal; and (3) not all visual information received by the eye is processed into meaningful information by the brain (Muntz, 1974).

Physiological data must be combined with behavioural experiments to provide some evidence of the extent to which animals use the information derived from their environment (Lomas et al., 1998). Only if an animal can reliably demonstrate visual discrimination in a controlled behavioural experiment, can we prove that the animal really can 'see' the visual stimulus.

### **Behavioural Studies**

Collecting behavioural data on vision requires the use of psychophysics, a branch of psychology that deals with the relationship between physical stimuli and their perception. Within psychophysics, there are three general methods for collecting

sensory information; (1) those that use pre-existing behaviour such as reflexes, (2) those that use classical conditioning of an existing behaviour, and (3) those that use operant conditioning to establish stimulus control of a newly trained behaviour (Lieberman, 1993).

Observing the effect of stimuli on a pre-existing behaviour can be useful to gain sensory information. For example, Hecht (1920) investigated the light sensitive behaviour of the long-neck clam (*Mya arenaria*). When illuminated by a light the animal could detect, it always responded by a retraction of its siphon. This response was reflex and reliable, so measurements could be taken of the latency of the reflex under various experimental conditions. Although useful for some animals, methods that use pre-existing behaviour have significant limitations because they rely on the unlikely assumption that an animal possesses a measurable response that correlates with the subject of concern (Stebbins, 1970).

Classical (Pavlovian) conditioning induces an increase in the probability of a response (e.g. saliva production) to a conditioned stimulus (e.g. a tone) due to pairings of the conditioned stimulus with an unconditioned stimulus (e.g. food) (Lieberman, 1993). Classical conditioning can be useful to gain sensory information because the conditioned stimulus can be manipulated to be anything of interest (there is no longer the requirement that the response be a reflex that is already in the animals' repertoire) (Stebbins, 1970). For example, Powers and Easter (1978) classically conditioned goldfish to discriminate between lights at varying wavelengths and intensities. The presentation of a light was paired with an electric shock, causing the heart rate of the fish to increase rapidly. After several presentations of the light and shock together, the presentation of the light alone was enough to elicit a response in heart-rate, indicating that the fish had detected the light. Although electric shocks are a powerful tool, their use as a conditioned stimulus raises some ethical issues, and is not usually considered for use in today's animal behaviour experiments. Even classical conditioning using a positive unconditioned stimulus, such as the presentation of food, entails the risk of habituation (a decrease in strength of the reflex due to repeated presentations of the stimulus by itself). But perhaps the biggest disadvantage of using Pavlovian

conditioning for sensory experiments is the problem of clearly defining a response which indicates a sensory effect in the first place (Stebbins, 1970).

Operant conditioning is a type of associative learning in which a trained response can be modified by its consequences (Lieberman, 1993). The most common operant method is the two-response forced-choice procedure. Subjects have to choose between two stimuli (e.g. a lit-light and an unlit-light) and responses (e.g. a lever press) corresponding to the 'correct' stimulus may result in reinforcement (e.g. food). Operant conditioning may be ideal for animal vision experiments because not only can the stimuli be manipulated, but so too can the response – it can be anything the experimenter chooses, within the animal's physical capabilities. The trained response may be an easily measurable behaviour such as pressing a button that can be repeated many times within a session and automatically recorded (Lieberman, 1993). However, the animal taking part in the operant procedure must be intelligent enough to learn the set task, and the reinforcement must be desirable enough so that the subject will work for access to it. Operant conditioning procedures have been used on ferrets in the past to study thresholds for pure tone detection (Kelly et al., 1986) and shape discrimination (Pollard et al., 1967), so this method may also be successful for studying visual frequency and intensity thresholds in ferrets.

## **Previous Studies on Ferret Vision**

Most ferret research has transpired because ferrets are useful surrogates for their close relatives the wild polecats, which are a protected species in Europe, and because feral ferrets are pests in New Zealand. The recent discovery that wild ferrets have a high prevalence of TB (Lugton et al., 1997a), and speculation that IR equipment may modify predator behaviour, have reinforced the need for further scientific information on ferrets. Ferret vision was studied extensively in the 1980s, but the topics chosen were mostly physiological, and only a few were behavioural (see below).

### **Physiological Studies on Ferret Vision**

Ferrets are commonly used as experimental subjects for anatomical and physiological studies of the eye because they are cheap, commercially available (although no longer in New Zealand), and the newborn ferret's visual system is still in a very immature state (Jackson and Hickey, 1985). The eyes do not function until 30 days after birth, and there is no visually evoked activity within the cerebral cortex until after that age (Rose, 1981). Although the ferret has laterally placed eyes, the triangular shape of the head still gives it a considerable degree of binocular vision, with receptive fields extending more than 20 degrees into the contralateral visual field (Law et al., 1988). This reflects the role of both wild polecats and feral ferrets as predators, using binocular vision to locate prey; and also as potential prey, using monocular vision to watch for the many four-footed and winged predators that hunt all small mammals in the northern hemisphere (Corbet and Southern, 1977).

Both cones (responsible for colour and detailed photoreception) and rods (responsible for detection of light and dark, shapes and movement) are present in the receptor layer of the ferret retina (Braekevelt, 1983). It can be difficult to study the nature of cone-based vision in nocturnal animals because their retinas characteristically contain only a relatively small proportion of cones (Jacobs et al., 2001). This is true for the ferret, in which rods predominate at a ratio of 50-60:1 (Braekevelt, 1983). However, the ferret retina still contains a total of about 1.3 million cones, including two types of cone pigment, and thus ferrets should have dichromatic (two-colour) vision. This means ferrets (like most non-primate mammals) can match any colour they see with a combination of no more than two pure spectral lights. This is inferior to trichromatic (three-colour) vision, by which most insects, marsupials, birds and primates (including humans) can match any colour they see with a combination of three spectral lights (Jacobs, 1981). The two types of cone pigment in the ferret retina include either short-wavelength (S) or long-wavelength (L) cones. The sensitivity of the S cones peaks at 430 nm (violet) and that of the L cones at 558 nm (greenish-yellow). L cones outnumber S cones by around 14:1 (Calderone and Jacobs, 2003).

The ferret's excellent night vision can be attributed to the tapetum lucidum (a reflecting layer absent in human eyes), which increases sensitivity in the dark by causing light to pass through the visual cells a second time (Tjalve and Frank, 1984). The tapetum can easily be detected as the 'eye-shine' reflected from the eyes of mustelids, cats and many other nocturnal animals in torchlight (Ali and Klyne, 1985; Deegan and Jacobs, 1996). Reflections from the tapetum are distinctly different in colour (often green) and character (gleaming as opposed to dull) from the 'red-eye' of humans and other animals with no tapetum.

The structure of the tapetum has been examined in ferrets by light- and electron microscopy. It is an iridescent triangular area in the rear pole of the eye above the optic disc. There is no difference in the number or morphology of the tapetum cells between the eyes of the pigmented and albino ferrets (Tjalve and Frank, 1984), although albino ferrets have some general defects in the visual field (Garipis and Hoffmann, 2003).

Meredith et al. (2000) investigated the ferret superior colliculus (SC), a part of the brain known for its role in initiating orienting behaviours. The aim was to discover whether a ferret's SC was dominated by inputs from distance sensors (vision and hearing) such as in the cat, or if it relies more on non-visual inputs (touch and pain receptors), as in the rat. The ferret SC was dominated (63%) by visual/auditory inputs rather than by somatosensory inputs, and it was concluded that the ferret employed strategies of both (rodent and cat) groups: during subterranean hunting ferrets would rely on non-visual signals to direct orienting; but above ground ferrets would depend mostly on their distance sensors to guide orientation and facilitate above ground hunting.

### **Behavioural Studies on Ferret Vision**

Few behavioural studies have been conducted to back up physiological studies on ferret vision. To my knowledge, no behavioural studies have ever been conducted to find the visual frequency or intensity thresholds of ferrets, although two experiments investigated the influence of visual stimuli on prey-catching behaviour (Apfelbach and Wester, 1977; Vargas and Anderson, 1998), one

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experiment used operant conditioning to investigate visual discrimination of basic shapes (Pollard et al., 1967), and one experiment used operant conditioning to test the abilities of several mustelid species to discriminate between different colours (Gewalt, 1959). These results are explained in further detail below.

Apfelbach and Wester (1977) analysed reflex behaviour of ferrets towards 16 'prey dummies' with different visual and tactile characteristics. Objects less than double the size of the ferret moving at a speed of 25 cm/sec to 45 cm/sec (about the escape speed of a mouse) reliably elicited hunting reactions. Stationary objects did not elicit any prey catching response, indicating that movement is an important stimulus for prey recognition. Objects with fur-like surfaces induced more biting than hard surfaces, and ferrets reliably aimed their bite at the anterior part of the prey dummies or at narrow areas such as a neck or leg. Objects brighter than the background were relatively more effective at eliciting prey capturing reactions than darker ones. Vargas and Anderson (1998) showed that juvenile ferrets that witness their mother performing a prey kill enhanced their own predatory skills. Together these experiments show that vision, including movement, size, orientation, brightness, texture and observation, all contribute towards prey recognition and elicitation of hunting behaviour in ferrets.

Pollard et al. (1967) used operant conditioning to train four ferrets to discriminate between upright and inverted triangles. Different aspects of the triangles (e.g. size, colour and rotation) were manipulated to determine exactly what parts of the triangle were important for discrimination. They found that some ferrets paid particular attention to the baseline of the shape, could transfer from filled to outlined figures, to figures of reduced size, and rotated figures. All four ferrets failed to discriminate between triangles when reduplicated or irrelevant material was added. The results of this experiment suggest that ferrets are capable of using visual cues in an operant task, and of generalisation (the ability to transfer learned recognition of some stimuli to similar stimuli) without deterioration in the performance of the task.

Gewalt (1959) used a two-choice operant procedure to train several mustelid species (including domestic ferrets, their close relatives the polecats, and stoats) to discriminate between coloured doors for food rewards. The colours red,

yellow, green and blue were the positive stimuli (gaining the animals reinforcement if chosen), and a grey of the same or similar brightness was the negative stimulus. Gewalt found that all three mustelids showed at least limited detection of the colour red, but had little to no detection of the colours yellow and green. Blue was reported to be detected solely by the polecat. However, in colour discrimination experiments such as this, it is extremely difficult to match exactly a colour stimulus with a grey stimulus, so brightness and saturation cues may have confounded the results.

Behavioural experiments on ferret vision are still required because vision is an important and underestimated sense used by ferrets to find and kill prey. Greater definition of the visual abilities of ferrets may contribute to identifying; (1) the most effective and cost efficient options for ferret control, and (2) options that should be avoided because they introduce danger for protected species.

It is especially important to determine the true capability of ferrets to detect light of different wavelengths and intensities, to rule out the possibility that they could use IR light as a beacon to find prey, or avoid traps triggered by IR beams. This information is important to the conservation of New Zealand's endemic wildlife and to halt the spread of TB.

## **Aims and Structure**

The purpose of this thesis is to provide baseline data for future studies by determining the range and sensitivity of light detection in the ferret. My main aims were to:

1. Assess whether ferrets are capable of operating a two-choice operant procedure with a visual discrimination: ‘follow-the-lit-light’;
2. Test for ‘stimulus generalisation’ over light stimulus properties;
3. Investigate whether ferrets can see IR light, and if so, establish the frequency range;
4. Determine what brightness threshold is required for ferrets to detect IR light; and
5. Eliminate the possibility of extraneous cues.

The thesis structure is outlined in the following paragraphs...

Chapter 1 reviews the available literature on spectral sensitivity in humans and animals, and examines physiological and behavioural studies in ferret vision. The need to test whether ferrets can detect infrared light and the adaptive advantages of this is discussed.

Chapter 2 reviews the training procedure used for the two-choice operant procedure. Ferrets were trained with a highly visible bright white light to press a lever under a lit light and not under an unlit light. Several modifications were made to the standard operant procedure to increase reliability of data.

Chapter 3 assesses generalisation across varied properties of visual stimuli by changing the light wavelength (colour) from white to blue, green, yellow, orange and red (lights within the ‘visible’ spectrum). Subsequently the red light was systematically dimmed to show generalisation across intensities and promote optimal eye positioning and light-searching behaviours under difficult visual circumstances (as proved successful by Vanstone, 2005).

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Chapter 4 reviews how the red LED was returned to full brightness and then extended into the near IR spectrum (frequencies exceeding about 700 to 750 nm). Two IR frequencies were tested; 870 nm and 920 nm. A frequency threshold was tested for each individual ferret. Dimming of detected IR frequencies to obtain an intensity threshold was also tried.

Chapter 5 introduces the possibility of ultrasound and sequential dependencies as extraneous cues and tests for the presence of these influences on the ferrets' ability to perform the visual discrimination task.

The final chapter (six) discusses conclusions on the visual frequency and intensity thresholds for detection of infrared light in ferrets. The general significance of these results for future technology for control of ferrets and for protection of non-target species is discussed.

## **Chapter 2**

### **Training**

#### **Abstract**

Five ferrets were trained in a two-alternative forced-choice operant procedure to press a lever under a lit-white-light. The lit-light initially appeared in a random series to the left or the right of a trial-starting device. Correct responses (a lever press under the lit-light) gained the ferret food reinforcement, and incorrect responses received no reinforcement. The ferrets had 17 to 40 sessions practice at lever pressing, and this stage of training was named F-level-1. Training the ferrets to only lever press under the lit-light was difficult, and additional procedural measures were taken to combat lack of subject motivation and attentiveness, the presence of response bias, inconsistent responding and satiation: at F-level-2 an inter-trial-interval (ITI) of 10 seconds was programmed after incorrect responses; F-level-3 incorporated a pre-determined pseudo-random series of reinforcement; F-level-4 introduced a variable ratio (VR  $1.5 \pm 0.5$ ) schedule that made the reinforcement leaner. Together, these procedural modifications helped to decrease side biases and increase accuracy from around chance levels (overall mean 58%) to near perfect levels (overall mean 89%), showing that the ferrets had successfully learnt to follow the light. All together, 71 to 101 40-minute sessions were required for each ferret to reach criterion at the final training level.

## **Introduction**

### **Operant Conditioning Definition**

Operant conditioning is a type of associative learning in which a trained response can be modified by its consequences (Lieberman, 1993). It involves voluntary (controllable; non-reflexive) behaviour that is strengthened if it is reinforced, and weakened if it is punished (or not reinforced).

Operant procedures can be used to learn sensory information from an animal's behaviour. This procedure is carried out in an 'operant chamber'. This is a three-dimensional box that accommodates several key components: a subject (often an animal); at least one stimulus; at least one response key; and a reinforcement mechanism. If the animal performs the desired behaviour (such as touching a response key when a sound or light stimulus is turned on) then the animal may receive reinforcement (such as food or access to a resource). If the animal performs an undesired behaviour (such as touching the key when the light or sound is off) then the animal may receive punishment (such as an electric shock) or no reinforcement. Over successive trials the animal may learn to touch the key when the light or sound is on, and not when it is turned off. Once this task has been learnt with a high degree of accuracy, the learned behaviour can be used to test different parameters of the stimuli (such as different intensities or frequencies of light or sound) to make judgments on whether an animal can see a light or hear a sound (Blough and Blough, 1977).

### **Operant Conditioning and Visual Stimuli**

Operant conditioning may be the most suitable procedure to obtain visual thresholds because responses can be trained to be convenient for the experimenter and properties of the stimuli (such as light frequency and intensity) can be manipulated independently. Operant conditioning is a widely used behavioural procedure, successful in gaining visual information on behaviour in a large number of species in the past. For example, Nye (1973) conditioned pigeons in an

operant task to discriminate image structure, motion, colour and luminance, in order to compare frontal and lateral visual fields. Rybarczyk et al. (2001) used operant conditioning to show that cows use visual cues, such as body height and facial characteristics, to discriminate between people. Visual cues were used in an operant conditioning experiment by Taira (1996), in which a rhesus monkey (*Macaca mulatta*) received grooming as a positive reinforcement for choosing a correct visual pattern over an incorrect pattern. And finally, Pollard et al. (1967) were successful in using operant conditioning with ferrets to investigate visual discrimination of basic shapes, proving that ferrets are capable of using visual cues in an operant task. Operant conditioning is an ideal procedure to present light stimuli to ferrets to determine their threshold for light detection.

### **Two-Alternative Forced-Choice Operant Procedure**

A common operant procedure is the two-alternative forced-choice procedure (Blough and Blough, 1977; Lieberman, 1993). This can be used to test a vast variety of stimuli, including olfactory (Extance and Goudie, 1981), auditory (Kelly et al., 1986) and visual (Neitz et al., 1989). In the two-response forced-choice procedure, subjects choose between *two* test stimuli presented *simultaneously* (e.g. a lit-light and an unlit-light). As in all operant procedures, responses (e.g. a lever press) corresponding to the 'correct' (lit) stimuli may result in reinforcement (e.g. food). The reinforcement strengthens the behaviour that preceded it (lever pressing under the lit-light), resulting in the animal readily and accurately performing the task to obtain maximum food rewards. The position of the correct stimulus should vary randomly or pseudo-randomly between the two test stimuli. Just guessing the correct stimulus over a large number of trials, without actually detecting it, should not result in a performance better than chance (about 50% correct). Genuine stimulus detection produces data extending from above chance levels to perfect performance (100% correct).

Having a three- or four-response forced-choice procedure extends the range between perfect and chance performance. For example, a three-response method results in chance levels at 33% (Blough and Blough, 1977). However,

there are disadvantages to having more than two stimuli and response levers. Firstly, it may be difficult to separate the stimuli in space and maintain a simultaneous view of all stimuli, and secondly, the subject may be closer to one or two stimuli than the rest, creating a position bias (Blough and Blough, 1977). For these reasons, a two-response forced-choice method with a sufficient number of trials is preferable.

### **Operant Conditioning Training (shaping)**

The teaching of the operant task is called ‘shaping’, a technique for training responses which are initially unlikely (Lieberman, 1993). For example, an animal may be trained to press a lever under a lit-light. The first step is to reinforce behaviour that is closest to the desired response (e.g. approaching a lever under a lit-light). As this behaviour is repeated, reinforcement is withheld until the animal achieves some closer approximation to the desired response; e.g. touching a lever; pressing down on a lever; pressing only the lever under the lit-light, and so on (Lieberman, 1993).

### **Including a Trial-Starter**

Frequently a third ‘trial-starting’ response is added to the two-response forced-choice procedure (Blough and Blough, 1977). This may be an additional signal (such as a light or sound) with a response lever below it. This is not another ‘choice’ between stimuli (chance is still set at 50%), but rather a signal close to a central key that must always be activated first before making a choice between the side stimuli.

The main purpose of a trial-starting stimulus is to indicate to the subject the initiation of a new trial, even if the tested stimuli are not detectible. For example, the trial-starter may remain a highly visible light source consisting of a known visible wavelength and intensity, while the side LEDs are permitted to change frequencies and intensities. The side LEDs may potentially become difficult to see or invisible to the animal. The highly visible trial-starter will

indicate to the animal that a trial has commenced, and it needs to make a left or right choice, even if it cannot see which side light is lit. This helps differentiate ‘no behaviour’ (which may or may not indicate the stimulus is invisible) from ‘wrong choices’ (a more reliable indicator that the animal cannot detect the stimulus (W. Temple, pers. comm.)).

A trial-starter also has several other advantages if it is placed centrally between the two test stimuli:

- 1) It can help eliminate response bias by ensuring that at the onset of each trial, the animal is placed roughly between the left and right response lever, orientated in the correct direction, and at approximately the same distance from both test stimuli. This is particularly advantageous for ferrets because their eyes are laterally placed (Law et al., 1988), so when they are in a central position, both side stimuli will potentially be in full view at their onset.
- 2) Forcing the animal to move back to the middle before making another choice may help stop the animal from continuously pressing one side lever, if it becomes content with reinforcement every few presses.
- 3) Having a trial-starter may help provide latency information that can be used to compare correct and incorrect choices.

Vanstone (2006) used a trial-starter in a two-response forced-choice procedure that tested light detection by possums. The operant chamber contained a centrally located, brightly-lit amber-coloured LED (trial-starting stimulus) with a trial-starting lever directly below. When the trial-starting LED was lit, the possum was required to press down on the trial-starting lever to commence a trial. Once it was pressed, the trial-starting LED was turned off, and either the left or right side (test LEDs) was lit up, and food reinforcement was available only for a lever press under the *lit*-side-light. This process constituted one trial, and was repeated multiple times throughout daily sessions. To obtain maximum reinforcements, the subjects simply had to ‘follow the lit-light’ from the trial-starting LED to a test LED and back. In subsequent sessions the lit-side-lights included some light wavelengths and intensities that were difficult for the possums to detect (including

low intensity IR LEDs). However, the central trial-starting LED remained a highly visible amber colour, so the possums continued responding (although less often than when the test stimuli were also highly visible), encouraged by the trial starting light. This allowed Vanstone to continue collecting data even when the possums could not tell which side light was lit. This was important because otherwise the possums may not have realized the apparatus was operating, and so stopped working for rewards.

### **Achievement of Good Stimulus Control**

Operant conditioning experiments require strong stimulus control to be successful. This comes from carefully planned reinforcement schedules and high-quality experimental design. For data to be reliable, procedural measures must be designed to combat any possible lack of subject motivation and attentiveness, response bias, inconsistent responding and satiation (see below).

### **Motivation**

Motivation depends partly on deprivation and partly on the attractiveness of the reinforcement. Primary reinforcers (those physically necessary for survival, such as food and water) are particularly effective because they require no special training and are highly desirable. A more attractive reinforcement (e.g. a preferred food) has more incentive to work for access to it. Setting experimental sessions shortly before daily feeding also helps motivation, since hungry subjects will have the most incentive to work for food (Lieberman, 1993).

### **Attentiveness**

A subject's failure to respond to a stimulus may result from a failure of attention as well as failure to detect the stimulus. Even with an appropriate reinforcement established, subjects may still need encouragement to pay careful attention to the task. The programming of aversive consequences for incorrect responses is one technique used to increase attentiveness. Electric shocks have been a popular

choice to alter behaviour of animals in the past (Ferrari and Todorov, 1980; Lewis et al., 1980; Powers and Easter, 1978), but their use as a conditioned stimulus is not usually considered in animal behaviour experiments of today.

A more humane aversive consequence may be a time-out, a period during which no stimuli are presented and responses are ineffective (Blough and Blough, 1977). Time-outs (also referred to as inter-trial-intervals (ITI)), are usually programmed to follow incorrect responses (e.g. a left lever press when the right light is on and vice versa). The purpose of the time-out is to decrease the likelihood of the behaviour that led to the incorrect response (Lieberman, 1993), and thus, increase accuracy. A changeover delay between trials operates in a similar manner to discourage rapid alternation between levers (Blough and Blough, 1977). Many experiments also demand responses that require a certain amount of effort to help increase attentiveness. For example, a certain force to activate a lever (as in Vanstone, 2005) and/or multiple responses per trial (Smith and Gantert, 2004). Increasing the effort required to obtain reinforcement means that it costs the animal more to make an incorrect choice, and therefore the animal may be less likely to make incorrect choices.

### **Response Bias**

Response bias (also called positional preference or side bias) is defined as the systematic tendency for a subject to favour responding in a particular way, due to non-sensory factors. Response bias is difficult to control and grows more profound as stimulus strength decreases (Blough and Blough, 1977). Several programming techniques are used to minimize response bias, involving manipulation of the reinforcement schedule set by the computer program. The reinforcement schedule determines when a response will be reinforced. Completely random trials are not desirable because a long series of identical trials may be generated, potentially strengthening a position preference. The choice of schedule has important consequences for how the animal responds. For example, a subject may develop a side bias because they are content to press the same-side lever multiple times and receive reinforcement half of the time. To combat this, a

pre-determined pseudorandom schedule of reinforcement can be introduced; after a certain number of consecutive reinforcements have been collected from the 'favoured' side, reinforcement can be programmed to next be due on the 'rejected' side. No reinforcement is allowed on the 'favoured' side until a correct lever press has been made, and reinforcement collected from the 'rejected' side. This forces the subject to change sides or receive no reinforcement (Blough and Blough, 1977).

### **Inconsistent Responding and Satiation**

Reinforcement schedules need to be carefully planned to obtain consistent responding from the subjects (to obtain meaningful latency data) and to make sure they do not become satiated before the end of a session. A ratio schedule of reinforcement can be introduced, where reinforcement depends on the number of responses that have been emitted.

A Fixed Ratio schedule (FR) is where the number of responses required for reinforcement is always the same. For example, FR three means that every third correct response will be reinforced. A disadvantage of a fixed ratio is that it can become predictable, as subjects learn they must lever press a certain number of times before reinforcement will appear. This potentially leads to long periods of no response immediately after reinforcement.

An alternative is the Variable Ratio (VR) schedule, where the number of responses required to obtain reinforcement varies across successive reinforcements. This schedule is often specified in terms of a mean value and range (Stebbins, 1970). For example, VR  $3 \pm 2$  could mean that an average of three responses (sometimes only one, sometimes five) is required for reinforcement. The advantage of a VR schedule is that reinforcement can occur at any time, so the unpredictability generates much more consistent responding. Gambling and lottery games are good examples of behaviour controlled by a reward available only on a variable ratio schedule (Rachlin, 1990).

Despite the effectiveness of all these measures, most animal subjects do not achieve 'perfect accuracy' - that is, on some trials they make errors even on

simple discriminations. Such errors are illustrated by data approaching an asymptote below 100% correct, even at the strongest signal values (Blough and Blough, 1977). It is difficult to estimate the degree of such inaccuracy, since the level of inaccuracy may vary with signal strength. For example, Vanstone (2006) found that when visual discrimination trials were difficult, responses became relatively few and data points more variable. However, incorporating the measures above should help improve data reliability and achieve better stimulus control.

## **Aims and Objectives**

This training was designed to assess: (1) if ferrets are capable of working in an operant chamber; and (2) if they are capable of learning a simple operant task: to press the lever under the lit-light for food rewards. If ferrets can perform this task, then investigating light frequency and intensity thresholds becomes possible using this method (investigated in Chapter 3).

To investigate these aims, my main objectives were to: (1) build a two-choice operant chamber in which the ferrets could press levers under a left or a right light to obtain access to food reinforcement; (2) develop a reinforcement desirable enough to induce ferrets to work for access to it; (3) train the ferrets to operate a centrally located 'trial-starter' before making a left or right choice; and (4) train the ferrets to lever press only under the lit-light, and not the unlit-light.

## **Methods**

### **Approvals**

Ferrets are Unwanted Organisms in New Zealand under the Biosecurity Act 1993, which means that buying or selling ferrets commercially is illegal unless an exemption permit has been issued by the Chief Technical Officer of Conservation,

Wellington. An exemption permit was first requested in September 2004 for the purpose of undertaking this research on the learning and sensory abilities of captive ferrets. Approval for the exemption permit was granted on 1<sup>st</sup> May, 2005, for a period of five years, expiring on 1<sup>st</sup> May 2010. Potential suppliers were Mystic Ferrets of Hamilton (the preferred option) and Dun-Edin Farm Ltd, of Dunedin. Subjects were not received until 8<sup>th</sup> August, 2005, due to untimely permit processes and the unforeseen withdrawal of co-operation by Mystic Ferrets.

Ethics permission was granted by the University of Waikato Animal Ethics Committee in February 2005 and extended until February 2007.

### **Subjects and Husbandry**

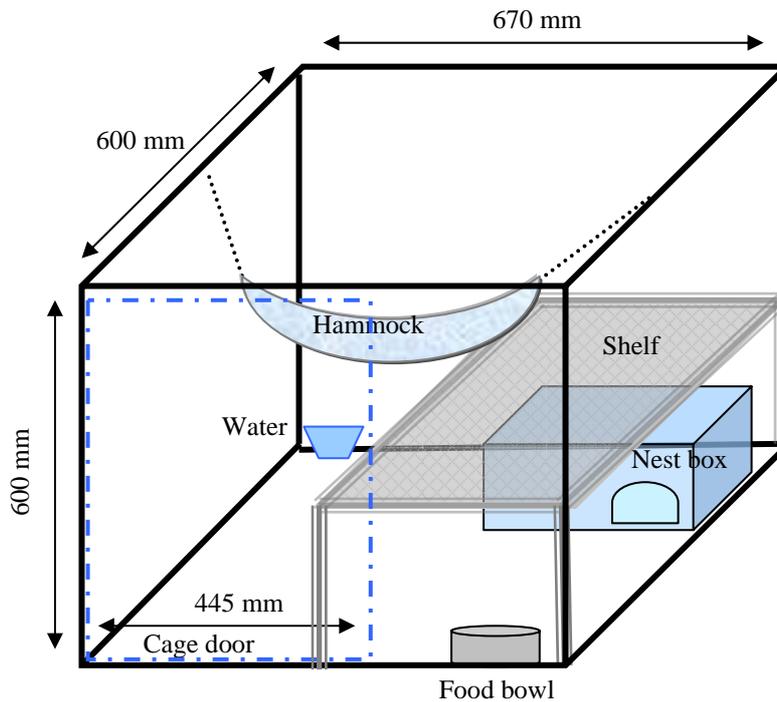
Six male ferrets served as subjects, named: Nero, Felix, Orion, Rex, Micci and Ajax. The ferrets were obtained from Dun-Edin Farm Ltd at approximately five months of age. They were from commercial stock bred to become tame pets, normally exported from New Zealand. They had their scent glands removed, were disease free, were all male and were all de-sexed to render breeding impossible (required under our permit). Only pigmented ferrets were used to ensure no eye-sight abnormalities, known in the albino ferret population (Garipis and Hoffmann, 2003).

The permit regulations stipulated that the room containing ferrets be sealed off by double doors, padlocked, held within a locked and monitored building, and that access be available only to people formally named on the exemption permit. The room temperature was kept between 17 and 22°C, controlled by a wall-fan in summer and a heat radiator in winter. A single 100 watt light bulb, situated in the centre of the room, operated under a reverse 12:12 hour light/dark cycle, slightly modified every three months to represent natural seasonal changes.

The ferrets lived singly in their own cages, placed in two tiers of three against a concrete block wall. Cages measured 670 mm long, 600 mm wide and 600 mm high, and were constructed from wire mesh sides and a metal frame. A 10 mm thick wooden door measuring 600 mm by 445 mm was situated at the front of

the cage, in the bottom left corner. Each cage had a wire mesh platform approximately 260 mm from the cage floor and measuring 320 mm wide and 600 mm long. A small rubber hammock was suspended from the roof of each cage and could be accessed via the platform. On the cage floor was a small plastic nest box measuring approximately 280 mm long, 270 mm wide and 135 mm high and filled with newspaper and/or hay (see Figure 1). Occasionally, behavioural enrichment items such as tunnels, balls, scratching paper and cat-toys were placed in the cage, but these were removed during experimental sessions.

Ferrets had continuous access to water via a valve connected to a main hose. Base food consisted of high-protein quality kitten dried food pellets ('crunchies'), of which several brands were trialled until the most readily accepted brand (Nutrients) was found. Supplementary food included 10 grams of Whiskers jelly-meat daily, one mouse (obtained frozen from the Small Animal House, Ruakura campus, Hamilton) every second day, and two drops of a commercial liquid vitamin supplement (Animal Farms LM Ferret Vitamins) when available. Ferrets were weighed weekly, and their diet was adjusted to keep each individual between 80% and 90% of its free-feed weight. On days where no mice were given, or when their weight was approaching 80% of their free-feed weight, they were offered about 30 to 45 grams of base feed. On days when the ferrets were fed a mouse or were approaching 100% of their free-feed weight, they received only about 15 to 30 grams of base feed.



**Figure 1.** Layout of each ferret's home cage. Note the position of the cage door (shown with a broken line), which was later modified to contain the experimental apparatus.

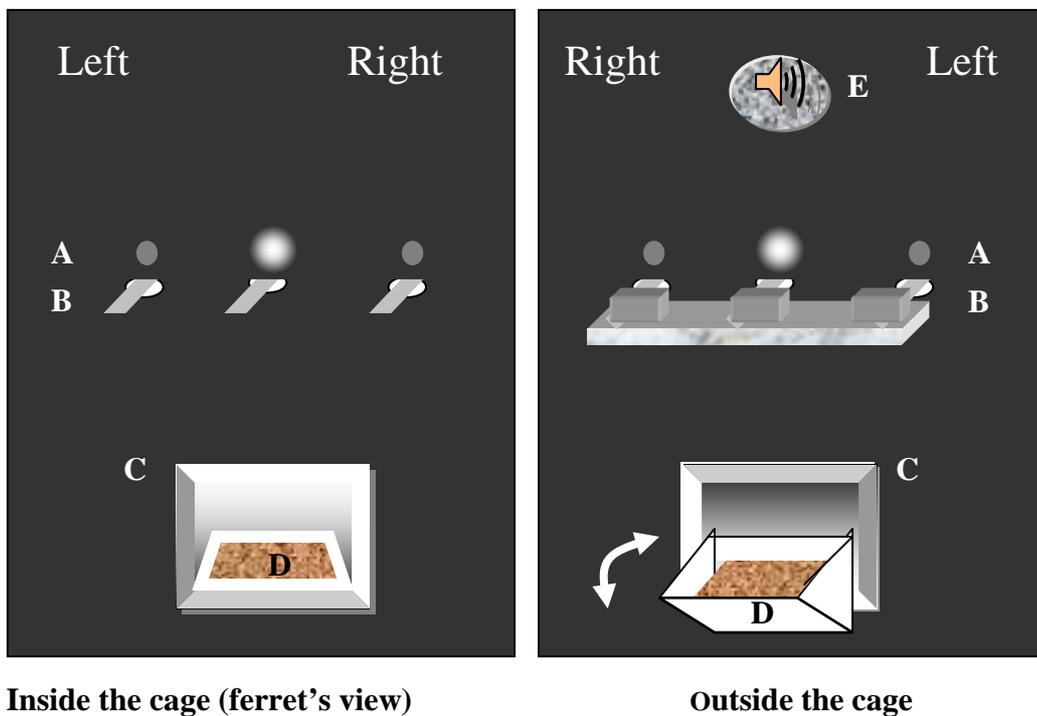
### Experimental Apparatus

An experimental panel was installed into the wooden door of each home cage. The panel contained three small holes (10 mm diameter) positioned 340 mm from the cage floor and eight cm apart (as shown in Figure 2, equipment A). Each hole was just wide enough to insert a single LED to produce a light stimulus.

Directly below each LED hole was a larger hole (15 mm diameter) through which a 25 mm long, three mm wide metal lever could be inserted to facilitate the ferret's responses (shown in Figure 2, equipment B). Levers were removable, and one, two or three levers could be installed at any one time.

Fifteen cm below the lever holes was a single large square hole (85 mm high and 125 mm wide), through which access to a magazine full of crunchies could potentially reinforce a correct behavioural response. The magazine could automatically swing upwards to give five seconds access to food and then swing backwards, putting the food just out of reach of the ferret (see Figure 2, equipment

C and D). A sound producing device was attached to the door of each cage, capable of emitting a short (0.05 seconds) loud 'beep' indicating the correct lever had been pressed and reinforcement was available (see Figure 2, equipment E). The experimental panel was linked via a series of wires to a computer MED/PC system in another room. The computer automatically controlled stimulus and reinforcement presentation and recorded the ferret's responses on the levers.



**Figure 2.** Control panel in cage door (painted matt black) with a view from inside the cage (left image) and outside the cage (right image). (A) Stimulus (three single LEDs) shown with centre LED lit with a white light; (B) three connected levers poking into the cage; (C) Hole for ferret to insert his head to receive reinforcement; (D) Food magazine full of crunchies, capable of automatically swinging forward to allow access to reinforcement, or swinging back to put reinforcement out of reach; (E) sound delivery device ('beep') for correct lever press.

### Procedure

Experiments were conducted in a darkened room during the night-time cycle of the ferrets' reversed 12:12 hour night/day regime. Ultimately, ferrets were to be trained to press the lever directly under the lit-light. This part of the study was done in four stages;

- 1) Ferrets learned to press levers for food rewards,
- 2) Operators searched for a more suitable reinforcer,
- 3) Ferrets learned to follow the light using a 'trial-starter' and receive reinforcement only for lever presses under the lit-light (F-level-1),
- 4) Ferrets learn to follow the light under differently programmed schedules:

- F-level-2: a time out (ITI seven seconds) was programmed to follow an incorrect lever press. This was designed as an aversive consequence to motivate the subjects to increase their accuracy and attentiveness to the task.
- F-level-3: a pre-determined pseudorandom series of reinforcement was introduced, so that no more than three consecutive reinforcements could be collected from one position (left or right). This was programmed to help limit side biases by forcing the animal to change sides or gain no more reinforcement.
- F-level-4: a variable ratio (VR 1.5) schedule was introduced to induce more consistent responding and reduce satiation.

### **Stage 1: Learning to Press Levers for Food Rewards**

#### *Establishing a reinforcement area*

The ferrets' food bowls were removed for an hour a day, during which time they were encouraged to eat their food from the automatic food dispenser. The reinforcement inside the food dispenser was Kitten Nutrients crunchies since they are small, palatable, healthy, easily dispensed and recommended by a vet experienced in ferret health. The food magazine was locked in the raised position to give constant access for 40 minutes a day over two weeks, and the ferrets were free to eat as much as they chose.

#### *Setting up equipment*

At 3:00 pm each day, all food was removed from the ferrets cage in order to ensure the ferrets were moderately food deprived by the time the behavioural shaping sessions began at 8:30 am the following morning, lasting for about 40 minutes per subject. The magazine was tilted away from the ferrets so they could smell the crunchies but could not have access. Three full-intensity white LEDs

were inserted into the LED holes. White LEDs were used at this stage rather than other colours, because it was not yet known what light frequency or intensity ferrets were capable of seeing, and white light incorporated the widest range of light wavelengths within the visible spectrum (400 to 700nm). To begin with, only the centre LED was lit and the side LEDs were turned off. Only the centre lever was inserted (in the lever hole directly under the lit-light). The side levers were removed at this stage.

### *Pressing one lever*

The first objective was to teach the ferrets to press a lever for food rewards. The food magazine could be controlled manually by an extended button that was held while watching a ferret's behaviour through the wire cage. If the ferret directed behaviour towards the lever, the button was pushed, the magazine raised towards the ferret, and it was allowed five seconds access to food. Initially the behaviour required included looking directly at the lever, touching the control panel near the lever or touching the lever with any body part. Over successive trials each ferret eventually learnt to squat on his hindquarters with his head raised towards the light and press down the lever with either his chin or front paw. Up to two ferrets had shaping sessions per day. Each ferret was worked on in turn to teach him to press the lever until they all could do the task.

After a maximum of 25 sessions, the button that manually operated the food magazine was no longer needed, as all the ferrets could press the lever by themselves. The food magazine was set to an automatic reinforcement function controlled by the computer. When the lever was correctly pressed (requiring a downward force of at least 0.25 N), the apparatus would 'beep' (indicating the reinforcement was available), and the subject had access to the food for five seconds. Unsuccessful attempts at pressing the lever resulted in silence and the food remaining out of reach. One ferret (Rex) stood on his nest box to get closer to the lever. At the end of a session the lever/s and reinforcement apparatus were withdrawn from the cage. All regular food was returned to the cage within two

hours of completion of a session, so that ferrets that did not complete many trials did not go hungry.

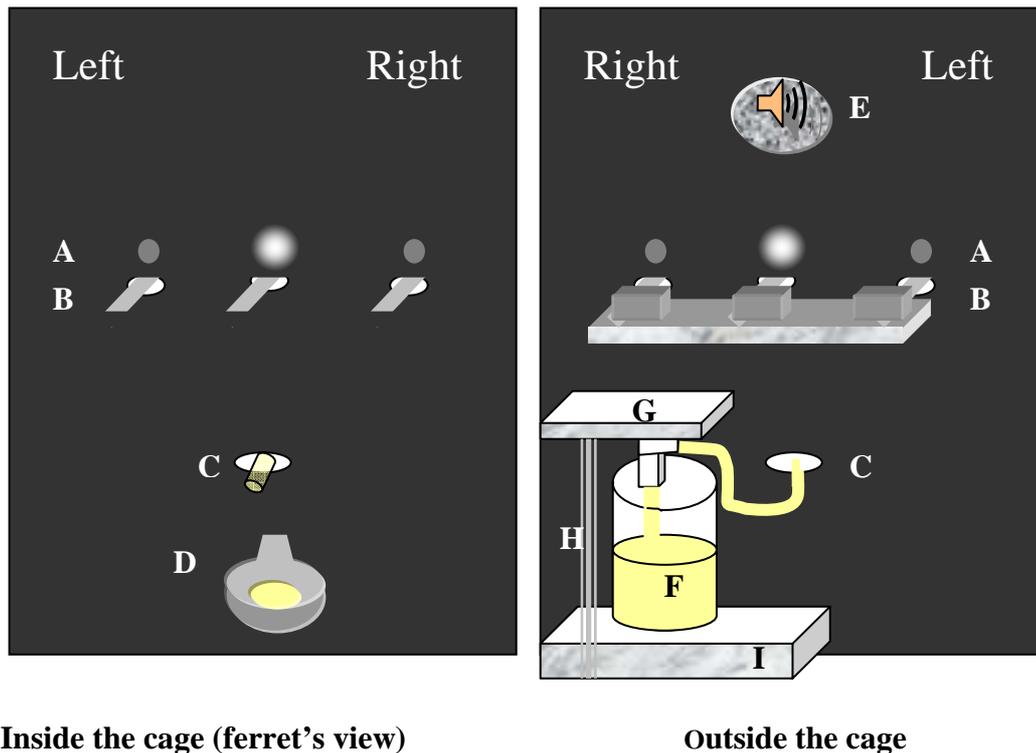
### **Step 2: Finding a New Reinforcer**

The disadvantage of the traditional food magazine containing hard kitten crunchies became apparent when one of the ferrets (Ajax) was regularly witnessed ‘cheating’ by forcefully shoving the magazine to make the crunchies bounce out. This gained him free access to food without lever pressing. Another disadvantage was that the ferrets could cache this reinforcement instead of eating it immediately. Furthermore, the ferrets were not completing many trials within the time allowed (one hour sessions). Extending the lever-pressing session for more than an hour per day, or depriving the ferrets of food for longer, did not result in the completion of more trials. Ferrets were becoming underweight, and lever pressing training had to stop for 30 days to allow extra feeding and weight recovery.

When the experiment resumed, the ferrets completed only a small number of trials (<30) per session. Fewer than 30 trials per session was insufficient, because their data may be skewed and unreliable. An alternative and more effective reinforcement had to be found.

Field trials conducted during a related program (King et al., in press) showed that wild ferrets readily take a mixture of fresh egg and flaxseed oil from the Scentinel®, a liquid dispensing mechanism, so a modified version of this method was developed for the captive ferrets (see Figure 3). The egg mixture was made of eight egg yolks and one tablespoon of flaxseed oil blended together. The liquid dispenser was a modified liquid-soap-dispensing bottle with a 150 mm hose attached to the end of the nozzle (see Figure 3, equipment F). When the ferret pressed the response lever, the mussel wire snapped downwards, pushing the metal plate onto the liquid dispenser (see Figure 3, equipment H and G). This squeezed a small amount (about 0.38 grams) of egg mixture down the hose. The tip of the hose poked through a small hole in the experimental panel, and directed the egg mixture onto a stainless steel soup spoon mounted on the door of the cage

(see Figure 3, equipment C and D). The ferret could lick up the egg mixture from the spoon. This reinforcement was more readily eaten by the ferrets than crunchies. Ferrets were willing to complete a sufficient number of trials per session (>30) for this reward, and therefore, the experiment could continue.



**Figure 3.** Control panel in cage door (painted matt black) with a view from inside the cage (left image) and outside the cage (right image). (A) stimulus (shown with centre LED lit with a white light and side lights unlit); (B) levers protruding into the cage; (C) tube for delivering reinforcement; (D) spoon to collect reinforcement; (E) sound ('beep') delivery device for correct lever presses; (F) liquid dispenser full of egg/linseed mixture; (G) plate that presses on dispenser; (H) muscle wire that contracts and pulls down plate, and (I) base that dispenser sits on.

### *Changing lever positions*

After it was established that the ferrets could press the centre lever, the task needed to be transferred to the left and the right levers (same task, different position; 80 mm left or right of the centre lever). Several sessions were trialled with the left lever and the left LED lit (all other LEDs off and levers out). Then just the right lever and the right LED lit (all other LEDs off and levers out). By

the end of this stage the ferrets had experienced pressing only one lever at a time, although it was not always in the same position.

### **Step 3: Using a Trial-starter (F-level-1)**

When the ferrets showed they could learn to follow the light stimulus presented in all three positions, the centre lever became the ‘trial-starter’. This meant that the ferret would have to press the centre lever first before making a left or right choice, as in a similar procedure used by Vanstone (2005).

To shape this behaviour, the ferrets were first given several sessions in which to practice with two levers (centre and right or centre and left). The lit-light alternated between the two positions, and the ferret would receive reinforcement if it pressed the lever under the lit-light. Once this was achieved, the third light and lever were added to the experimental apparatus. The ferret was shaped to follow the light, and the light was programmed to always return to the centre. For example: the middle light would turn on (middle lever press required); then the left light (left lever press); middle light (middle lever press); right light (right lever press)... etc. The side light was controlled in a semi-random manner so that it would not appear more than three consecutive times in one position (left or right). This level of shaping was named F-level-1.

A daily routine established, allowing each ferret to get regular practice at the F-level-1 task. Because ferrets are mainly nocturnal, the day began with the ferrets’ room entering the dark phase of the reverse 12:12 hour light/dark regime. The dark phase was set to commence at least an hour before the test session, to ensure the ferrets’ eyes had properly adjusted to the dark. Daily sessions lasted 40 minutes for each subject, and consisted of as many trials as the ferret could complete within that time.

Sessions started when all three levers were inserted into the panel. One bright white LED was used for each position (mid, left and right) and remained un-lit until a trial started. The liquid dispenser was inserted into the panel ready to give out reinforcement. A test session was run to ensure all equipment was working. Ferrets had access to all levers, and the lit-light switched between the

middle and then left or right position. Only one light was on at any time. The ferret would press the middle lever to start a trial. Correct lever presses (e.g. a centre lever press followed by a left lever press when the left light is on, or a right lever press when the right light is on) resulted in the ferret receiving one reinforcement (0.38 grams of egg mixture). If the wrong lever was pressed there was no reinforcement and no penalty. The centre light would turn back on and the ferret was free to start another trial immediately by pressing the centre lever.

Subjects remained at F-level-1 until they were reliably completing at least 30 trials per session. This level was important to let the subjects gain experience in receiving reinforcement by lever pressing. The disadvantage of letting them stay too long at this level was that accuracy was poor, since ferrets could still receive a large amount of reinforcement for random lever pressing. Reinforcement would therefore likely be distributed at about chance levels (50% of the time). If ferrets did not improve in accuracy over time, further measures would be required to increase their attentiveness and reduce any response bias.

#### **Step 4: Changing the Programmed Schedule**

Different schedules (named F-level-2, F-level-3, and F-level-4) were required to motivate the ferret to press only the lever under the lit-light. Each level incorporated the rules from the last level, but was extended to include more rules. These modifications to the operant procedure are summarized in Table 1.

##### ***F-level-2: increasing attentiveness***

When a humane aversive consequence was needed, a time-out (ITI 10 seconds) was introduced after incorrect responses. This meant that when the ferret chose the wrong side (pressed the lever under the unlit-light), it had to wait 10 seconds before starting another trial. No lights were lit and responses would not gain the ferret any reinforcement. Training at this level was important to establish motivation to press the lever under the lit-light. The ferret remained on this level for up to ten sessions, unless a side bias had developed (e.g. if the ferret became

content with pressing just the centre and one side lever and getting reinforcement half of the time), in which case the ferret was advanced to F-level-3 before ten sessions had been completed.

### ***F-level-3: reducing response bias***

To prevent the development of side biases, a program was developed that required the ferret to change sides to get his next reinforcement. No more than three consecutive reinforcements could be collected from one side before the next reinforcement was assigned to the next *correct* lever press on the other side. For example, if three consecutive reinforcements had already been collected on the right side, there would be no more reinforcement for responses on the right until a correct left response had been made and reinforcement collected. This stopped the ferret from using only one side lever. Subjects remained at this level of training for at least five sessions.

### ***F-level-4: consistent responding and reducing satiation***

At first, the reinforcement schedule involved continuous reinforcement: every correct response was reinforced. However, experience showed that a leaner schedule was needed to get more trials out of each ferret before it became satiated. A VR schedule was introduced, in which the number of responses required for reinforcement varied across successive reinforcements. This meant that reinforcement would not reward every correct lever press, although the equipment would still 'beep' to indicate a correct response. Reinforcement was programmed for VR 1.5, varying randomly across a range of  $\pm 0.5$  (reinforcements could be received after one or after two correct trials, but was received on average after every 1.5 correct trials). The unpredictability of this reinforcement was expected to produce more consistent responding, since reinforcement could result after any correct lever press. Under this leaner schedule, the ferrets should complete more trials before becoming satiated, and had the potential to keep working for the full 40 minutes of the session.

Additionally, responding with an incorrect lever press would mean that the ferret would not receive reinforcement (as programmed in F-level-1) and would have to wait for a time-out of five seconds (as programmed in F-level-2). Neither would they receive reinforcement for continually pressing the same-side lever (as programmed in F-level-3). Together, these rules were formed to shape the ferrets behaviour into pressing only the lever under the lit-light. These procedures are summarized in Table 1.

**Table 1.**

Summary of modifications to the operant procedure to teach ferrets to only press the lever under the lit-light.

	<b>Shaping</b>	<b>F-level-1</b>	<b>F-level-2</b>	<b>F-level-3</b>	<b>F-level-4</b>
<b>Number of stimuli and response levers</b>	One, then two.	Three (including a central trial-starter)	Three (including a central trial-starter)	Three (including a central trial-starter)	Three (including a central trial-starter)
<b>Reinforcement</b>	Crunchies (five seconds access), then egg and flaxseed mixture	Egg mixture (0.38 g)	Egg mixture (0.38 g)	Egg mixture (0.38 g)	Egg mixture (0.38 g)
<b>Aversive consequence for incorrect response</b>	None	None	ITI seven seconds	ITI seven seconds	ITI seven seconds
<b>Forced to change sides</b>	No	No	No	Yes, after a pre-arranged pseudo-random series of three consecutive reinforcements	Yes, after a pre-arranged pseudo-random series of three consecutive reinforcements
<b>Continuous reinforcement for correct lever presses</b>	Yes	Yes	Yes	Yes	No VR 1.5 (leaner schedule)

### **Data Collection**

Data were collected instantaneously by a computer, using a program written in MED-PC by Jennifer Chandler, Electrical Technician and Biologist, Department of Psychology, the University of Waikato. Data collected included: ferret identification; date; schedule of reinforcement; total trials per session; total number of left and right reinforcements over the session; number of correct left, correct right, incorrect left and incorrect right lever presses; percent correct lever presses over the session; and total time of session.

### **Data Analysis**

The most important data collected for this experiment were the percent correct lever presses, because it is the most likely to demonstrate real visual perception. At the beginning of training (F-level-1), only poor accuracy (around 50%) would be expected because the ferrets would not yet have learnt the task of following the lit-light to maximize their food rewards. Towards the end of training (F-level-4), accuracy would be expected to approach 100%, indicating that the ferrets are following the lit-light. The overall mean and confidence interval for percentage of correct responses could be compared for all sessions in that training level for each ferret. Confidence intervals (95%) were calculated using the following equation, where P is the proportion of correct responses and N is the total number of trials in a training level.

$$P \pm 2 \sqrt{\frac{P(1-P)}{N}}$$

Another parameter of interest was whether the different levels of training may have affected any learned or natural side biases inherent in individual ferrets. To investigate this, log ratios of the total number of left trial responses over the total number of right trial responses for each training level (F-level-1 to F-level-4) were compared for each ferret.

### **Criteria for ‘Seeing the Light’**

#### ***Pass criteria***

Criteria were established to decide whether a subject had detected a given light wavelength. An accuracy of at least 75% over four consecutive (or five out of six) sessions was required, with a minimum of 30 trials per session. Sessions in which ferrets completed fewer than 30 trials were generally not included, due to the risk of skewed data. However, if a subject repeatedly completed less than 30 trials per session, then allowances were made to pass that animal provided more (at least ten) consecutive sessions were undertaken, and an accuracy of at least 75% was still achieved.

This protocol assumes that, if an animal can reach the ‘pass criteria’ for a light wavelength, it is almost certain that the animal can see the light.

#### ***Fail criteria***

A score centring around 50% during at least ten consecutive sessions indicated that an animal may be unable to discriminate a lit-light from an unlit-light. If an animal showed signs of improving in accuracy, then more sessions were carried out until no further improvement was observed. No minimum amount of trials per session could be enforced, because a light that is difficult or impossible for the animal to detect would naturally result in fewer responses.

If an animal fails to show that it can see a given light wavelength when tested by this method, it does not prove beyond a doubt that the wavelength is invisible to the animal. The animal may choose not to respond, or to respond incorrectly, or may even receive sensory stimulation for the act of lever pressing itself.

## **Results**

Ferrets immediately learnt to eat the kitten crunchies from the hopper-shaped reinforcement area when given the opportunity. They took between three and 25 sessions of shaping to learn to press a centrally placed lever to gain access to kitten crunchies (see Table 2), However, they completed few trials per session (often less than 30), so collected insufficient food to maintain their weight. Mean body weight fell below 80% of their free-feed weight, so the experiment was stopped on 21<sup>st</sup> December 2005 to give time for their weight to re-stabilize.

Once their weight had recovered, training resumed using a highly desirable and calorie-rich egg yolk and flaxseed oil mixture as reinforcement. This was successful in motivating the ferrets to work, and to partake in more than 30 trials per session. Five of the ferrets continued with shaping, but one ferret (Micci), who had had ongoing liver and weight problems since arrival, was removed from the experiment permanently on 21<sup>st</sup> December 2005. Micci was put down on December 24<sup>th</sup> 2006 due to liver failure.

The remaining five ferrets were given experience of lever pressing in all three (middle, left and right) positions. When a single lever was moved from the central position and replaced in one of the side positions (80 mm left or right of the centre lever position), most of them immediately lever-pressed without having to re-learn the task, but Ajax required further practice sessions to learn to move his body left and right (see Table 2).

Table 2.

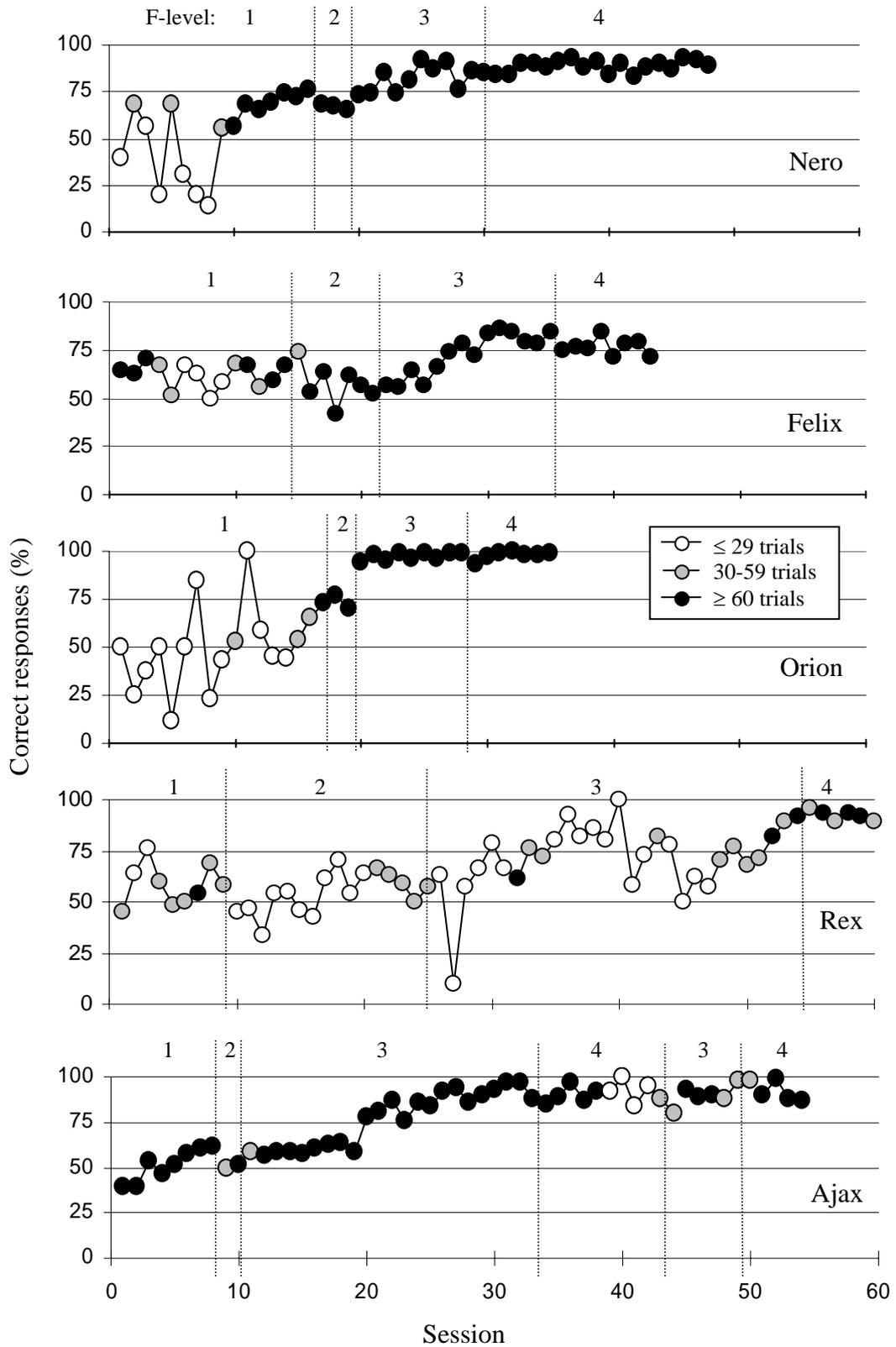
Number of shaping sessions required to teach each ferret to press one lever. Note: first the ferrets were taught to press the centre lever, and then the centre lever was removed and placed in either the left or right position.

Ferret	Lever position		
	Centre	Left	Right
Nero	3	1	1
Felix	8	1	1
Orion	15	1	1
Rex	7	1	1
Micci	8	NA	NA
Ajax	25	3	4
OVERALL	11	1.4	1.6

Once the ferrets could lever press one lever in all three positions, two and then three levers were presented simultaneously. In F-level-1 the ferrets did not immediately learn to press the lever only under the lit-light. Ferrets pressed the centre lever, and then pressed the left or right lever randomly, regardless of which side the light appeared. This reaction prompted the development of the subsequent training conditions (F-level-2 to F-level-4).

Figure 4 presents the overall percentages of correct responses for the four training levels for each ferret. Vertical dotted lines represent a change in the training condition. During F-level-1, mean accuracy was generally low (close to chance levels) and data points were variable. The mean correct lever presses for each ferret at F-level-1 ranged between  $52\% \pm 3$  and  $65\% \pm 3$  (see Table 3). As each learning stage progressed (F-level-1 through to F-level-4) the accuracy of each ferret generally increased. However, it should be noted that when F-level-2 (the ‘time-out’) was introduced, accuracy generally decreased. F-Level-4 (the addition of a VR 1.5 schedule of reinforcement) was introduced to increase the number of trials per session and generate more consistent responding. This coincided with an increase in the number of trials per session for Rex. Nero, Felix

and Orion were already completing at least 60 trials per session before the introduction of F-level-4. Ajax became sick (for reasons unrelated to the experiment) and was off his food for sessions 39 to 44. This could explain the sudden decrease in trials per session during this period. To allow him more reinforcements, Ajax was returned to F-level-3. Once his responding had increased again, he was replaced back on F-level-4. For this reason, there are double categories for F-level-3 and -4 in Figure 4.



**Figure 4.** Mean percentage of correct responses per session for each ferret over each training level. Training levels are separated by vertical dotted lines and displayed above each graph. The number of trials per session are displayed in the key.

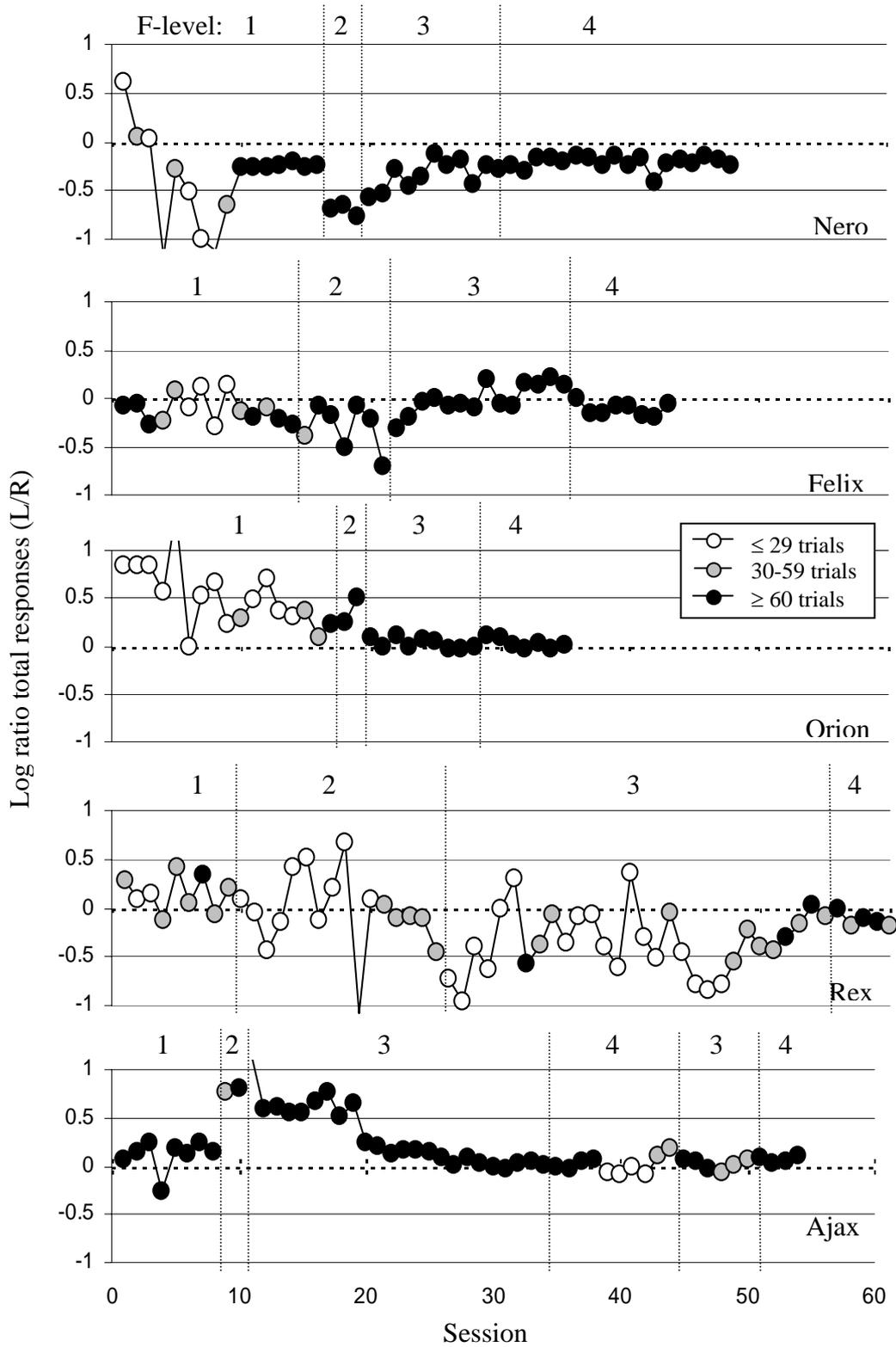
**Table 3.**

**Average percentage (%) correct and 95% confidence intervals for each ferret with the follow-the-light task (F-level-1), and with the added procedural modifications (F-level-2 to F-level-4). Note that F-level-4 is shown twice for Ajax, as the first set of data was based only on a small number of trials.**

	<b>Mean correct responses (%)</b>			
	<b>F-level-1</b>	<b>F-level-2</b>	<b>F-level-3</b>	<b>F-level-4</b>
Nero	65 ( $\pm$ 3)	67 ( $\pm$ 5)	83 ( $\pm$ 2)	89 ( $\pm$ 1)
Felix	64 ( $\pm$ 4)	57 ( $\pm$ 4)	73 ( $\pm$ 2)	76 ( $\pm$ 3)
Orion	53 ( $\pm$ 5)	74 ( $\pm$ 8)	97 ( $\pm$ 1)	98 ( $\pm$ 1)
Rex	56 ( $\pm$ 6)	56 ( $\pm$ 5)	77 ( $\pm$ 3)	92 ( $\pm$ 3)
Ajax	52 ( $\pm$ 3)	51 ( $\pm$ 9)	80 ( $\pm$ 1)	91 ( $\pm$ 3); 91 ( $\pm$ 2)
Overall accuracy (%)	58	61	82	89
N (total no. trials)	3571	1537	8193	5184

By the end of stage F-level-4 of training, all ferrets had successfully met the criteria for demonstrating that they had learned the follow-the-light task; an accuracy of at least 75% over four consecutive sessions including at least 30 trials per session. Percent correct lever presses were approaching 100 %, with the mean for each ferret ranging between 76% ( $\pm$  3) and 98 % ( $\pm$  1) (see Table 3).

Figure 5 shows the Log ratios of the total number of left trial responses over the total number of right responses for each level of training, plotted against session number for each ferret. The horizontal dotted line represents an equal number of trials on the left and right side (i.e., no side bias). Data above zero represents a left side bias, and data below zero represents a right side bias.



**Figure 5.** Log ratio of the total number of left trial responses over the total number of right trial responses for each level of training for each ferret plotted against session number. Data above zero represents a left side bias, and data below zero represents a right side bias.

During F-level-1 and F-level-2, data points were more variable than in subsequent F-levels. All ferrets had large and inconsistent side biases; Orion and Ajax generally had stronger left biases than right; Nero and Felix generally had stronger right biases than left and Rex had strong side biases for both sides in different sessions. An immediate increase in side biases at the onset of the F-level-2 training level (see Figure 5) coincided with a decrease in accuracy in the same sessions for Nero, Orion and Ajax (Figure 4).

F-level-3 was introduced to decrease side biases by forcing ferrets to have to change sides or receive no further reinforcement. Figure 5 shows that this program generally had the desired effect; within ten sessions, all ferrets except Rex had substantially reduced their side biases. Rex still had a strong side bias, generally to the right side, although this was considerably reduced within 28 sessions. By the final training level (F-level-4), any side biases were relatively small and insignificant for all ferrets.

In total, it took the ferrets between 71 and 101 40-minute sessions from learning to eat from the reinforcement area to completing training at F-level-4. Orion got through his training sessions the fastest, and Ajax took the longest (see Table 4).

**Table 4.**  
**Number of sessions completed at each training level by each ferret.**

<b>Training level</b>	<b>Number of sessions</b>				
	<b>Nero</b>	<b>Felix</b>	<b>Orion</b>	<b>Rex</b>	<b>Ajax</b>
Eating from the reinforcement area	14	14	14	14	14
Shaping to lever press	12	17	22	16	32
F-level-1	16	14	17	9	9
F-level-2	3	7	2	16	2
F-level-3	11	14	9	30	30
F-level-4	18	8	7	5	14
Total sessions	74	74	71	90	101

## **Discussion**

### **Using an Operant Procedure**

The first aim of the training was to assess whether ferrets are capable of using an operant chamber with visual stimuli. Researchers have had success in using operant procedures with ferrets in the past: Kelly et al. (1986) trained ferrets to touch their nose to a right copper spout if a tone was present, and left if no tone was present, for water reinforcement; and Pollard et al. (1967) trained ferrets to discriminate between different shapes mounted on small doors and to open the doors with sideways jerks of their heads to retrieve meat reinforcement. However, nobody has ever trained ferrets in an operant procedure to follow a lit-light. This was necessary to lead towards testing their visual thresholds for different light frequencies and wavelengths.

The five ferrets used in these trials were difficult to train to lever-press for three reasons: (1) the reinforcement initially chosen was not desirable enough; (2) the required task (pressing a response lever) was not a natural activity for ferrets; and (3) the schedule of reinforcement was not initially successful.

### ***Reinforcement***

Kitten crunchies were used for reinforcement at first, because they are small, palatable, healthy, and most important, easily dispensed by the automated training equipment initially used in this study. Although the ferrets readily ate crunchies when given free access, they did not desire this reinforcer strongly enough to work long enough for access to it. Another disadvantage was that the dry pellets were removable and could be cached instead of eaten immediately. To learn the task, it was important that the ferret had immediate reinforcement to strengthen the association between pressing the lever under the lit-light and being reinforced (Malott and Trojan-Suarez, 2004). An alternative, more desirable and less portable reinforcement was necessary.

In the past, water has been used as a very successful reinforcer to motivate ferrets to work. Kelly et al. (1986) deprived ferrets of water for 22 hours, and ferrets had to work in an operant chamber for their total daily ration. However, ferrets have a short digestive tract (Wellstead, 1981), and depriving them of access to water for long periods of time may affect their ability to digest food, especially when their staple diet is dried kitten crunchies. Water as a reinforcement was not an ethical option in this study. An egg and flaxseed oil mixture was finally chosen as reinforcement since it was healthy, small measures could be dispensed at a time, and ferrets were highly motivated to work for access to it. Once the reinforcer was changed from crunchies to the egg and flaxseed oil mixture, ferrets worked steadily, and often completed more than 60 trials per session.

The dramatic effect of the change from crunchies to an egg mixture has important implications for understanding the behaviour of wild ferrets. Ferrets will work harder to gain access to a more highly desirable food (as demonstrated

above). This food could in fact be an egg of an endangered, ground-dwelling bird in the wild. Ferrets are intelligent hunters adapted to having to solve access problems (as verified by Ajax learning to ‘cheat’ to gain access to the crunchies). The combination of an intelligent, fast-learning predator and a highly desirable food source may result in the predator using any means possible and all available cues to locate and access the food. If ferrets can see the monitored nest areas lit up by IR light at night then it is feasible that ferrets could learn to associate ‘food’ with night-time lights and use this information to locate and access their prey.

### ***Response levers***

Levers were chosen to measure responses because they are commonly used in operant procedures involving animals with dexterous paws such as rats (Hyytia and Sinclair, 1990), mice (Elmer et al., 1987), and possums (Signal, 2002). It took between three and 25 sessions of shaping for all five ferrets to learn to press a lever (see Table 2). In an operant chamber, data can be generated quickly and recorded instantaneously – a great advantage in a study required to complete a large number of trials within strict time limits.

On the other hand, a more natural, species-specific behaviour may have been more appropriate. Ferrets have been trained in an operant procedure to push their head through a door (Gewalt, 1959; Pollard et al., 1967) or touch their nose to a spout (Kelly et al., 1986). Ferrets can be easily and reliably trained to work with hunters to flush rabbits out of their holes, in a sport called ‘ferreting’ (Wellstead, 1981). Moving through tunnels is a more natural behaviour for this species than lever pressing, and this behaviour can be easily transferred to laboratory experiments. For example, Cloe et al. (2004) used a Y-maze to study olfactory mate recognition in ferrets. Subjects began in a starting box, and on release, chose between two enclosed tunnels (the two arms of the ‘Y’). At the end of each tunnel was a different stimulus (odours emitted from anal scent glands of opposite or same sex ferrets). A similar method using a Y-maze was considered for this study, on the grounds that it might have been more appropriate than an operant chamber to test ferret vision. The two tunnel-like arms of the Y could

have contained either a lit- or unlit-light, and ferrets could have been reinforced for choosing the arm with the lit-light. Although moving through tunnels would have been an ideal behaviour to make use of for this study, it would require handling and transporting the animal from its home cage to the Y-maze apparatus. The strict permit requirements issued by DOC meant that the ferrets were not permitted to leave their home cages except for emergency vet visits.

### ***Reinforcement Schedule***

The second aim of the study was to assess whether ferrets are capable of learning a simple operant task: press the lever under the lit-light for food rewards. The initial schedule of continuous reinforcement was unsuccessful at training ferrets to do this because they simply pressed levers randomly. There were no aversive consequences for incorrect lever presses, and no incentive to improve because the ferrets still received reinforcement about half of the time.

Three subsequent reinforcement schedules were programmed to teach the ferret to only press the lever under the lit-light (see Methods). At F-level-2, which added an aversive consequence of a ten second time-out after incorrect responses, Orion's accuracy for lever-pressing under the lit-light increased to near perfect levels. However, the time-out had the opposite effect for the other ferrets - most of them decreased in accuracy. The decrease in accuracy correlated with a strengthening of side bias, so a program had to be developed to reduce side bias.

F-level-3 added a pre-arranged pseudorandom series of reinforcement to force ferrets to change sides after receiving three consecutive reinforcements on one side. If the subject did not change sides then it received no further reinforcement. This procedure may reduce, but does not eliminate position bias (Blough and Blough, 1977). For Nero, Felix and Ajax, the combination of a time-out for incorrect responses and a pseudo-random series of reinforcement correlated with a steady increase in accuracy and decrease in position bias over subsequent sessions. However, Rex still had a right-side bias and low accuracy (see Figures 4 and 5).

It is not obvious why animals develop or maintain response biases in operant procedures. In this study, left and right levers were equally spaced from the centre lever, required an equal amount of force to activate, issued an equal number of reinforcements, and presented the lit-light an equal number of times over a session. However, all ferrets were shaped to lever-press in a particular order: centre, left and then right. This might explain a left-side bias (as shown by Orion and Ajax), although this does not explain why positional preferences occasionally alternated between both the left- and the right-side for these ferrets, or why Nero and Felix had stronger right-side biases than left.

F-level-4 introduced a VR 1.5 ( $\pm 0.5$ ) schedule of reinforcement to decrease predictability of reinforcement and establish a leaner schedule to get more trials out of each ferret before it became satiated. Under this regime, Orion, Rex and Ajax generally increased in the number of trials completed per session. Nero and Felix did not change their response rates. The VR schedule was not further increased from VR 1.5 as it was feared that the ferrets might stop lever pressing if the cost (effort) for obtaining the food became too great.

By the end of adding different training schedules, all ferrets were lever-pressing under the lit-light with a mean accuracy of between 76% ( $\pm 3$ ) and 98% ( $\pm 1$ ) (Table 3). All ferrets had reached pass criteria, indicating they had successfully learnt the task. This was considered to be an easy discrimination since the light was bright and of a wavelength known to be visible to these ferrets (white; 400 nm to 700 nm). The ferret's inability to reach 100% accuracy reflects other research where correct responses reach an accuracy level close to, but not exactly 100%, even on the easiest discrimination (Signal, 2002; Vanstone, 2006). Reasons for close to, but not perfect accuracy in this study are likely to include: (1) not having a fixed retinal position (Muntz, 1974) (the ferrets were free to move their heads); (2) Motivation to obtain reinforcement and attentiveness towards the task is not likely to be perfect all the time; (3) Side bias can be reduced, but not completely eliminated (Blough and Blough, 1977); and (4) There may be unintended reinforcements for both (correct and incorrect) choices such as stimulation from lever pressing alone.

Lengthy training is typical of operant procedures with non-human subjects. For example, Entsu et al. (1992) used three cows in an operant procedure which required up to 40 daily sessions of 30-trials each to reach a pass criterion of only a 70% correct. In this study it took the ferrets up to 101 40-minute sessions before all five ferrets were pressing the lever under the lit light with a high degree (<75%) of accuracy (see Table 4). Lengthy training and testing periods usually mean that sample sizes remain small, and experimenters must be patient for reliable results.

## **Conclusion**

Subjects must be trained to perform an operant task with a high degree of accuracy before properties of the stimulus (e.g. frequency and intensity) can be manipulated. Training the five ferrets was time consuming and difficult, and modifications to the standard operant procedure were required to enable them to learn the task. With the help of shaping to lever press, a time-out for incorrect responses, pseudo-random reinforcement, and a VR schedule, all five ferrets eventually (over a total of 101 sessions) learnt to correctly and reliably respond to the bright white LED. In general, as each learning stage progressed (F-level-1 through to F-level-4) the accuracy for each ferret increased, and side bias decreased. By the end of stage F-level-4 of training, all ferrets had met the criteria for the follow-the-light task. Establishment of this reliable skill was the essential pre-requisite for investigating the light frequency and intensity thresholds of ferrets.

## **Chapter 3**

# **Stimulus Generalisation**

### **Abstract**

The two experiments described in this chapter were designed to investigate whether five ferrets, already trained to press a lever under a lit white light (see Chapter 2), could generalize their learned response to related but different light stimuli. The experiments tested generalisation across wavelengths (i.e., colours) of a given brightness, and across brightness values for one wavelength (red).

In the first experiment, all variables other than the left and right (test) stimuli remained the same as in training (Chapter 2). Over 23 to 37 sessions, the test stimuli were changed from white LEDs to different coloured LEDs, representing in turn a series of light wavelengths in the ‘visible’ spectrum (peak wavelengths at: 474 (blue), 521 (green), 593 (yellow), 610 (orange), and 635 nm (red)). This part of the experiment was not designed to distinguish whether or not ferrets could ‘see’ those particular colours as different from each other, but instead to determine if they could detect different coloured lights at all, and transfer their already learned task between the different wavelengths. All five ferrets easily transferred the task from one colour to another. The overall mean accuracy of their responses to each colour varied between 92% and 84%. Accuracy always exceeded the pass criteria (defined as at least 75% correct responses over four consecutive (or five out of six) sessions, with a minimum of 30 trials per session), showing that the ferrets were still successfully following the lit-light, and their performance of the learned task was not disrupted by changes in wavelength. This test established that the ferrets could perform the task in response to a red stimulus, which was important because visible red is the closest wavelength to IR.

In the second experiment, the white trial-starting LED was replaced with a red LED, and the two side LEDs were also red. The brightness of the two test LEDs was halved after every ten trials, up to nine times a session. All five ferrets

easily transferred the learned task between the different intensities, with overall mean accuracies of between 88 ( $\pm 3$ ) and 78% ( $\pm 4$ ), even at the dimmest level.

Stimulus generalisation across different wavelengths and intensities is a necessary pre-requisite before changing the test LEDs to potentially invisible IR light wavelengths. These experiments demonstrated that the five ferrets were ready to proceed to the next stage of the visual perception test program.

## **Introduction**

### **Stimulus Discrimination**

In previous two-choice training sessions (Chapter 2), five ferrets had been taught to press the lever under a lit-LED. This LED emitted a high intensity white (400 to 700 nm) light, highly detectible to ferrets. However, it was not known whether the ferrets would continue to perform this learned response if the properties of the test stimulus (light frequency and intensity) were changed. It was important to show that the ferrets could generalise between different light stimulus properties before a potentially invisible IR light (long-wavelength, low-intensity) was introduced. This procedure ensured that any later failure by the ferret to perform the task could not be attributed to inability to generalize the stimulus rather than inability to see the light.

### **Stimulus Generalisation**

Stimulus generalisation is defined as the tendency to transfer a learned response towards a training stimulus to other, similar stimuli (Lieberman, 1993). In operant procedures designed to investigate sensory abilities, it is important to show that the subject can still perform the learned response after general changes in the properties of the stimuli. Stimuli used to *train* an organism should be highly detectible, to ensure there is a strong association between the response and the reinforcement (Malott and Trojan-Suarez, 2004). For example, a light should be

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sufficiently bright, and of a known visible wavelength. But potentially invisible *test* stimuli cannot be examined until it has been shown that an animal trained to respond to one form of visible light can transfer the learned task (generalise) to other visible lights.

For example, Vanstone (2006) used possums trained to lever press under a bright amber light to investigate whether they could also see potentially invisible IR lights. The possums were first required to demonstrate that they could see other wavelengths (blue, blue-green, orange and red) to show that the change in conditions (particularly wavelength) would not disrupt their performance of the learned task. Then when an IR light was introduced, it was unlikely that a failure to lever-press under the potentially invisible stimulus was due to the possum's inability to generalise between different wavelengths.

### **Method of Limits**

If the spectral sensitivity thresholds of the subject species are not known, it can be difficult to choose an appropriate sequence of test stimuli for generalisation tests. If wavelengths or intensities are randomly chosen, there is the danger of inadvertently presenting the subjects with an invisible stimulus, and falsely concluding that the subjects cannot generalise between similar stimuli.

One method for choosing stimuli is the 'method of limits', invented by Wilhelm Wundt, a founding father of experimental psychology. In the 'descending method of limits', some property of the stimulus starts out at a level high enough for reliable detection (e.g. a high-intensity light of a known visible wavelength), and then this level is gradually decreased until response accuracy decreases (e.g. the light wavelength or intensity is manipulated until accuracy is affected) (Stebbins, 1970). Descending order is the most popular procedure since it is best to start with an easy discrimination task and then proceed to tasks that are more difficult (Blough and Blough, 1977). Using this method, stimulus generalisation can be tested without the risk of unknowingly presenting invisible stimuli.

Systematically decreasing the intensity of an LED can also teach animals behaviours that can help them to detect stimuli that may be difficult to see. For example, Vanstone (2006) found that four possums trained with high accuracy to lever-press under full-intensity (100% on) red (635 nm) LEDs, did not maintain such high accuracy when presented with lower intensity IR (870 nm) LEDs. Before concluding that this was because the possums could not see dim lights, an alternative explanation, that the possums were inexperienced in performing the learned task with a dim light source, had to be eliminated. After the possums were given ‘dimming training’ with red lights of systematically decreasing intensities, two of them performed with higher accuracy in subsequent IR trials. Vanstone’s results suggest that the possum’s experience with slowly dimming lights helped them to learn behaviours that aided their ability to detect a dimmer light. This course of action helped eliminate procedural errors, unrelated to the visual abilities of the animal.

## **Aims and Objectives**

This experiment was designed to assess whether ferrets could generalize between visual (light) stimuli of varying properties. Particularly, to see if ferrets could transfer the task of following a full-intensity white light to following a full-intensity light of increasing peak wavelengths (experiment 1); and if ferrets could follow a red light as it slowly and systematically decreased in intensity (experiment 2).

To investigate these aims my objectives were to: (1) source various coloured LEDs and use a spectrometer to record their spectral properties; (2) test detection of light wavelengths, by observing the effect on the ferrets’ accuracy in the ‘follow the lit-light’ task when presented with different coloured side LEDs; and (3) test detection of light intensity by observing the effect on the ferrets’ accuracy when presented with systematically dimming side LEDs .

## **Experiment 1**

### **Generalisation of Wavelengths**

#### **Methods**

##### **Subjects and Husbandry**

This experiment used the same five subjects (Nero, Felix, Orion, Rex and Ajax) and husbandry as described in Chapter 2.

##### **Experimental Apparatus**

This experiment used the same experimental apparatus employed in training (with the liquid dispenser and the white central trial-start signal light), except that the white test LEDs at either side of the apparatus were replaced with LEDs of different colours (peak wavelengths). The five colours chosen span the spectrum of light visible to humans; blue; green; yellow; orange; and red. They were sourced from Ultra-Bright and were similar to the coloured LEDs used in Vanstone's (2005) possum vision experiment. Table 5 shows the spectral properties of each coloured LED as specified by the manufacturer and measured with a spectrometer. The spectrometer measured the relative intensity of each LED compared to the Blue (474 nm) LED. The blue LED was chosen as a point of reference because it was the highest intensity LED and the first colour tested in the operant chamber with the ferrets. After blue, the highest intensity LED was orange, then yellow, white, green and red. The relative intensities and wavelength curves are graphed in Appendix 1.

**Table 5.**  
Spectral properties of each coloured LED, including the visible colour of light, peak and range of wavelengths, and relative intensity.

<b>Visible colour of light</b>	<b>Peak wavelength as specified by manufacturer (nm)</b>	<b>Peak wavelength as found by spectrometer (nm)</b>	<b>Spectral bandwidth limits as found by spectrometer (nm)</b>	<b>Relative intensity as measured with spectrometer</b>
White	Not specified	551	400-700	482
Blue	470	474	440-540	3292
Green	524	521	475-600	395
Yellow	590	593	550-610	1523
Orange	612	610	575-640	2150
Red	635	635	590-710	257

### **Procedure**

The learned task had already been established in training: press the lever under the lit-light. The test procedure required the same schedules as used in the final stage of training (F-level-4): the middle lever must be pressed to start a trial; the reinforcement schedule was VR 1.5 ( $\pm 0.5$ ); reinforcement was semi-random (not repeated more than three consecutive times on one side); and a ‘time-out’ of 10 seconds followed an incorrect response. Experiments were conducted in a darkened room during the night-time cycle of the ferrets’ reversed 12:12 hour nigh/day regime.

The ferrets were previously trained with bright white LEDs, serving both as the trial-starting signal and as the side (test) stimuli. Because we knew ferrets could see bright white light (Chapter 2), it was important that a white LED would continue to serve as an indicator that a new trial has started, in case the test stimuli became invisible at some stage. The peak wavelengths of the left and right (test) LEDs changed as the experiment progressed. The first peak wavelength to be tested was 474 nm (blue) because this was the brightest coloured LED available

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(relative intensity 3292), and blue lies between violet (430 nm) and greenish/yellow (558 nm); the two peak wavelengths that the retina of the ferrets can maximally absorb (Calderone and Jacobs, 2003).

A method of limits was used to determine the next peak wavelength to be tested. The stimuli were presented in sequential order from shortest to longest wavelength: blue, green, yellow, orange, and red. The ferrets' ability to transfer the task between lights of different wavelength and intensities was recorded automatically (as outlined in Chapter 2). When a ferret met the 'pass criteria' defined below, then the next coloured LED was inserted into the operant chamber.

### **Criteria for 'Seeing' the Light**

#### ***Pass criteria***

The criteria used to define a 'pass' required an accuracy of at least 75% over four consecutive (or five out of six) sessions, with a minimum of 30 trials per session. Sessions in which ferrets completed fewer than 30 trials were generally not included, due to the risk of skewed data. However, if a subject repeatedly completed fewer than 30 trials per session, then allowances were made to pass that animal provided: (1) at least ten consecutive sessions were undertaken; and (2) an accuracy of at least 75% was still achieved.

This procedure accepts as almost certain that, if an animal can reach the 'pass criteria' for a given light wavelength, that animal can see the light. This is because it is highly unlikely that a subject could meet pass criteria by chance.

#### ***Fail criteria***

As in training (Chapter 2); a score close to around 50% during at least ten consecutive sessions was interpreted to indicate that a given animal may be unable to discriminate a lit-light from an unlit-light. If that animal showed signs of improving in accuracy, then more sessions were carried out until no trend was

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observed. No minimum amount of trials per session could be enforced because ferrets generally completed few trials when stimuli were difficult to see.

This procedure accepts the fact that an animal failing to demonstrate that it can see a given light wavelength has not proven beyond a doubt that that wavelength is invisible to the animal. The animal may choose not to respond, or chose to respond incorrectly.

### **Data Collection**

Data were collected instantaneously by a computer, using a program written in MED-PC by Jennifer Chandler, Electrical Technician and Biologist, Department of Psychology, the University of Waikato. Data collected included: ferret identification; date; wavelength and intensity of the LED; schedule of reinforcement; total trials per session; total number of left and right reinforcements over the session; number of correct left, correct right, incorrect left and incorrect right lever presses; percent correct lever presses over the session; and total time of session.

### **Data Analysis**

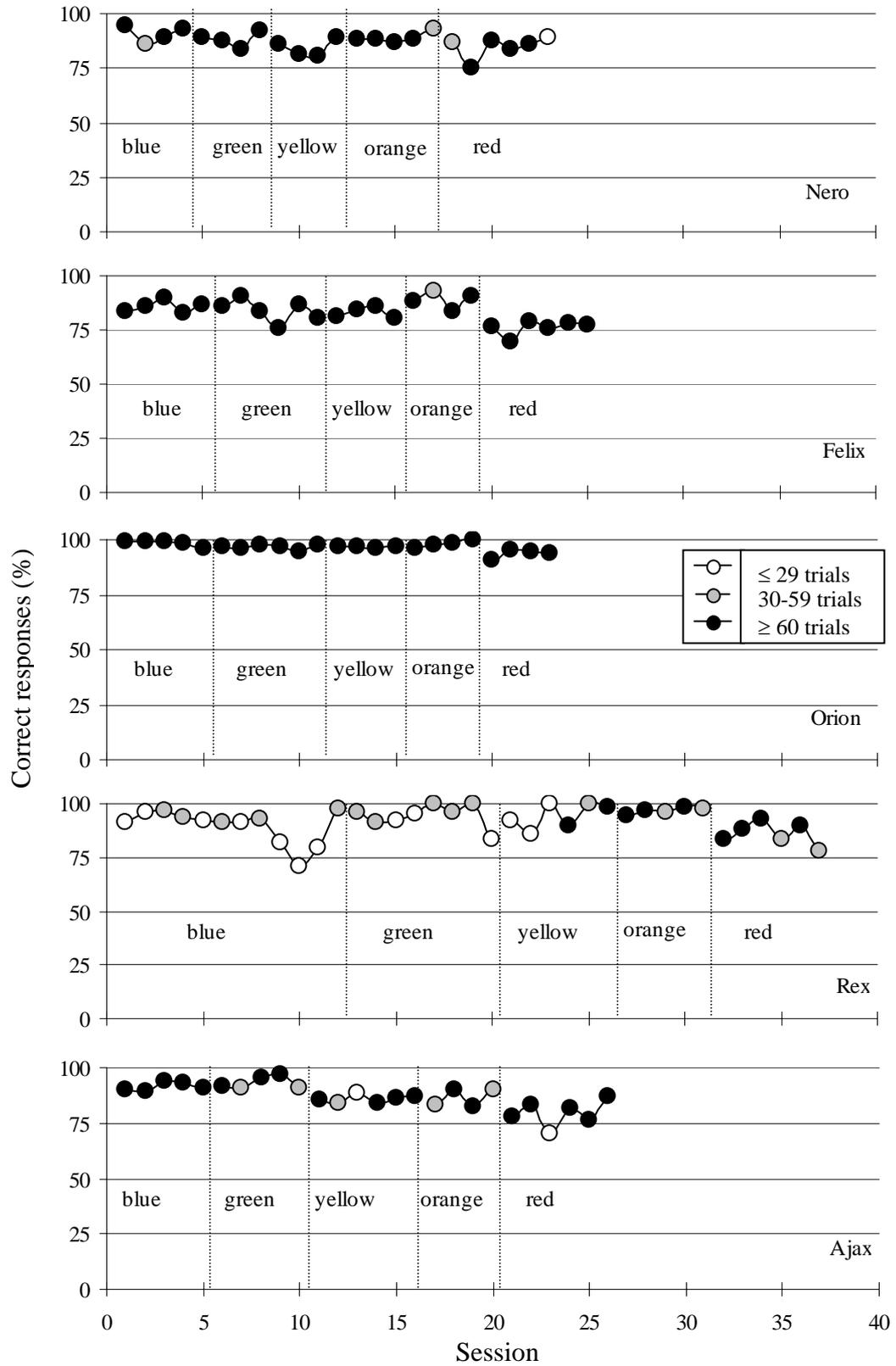
The most important data collected for this experiment were the percent correct lever presses, because this figure is the most likely to demonstrate real visual perception. If the ferret cannot detect a wavelength or intensity, then left and right lever pressing would be expected to centre around chance levels (50%). Light detection should produce data that approaches 100% accuracy. The overall mean and confidence interval for percentage of correct trials for each colour condition and dimming level was compared for all sessions for each ferret.

## **Results**

More than 30 trials per session were completed by Felix and Orion, and frequently completed by Nero and Ajax. Rex often completed fewer than 30 trials per session, so had to undertake more sessions to make up the number of trials and increase the reliability of the data.

All ferrets successfully continued the task of following the lit-light when the left and right LEDs were changed from white to other colours in the human visible spectrum (see Figure 6). Blue, green and orange LEDs produced the highest overall accuracies of 92%. Yellow LEDs also produced a high overall accuracy of 89%. Although the red condition did not reach the same level of accuracy as in the other colour conditions, all ferrets still met pass criteria within six sessions and reached an overall accuracy of 84% (see Table 6). The overall accuracy for the white condition in Chapter 2 was 89%. The results show little difference between accuracy levels over the different coloured conditions, indicating that all five ferrets could generalize between all of the wavelengths that were presented to them.

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**Figure 6.** Mean percentage of correct responses per session for each ferret over each colour condition. Colour conditions are separated by vertical dotted lines. The number of trials per session is divided into three categories as shown in the key.

**Table 6.**  
**Mean correct lever presses and 95% confidence limits for each coloured LED for each ferret.**

	Mean correct responses (%)				
	Blue	Green	Yellow	Orange	Red
Nero	91 ( $\pm$ 4)	88 ( $\pm$ 4)	85 ( $\pm$ 4)	89 ( $\pm$ 3)	84 ( $\pm$ 4)
Felix	86 ( $\pm$ 3)	84 ( $\pm$ 3)	83 ( $\pm$ 4)	89 ( $\pm$ 3)	76 ( $\pm$ 4)
Orion	99 ( $\pm$ 1)	97 ( $\pm$ 1)	97 ( $\pm$ 2)	98 ( $\pm$ 1)	94 ( $\pm$ 2)
Rex	91 ( $\pm$ 3)	95 ( $\pm$ 3)	95 ( $\pm$ 3)	97 ( $\pm$ 2)	87 ( $\pm$ 4)
Ajax	91 ( $\pm$ 3)	93 ( $\pm$ 3)	86 ( $\pm$ 3)	87 ( $\pm$ 4)	81 ( $\pm$ 4)
OVERALL MEAN	92	92	89	92	84
N (total no. trials)	2447	2293	1922	1848	2193

## **Experiment 2**

### **Generalisation of Intensity Levels**

This experiment was designed to test generalisation across different levels of light intensity, and to give ferrets experience in actively searching for a dim light. It was predicted that, as stimulus intensity decreased, so would the ferret's accuracy. As a result, it was anticipated that subjects would have to learn behaviours to help them to see the dim light, in order to continue receiving the same number of reinforcements. Such behaviours could possibly include optimal body and eye positioning (e.g. positioning body and face closer to the LEDs), and actively searching for the lit-light (e.g. learning to take a second look before making a left or right choice). Vanstone's (2005) experience with possums suggested that these skills could increase the probability of detection of the IR LEDs.

## **Methods**

The same experimental apparatus (with the liquid food dispenser) and the same five subjects (Nero, Felix, Orion, Rex and Ajax) and husbandry were used, as in training (Chapter 2). The difference here was that all three LEDs inserted into the operant chamber (one each for the left, right and centre position) were red (635 nm). The single white trial-starting LED was replaced with a single red LED because (1) the wavelength tests described above had already shown that ferrets could reliably perform the task with a red stimulus, and (2) the red LED had the closest peak wavelength to the IR (870 nm) LED, so the move into the IR spectrum (in Chapter 4) would require as small a change as possible.

After confirming that the ferrets could continue to detect the red stimuli with high accuracy (meeting the pass criteria defined above), a dimming computer program (named Red-dim) was added. The pass criteria were the same as in the wavelength tests (see above), except that no minimum number of trials per session was imposed, and any dimming level less than 100% intensity did not have to meet pass criteria because it was not known whether a dimming level was even visible to the ferrets, and if they could not see it, it was hoped that they would learn to over time.

### **Red-dim Program**

This program was developed by lab technician Jennifer Chandler, originally designed for Signal's (2002) experiment with possums, to allow a bright versus dim discrimination. It creates a simple and controlled method of reducing the brightness of LEDs in small, even steps. The dimming program operates by turning the LED on and off at a rate far higher than any animals' (possums and humans) 'critical flicker frequency' (CCF) (Signal, 2002). The CFF is the threshold frequency of an intermittent light source, defined as the point at which the light appears half the time as flickering and half the time as continuous. It is assumed in this study that the dimming program also operates at a rate far higher

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than a ferret's CCF. This means that when the light is flickering, it always appears still to the animal.

The program divides 0.025 of a second into 255 equal parts that can be individually turned on or off. To dim the light, the 'off' proportion of the time is increased and the 'on' proportion is decreased. This ensures that only a measured portion of the original (100% on) energy to be emitted from the LED, so it appears dimmer than previously. This method was used to dim the red test LEDs in gradual and controlled steps, while maintaining a still light.

### **Procedure**

The ferrets had already learned the required task, which was to press the lever under the lit-light. The schedule was the same as in the final stage of training (F-level-4): the middle lever must be pressed to start a trial; the reinforcement schedule was VR 1.5 ( $\pm 0.5$ ); reinforcement was semi-random (not repeating more than three consecutive times on one side); and a 'time-out' of 10 seconds followed an incorrect response. Experiments were conducted in a darkened room during the night-time cycle of the ferrets' reversed 12:12 hour night/day regime.

Each Red-dim session consisted of a single red LED in all three (left, centre and right) positions. All sessions began with the LEDs at full (100% on) brightness. Once the ferret had completed ten trials at this brightness level and achieved an accuracy of 60% or better, the intensity of the side LEDs were halved (meaning a flicker rate of 50% on). Nine dimming levels were tested by halving the intensity of the red LED nine times. Each ferret was advanced to the next dimming level only when it had achieved an accuracy of 60% correct responses or better at the current level. The final ten trials returned the brightness level to '100% on' to re-establish the learned task and to check that the ferrets were still performing the task as accurately as at the beginning of the session. This made a total 100 trials per session per ferret. The trial number, flicker speed, percent on and off, and relative intensity of each dimming level are presented in Table 7.

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Table 7.

Trial number, flicker speed, percent on and off, and relative intensity for each dimming level.

Trial number	Number of 'on' units (per 0.025 of a second)	Percent 'on' (%)	Percent 'off' (%)	Relative intensity
1-10	255.00	100.00	0.00	257.00
11-20	127.50	50.00	50.00	128.50
21-30	63.75	25.00	75.00	64.25
31-40	31.88	12.50	87.50	32.13
41-50	15.94	6.25	93.75	16.06
51-60	7.97	3.13	96.88	8.03
61-70	3.98	1.56	98.44	4.02
71-80	1.99	0.78	99.22	2.01
81-90	1.00	0.39	99.61	1.00
91-100	255.00	100.00	0.00	257.00

If a ferret could not complete 100 trials per session, then the number of intermediate dimming levels was reduced, so that the ferret could complete all the trials to the end of the second 100% brightness level. Red-dim training did not stop until each subject had experienced at least 40 trials with every dimming level *and* had met the pass criteria with the first and last dimming level ('100% on'). Performance at any dimming level less than '100% on' did not have to meet pass criteria.

#### Data Collection and Analysis

Data were collected automatically as in Chapter 2. Information recorded included: ferret identification; date; schedule of reinforcement; colour or dimming level of the LED, total trials per session; total number of left and right reinforcements over the session; number of correct left, correct right, incorrect left and incorrect right lever presses; percent correct lever presses over the session; and total time of

session. Data were analysed graphically and using the overall mean and 95% confidence intervals, as in Chapter 2.

## **Results**

Ferrets took between ten and 32 sessions to complete Red-dim training. Each ferret had at least 40 trials on each dimming level. Figure 7 Shows that the ferrets' overall accuracy decreased only slightly as the intensity of the left and right LEDs were halved nine times. A regression line shows a shallow linear decrease in response accuracy as intensity is reduced (slope = -1.17), The negative slope is highly significant ( $P < 0.000$ ), but all mean data points are still above 75%. The overall measure of how good the straight-line fit is ( $R^2$ ) = 90.3%. The overall mean accuracy for 100% intensity (100% on) was 87% ( $\pm 2.5$ ), and the overall mean accuracy for the dimmest intensity level (0.39% on) was 78% ( $\pm 4.0$ ), (see Table 8).

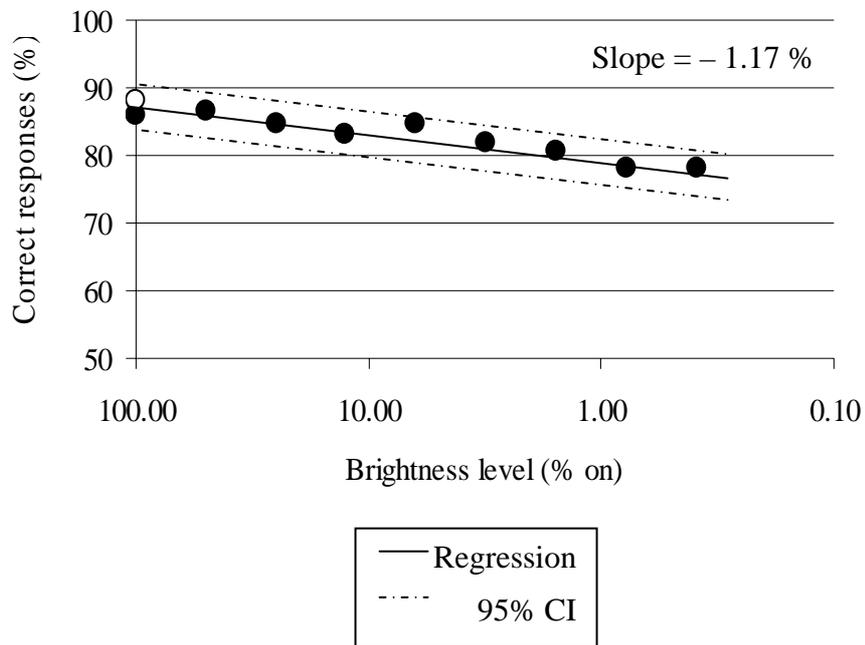


Figure 7.

Mean percentage of correct responding plotted against the nine descending dimming levels of the Red-dim program for all five ferrets (data pooled). The open circle (o) at the 100% brightness level represents the return to the '100% on' brightness level for the final ten trials. A regression line is shown with 95% confidence intervals and a slope of -1.17% over each of the nine decreasing brightness levels.  $R^2 = 90.3\%$ .  $P < 0.000$ .

**Table 8.**

**Flicker rate, relative intensity and overall mean accuracy and 95% confidence limits of lever pressing under a lit red light for five ferrets. Data is displayed over nine descending intensity levels, and then the LED is returned to 100% intensity.**

<b>Brightness (% on)</b>	<b>Relative Intensity of red LED</b>	<b>N (total number of trials)</b>	<b>Overall mean accuracy for all ferrets (%)</b>
100.00	257.00	950	86 ( $\pm$ 2)
50.00	128.50	400	87 ( $\pm$ 3)
25.00	64.25	370	85 ( $\pm$ 4)
12.50	32.13	370	83 ( $\pm$ 4)
6.25	16.06	560	85 ( $\pm$ 3)
3.13	8.03	530	82 ( $\pm$ 3)
1.56	4.02	790	81 ( $\pm$ 3)
0.78	2.01	626	78 ( $\pm$ 3)
0.39	1.00	386	78 ( $\pm$ 4)
100.00	257.00	650	88 ( $\pm$ 3)

## **Discussion**

Ferrets have been tested for generalisation over visual stimuli in an operant conditioning procedure in the past. Pollard et al. (1967) trained ferrets to discriminate between upright and inverted triangles, and showed that they could generalize over visual stimuli when some properties of the triangles were manipulated. Pollard's ferrets could transfer from filled to outlined figures, figures of reduced size and different painted colours. The results of this experiment suggest that ferrets are capable of generalisation over particular visual properties, without deterioration of performance of the task.

On the other hand, Chapter 2 of this study reports that moving the lever 80 mm to the left- or right-side LED position in the operant chamber could disrupt our ferrets' ability to perform the learned task. Pollard et al. (1967) also found that

their ferrets did not generalise over visual stimuli when irrelevant material was added. There is therefore a risk that other general response or stimulus properties (e.g. a change in stimulus wavelength or intensity) could disrupt the ferret's ability to perform the task.

The two experiments described in this chapter were designed to determine whether these ferrets could generalise between stimulus wavelengths and intensities. The next stage of the testing program will introduce a potentially invisible IR light, and it is critical to have established beforehand that the animals will not be confused by the change in stimulus, but will continue to perform the task if they can see the light.

### **Wavelength Detection**

During this experiment, the white test LEDS were replaced by LEDs emitting light of different colours, gradually moving towards the longer-wavelength spectrum. As peak wavelengths shifted from white to blue, green, yellow, orange and red, accuracy remained high (at or above pass criteria) for all colour conditions, except red (as shown by Figure 6 and Table 6). Overall, accuracy for the red colour condition (84%) did not reach the same level as in the other colour conditions (with overall mean accuracies between 89% (yellow) and 92% (blue, green and orange)). However, pass criteria were still met within six sessions, indicating that the ferrets could still see the red light most of the time.

Ideally, when changing wavelengths in an operant procedure, all other variables, including intensity, remain the same. This was not the case in this experiment, as all LEDs were presented at full brightness. The natural physical properties of the LED crystal when run with 20 volts mean that relative intensity differed between LEDs of different wavelengths with blue being the brightest, and red the dimmest (see Table 5). Procedures could have been introduced to manipulate intensity so that all coloured LEDs emitted the same intensity of light, but this was unnecessary here. The fact that the light stimuli were different in intensity as well as wavelength, and that the ferrets may have been detecting variation in brightness as well as colour, was not important. The results of this

experiment were not used to infer whether ferrets could 'see' the particular light wavelengths presented to them as different from each other, but rather to see whether they could detect them at all. If the ferrets could reliably detect the given light wavelength, then it is within their visual spectrum, whether they can see it as bright or dim; in colour or not.

The reason for the less accurate performance during the red condition may have been that the relative intensity of the red LED was less than that of the other coloured LEDs (shown in Table 5). It is unlikely that the lower accuracy in the red condition meant that the ferrets were unable to detect the red wavelength itself, as the ferrets could still respond with a high (>75%) level of accuracy, which would be impossible if the wavelength was not in their visible spectrum, no matter how bright the LED.

### **Colour Vision in Ferrets**

This study was not designed to test what colour ferrets can and cannot see. It was done merely to show that ferrets could transfer the task of pressing a lever under a white light to pressing a lever under a light of a different peak wavelength and/or intensity, as a necessary pre-requisite to testing IR wavelengths in Chapter 4.

There is some (very limited) evidence that ferrets can perceive the colour red as different from grey (Gewalt, 1959). However, Gewalt found no evidence that ferrets could perceive any other colours (blue, green and yellow) that were presented on coloured cards. In this study, coloured lights (blue, green yellow, orange and red) were presented to ferrets and they could detect all of the colours. However, this does not mean that they see the lights as coloured, as they may have been detected solely by their brightness compared to the unlit-LED. And even if the ferrets did detect some colour, it does not mean they would perceive those colours in any way that humans would recognise. Colour experience varies between different species and individuals, and humans can never truly appreciate what it is like to see the world as other species do (Lomas et al., 1998). There is still little evidence about the roles of color perception in non-human mammals, especially non-primates. The cones in a ferret's retina peak at 430 nm (violet) and

### Chapter 3: Stimulus Generalisation

558 nm (greenish-yellow); the greenish-yellow cones far outnumber the violet cones (14:1) (Calderone and Jacobs, 2003). Conversely, the cones in human eyes peak at 460 nm (blue), 530 nm (green) and 650 nm (red) (Jacobs, 1981), and human retinas contain many more cones than ferret retinas. Furthermore, visual perception depends not only on the interaction of light illuminating the retina, but also on the neural processing in the brain permitting light to be perceived as a certain image or colour. There is no reason why ferrets should experience the colours violet and greenish-yellow in the same way humans do.

Ferrets are nocturnal burrow-hunters (Alterio and Moller, 1997) and would have little use for colour vision when hunting underground. Colour vision may even be a disadvantage to nocturnal animals, because colour can obscure and overpower other visual properties of an object. Light and dark, shapes and movement are more easily perceived when the brain does not have to process additional un-necessary information about colour. For example, human colour vision extends almost to full darkness when time is allowed for full dark adaptation (Sjostrand, 2003), which might be expected to aid night vision. Yet colour-deficient war-time observers could penetrate camouflage that deceived the normal (colour-seeing) observer. Morgan et al. (1992) followed up this idea, and found that colour can interfere with segregation based upon texture. If visual acuity in ferrets is controlled in the same way, a ferret hunting in dim light may be better able to detect the shape and movement of potential prey without the perception of colour. Ferrets are not strictly nocturnal, because they are occasionally seen actively hunting during the day (Peach, 2005). The presence of cones in ferret retinas may merely enable daylight vision, regardless of whether they can perceive differences in colours of light to some degree.

#### **Light Intensity**

The Red-dim program tested the ability of ferrets to generalize their learned responses across decreasing levels of light intensity. This exercise also gave the ferrets experience in searching for a light that was more difficult to see. Although the red (635 nm) light was systematically dimmed so that the intensity halved nine

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times, all five ferrets easily transferred the learned task through the sequence of intensities, with high accuracy (between  $88\% \pm 3$  and  $78\% \pm 4$ ) even at the dimmest level (0.39% on). When the stimuli returned to 100% brightness for the second time in each session, their response accuracy increased to levels comparable to those at 100% brightness at the beginning of the session (see Figure 7, open circle data point). This suggested that the ferrets had not lost their ability to perform the learned task, and it was indeed the reduced light intensity that was responsible for any decrease in performance.

The ferrets' ability to achieve such high accuracy at low illumination may be attributed to the tapetum lucidum (the reflecting layer present in the ferret eye), which increases visual sensitivity in the dark by causing light to pass through the photoreceptors a second time (Tjalve and Frank, 1984). These results support the general claim that ferrets have better vision in the dark (Corbet and Southern, 1977), an asset ferrets could use to hunt above ground at night and in rabbit burrows at any time of day.

#### **Implications for Conservation**

The results of these experiments have important implications for conservation: Around dusk the light level changes rapidly, decreasing by about a log unit every 10 minutes (Lythgoe, 1979). Potential prey animals such as diurnal ground-dwelling birds may be at a visual disadvantage during this time of day. The ferret would be able to use the small amount of available light to see the bird, but the bird (with few rod photoreceptors and no tapetum lucidum) may not be able to see the ferret coming. This disparity could contribute to the predation of New Zealand's endangered ground-dwelling birds.

This wavelength and intensity information also has important implications for the choice of predator or prey monitoring equipment used for conservation. If ferrets can easily detect a low-intensity red light, then they can probably see the faint red glow emitted from some IR monitoring equipment used for conservation. Equipment with an IR light source that bleeds into the red spectrum is commonly reported (S. Cockburn, pers. comm., September 2006; Saunders & Maloney,

2002; Brown et al. 1998). This happens when (1) the IR wavelength used is too close to the visible spectrum (e.g. more than, but close to 700 nm); (2) The manufacturer of the IR equipment states a peak wavelength well into the IR spectrum, but the LED actually emits a wide range of wavelengths overlapping into visible red; or (3) an IR filter is used, which may degrade over time, allowing a small amount of red light to penetrate the filter. Any of these scenarios could risk detection of the equipment by ferrets, and potentially attract them to visit places where their attention is least wanted.

## **Conclusion**

It has been established that ferrets can generalise over different light wavelengths and intensities, with little to no disruption to the performance of their learned task: follow-the-lit-light. Now it is reasonable to assume that they could transfer the task to an IR light if it is visible to them. This possibility is investigated in Chapter 4.

## **Chapter 4**

### **Detecting Infrared Light**

#### **Abstract**

The two experiments in this chapter were designed to investigate whether five ferrets, already trained to press a lever under a lit-light of varying properties (see Chapters Two and Three), could continue to perform the follow-the-lit-light task when the test stimuli were changed from LEDs that emit ‘visible’ wavelengths to LEDs that emit supposedly invisible IR wavelengths. The experiments tested IR wavelength thresholds and, if applicable, intensity thresholds for the highest-wavelength IR light that was detected.

In the first experiment, several conditions (named Condition A to G) examined a series of gradually changing wavelengths and/or intensities of the test LEDs, while always keeping the central trial-starting LED at a known visible wavelength and intensity. In Condition A, a resistor was added to the three single visible red (635nm) LEDs to dim them slightly and aid dark adaptation in future conditions. In condition B, the central trial-starting LED remained red, and the test stimuli were changed to single IR (870 nm) LEDs. Two ferrets (Orion and Rex) continued to detect the lit-light with high accuracy ( $77\% \pm 4$  and  $72\% \pm 2$ ), indicating they could see IR light at this wavelength. When the red trial-starting light was also changed to a single IR (870 nm) LED (Condition F), Orion and Rex’s accuracy increased further, reaching a mean accuracy of  $84\% (\pm 1)$  and  $78\% (\pm 3)$  respectively. Following this, Orion was given a single IR (920 nm) LED (Condition G), but his response accuracy decreased to chance performance ( $48\% \pm 9$ ), indicating that this wavelength was not visible to him. Of the remaining three ferrets, Ajax could not reach the set pass criteria for single IR (870 nm) LEDs (Condition B), but could reach threshold criteria (continually achieving above 50%). Nero and Felix failed to continue the follow-the-lit-light

task with IR (870 nm) stimuli (Condition B), even when the LED's intensity was multiplied by six (Condition E).

The second experiment included only the two subjects (Orion and Rex) that had shown they could see a single IR (870 nm) light in all three positions with a high degree of accuracy (Condition F). Attempts were made to systematically dim the side (test) lights using the Red-dim program (described in Chapter 3) to obtain an intensity threshold. However, this failed after the ferrets stopped completing enough trials, even on the brightest (full intensity IR 870 nm) level. The reason for this was not known, as both ferrets could perform this task (with a full intensity IR LED) in Condition F prior to adding the Red-dim program. However, the fact that some of the ferrets could detect the IR LEDs prior to adding the Red-dim program was well established and has important implications for conservation.

## **Introduction**

### **IR Light Used in Conservation Projects in New Zealand**

IR light is commonly used to light up areas of conservation interest at night, and to record predator or prey behaviour (Brown et al., 1998; Innes et al., 1996; Laurance and Grant, 1994; Sanders and Maloney, 2002; Savidge and Seibert, 1988). Most experimenters that use IR light for this purpose do not publish the spectral properties of the light emitted from their equipment. Only two published papers from New Zealand, by Innes et al.(1994) and Johnston et al. (2003), state the peak wavelengths (reported to be between 830 nm and 950 nm) emitted from the IR equipment used in their experiments.

Even if the peak IR wavelength is stated, ‘bleeding’ into the red spectrum is not usually measured or properly considered. For example, a light source consisting of IR LEDs with peak wavelengths of 870 nm, may in fact emit wavelengths that range from 670 to 900 nm, including a large amount of red light. Conservation devices with IR LEDs that bleed into the red range of wavelengths are commonly used in New Zealand (Cockburn, pers. comm., September 2006). This is an indication that the IR light source is not only emitting IR light, but visible red and/or orange light also. Table 9 summarises the details of the conservation projects mentioned above, including: the type of IR equipment; purpose of its use; peak wavelength and range (if known); and any reports of visible light being emitted.

**Table 9.**

**Summary of published material from New Zealand involving the use of IR equipment where either the spectral properties of the IR source or bleeding of red light are reported.**

<b>Purpose of use of IR equipment</b>	<b>Type of IR Equipment</b>	<b>Peak IR Wavelength (nm)</b>	<b>Visible light bleeding from IR source?</b>	<b>Reference</b>
Detecting predators at Kokako nests.	Clusters of 24-36 LEDs and active laser beam	Cluster: 880 Laser: 830	Yes; described as faint small orange and red glows.	(Innes et al., 1996)
Predator and parent bird observations.	Clusters of LEDs, laser diodes and collimated laser beams	Not reported	Yes; described as a faint red glow.	(Brown et al., 1998)
Identifying the cause of mortality of ground nesting birds.	Cluster of 30-48 LEDs	Not reported	Yes; described as a dull red glow.	(Sanders and Maloney, 2002)
Monitoring bird and predator behaviour.	Cluster of five LEDs	950 nm (spectral bandwidth of 55 nm)	None reported.	(Johnston et al., 2003)
Supplying and installing IR equipment for D.O.C, New Zealand.	Large white spotlights with IR filters from 'Lightforce', model SSH415U	870 to 950 nm	Yes; described as a red glow visible in the dark.	Cockburn, pers. comm., September 2006

### **Variables That May Affect a Ferret's Ability to See IR Light**

It is commonly assumed, but not known for sure, that most mammalian predators (including ferrets) cannot detect IR light. However, there are several problems with this assumption: (1) The exact spectral sensitivity thresholds for ferrets are not known. (2) The eyes of ferrets are structurally different from human eyes because ferrets have a tapetum lucidum, increasing visual acuity in dim light. (3) IR equipment that specifies discharge of a certain peak wavelength can still emit a wide range of wavelengths. (4) Filters and IR LEDs are known to degrade over time, and then they emit proportionately more light that contains visible wavelengths (S. Cockburn, pers. comm., September 2006). (5) If the intensity of the IR light is increased (e.g. when run at high voltages), then proportionally more visible light is released through an IR filter (S. Cockburn, pers. comm., September 2006). These variables increase the potential risk of IR equipment used for conservational purposes in New Zealand becoming visible to ferrets.

### **Measuring Light Detection**

There are diverse criteria for proving light detection in animals. In this thesis, the criterion required to prove that the subject has detected a light was always set at an accuracy of 75%. However, an animal that responds with an accuracy of between 50 and 75%, can probably still detect the light, although with lower accuracy. This could mean that the animal is at its 'threshold' of light detection. An absolute threshold is the lowest level of stimulation that can be detected. For example, in an experiment on sound detection, a sound with varying levels of volume may be presented. The lowest level that a subject is able to hear is the absolute threshold (Heffner and Heffner, 1985; Kelly et al., 1986). Stimuli with intensities below the absolute threshold are not detectable, and those above are detectable. However, stimuli at values close to the threshold will often be detectable some proportion of the time, but not all the time. Consequently, a threshold is defined as the point at which a stimulus is detected on some predetermined proportion ( $p$ ) of all possible occasions often represented by a  $p$  level of 50% or higher.

## **Aims and Objectives**

This experiment was designed to assess whether the visual detection of ferrets extends into IR light wavelengths (experiment 1) and if so, at which intensities (experiment 2). To investigate these aims, the objectives were to: (1) source various IR LEDs and use a spectrometer to record their spectral properties; (2) test the detection by ferrets of IR light wavelengths, by observing the effect on the ferrets' previously established accuracy in a follow-the-lit-light task when presented with 870 and 920 nm LEDs; (3) if an IR wavelength is visible to a ferret, establish the intensity thresholds for that wavelength.

## **Experiment 1**

### **Infrared Wavelength Detection**

#### **Methods**

##### **Subjects and Husbandry**

This experiment used the same five subjects (Nero, Felix, Orion, Rex and Ajax) and husbandry as described in Chapter 2 and Three.

##### **Experimental Apparatus**

This experiment used the same experimental apparatus employed in Chapter 2 and Three (with the liquid dispenser) except that a resistor was added to the red (635 nm) central trial-starting stimulus to reduce the intensity. The resistor increased the resistance in the circuit by 1000 ohms, reducing the voltage to the red LED. This was done because these ferrets had already shown that they could see low intensity red lights (Chapter 3) and because it was suspected that if the central trial-starting light was left too bright, it might affect the ferrets' ability to see the relatively less bright side (test) stimuli. Test stimuli were full intensity IR LEDs of either 870 nm or IR 920 nm. These IR LEDs were the closest available to the red wavelength, which ferrets could certainly see (Chapter 3). Table 10 shows the spectral properties of each LED as specified by the manufacturer and measured with a spectrometer. Intensity was measured relative to the blue LED as in Chapter 3.

**Table 10.**  
Spectral properties for each LED including: the visible colour, peak and range of wavelengths and relative intensity of the light emitted from each LED.

<b>Visible colour of light</b>	<b>Peak wavelength as specified by manufacturer (nm)</b>	<b>Peak wavelength as found by spectrometer (nm)</b>	<b>Range of wavelengths as found by spectrometer (nm)</b>	<b>Relative intensity as measured against the blue LED (3292)</b>
Red	635	635	590-710	257
IR 870	870	871	800-910	212
IR 920	920	956	870-1120	70

### **Procedure**

The ferrets' task was the same as in the previous experiments: press the lever under the lit-light. The procedure involved the same schedules as in previous experiments; the middle lever must be pressed to start a trial; VR 1.5 (one reinforcement received on average after every 1.5 correct responses); semi-random reinforcement (not repeating more than three consecutive times on one side); and a 'time-out' after incorrect lever presses of ten seconds. As in Chapters Two and Three, experiments were conducted in a darkened room during the night-time cycle of the ferrets' reversed 12:12 hour nigh/day regime.

In addition to these procedures, an 'abort program' was introduced, whereby if a ferret did not respond within 60 seconds, the trial would be aborted and the next trial would begin. This was necessary because in some conditions (explained below) multiple LEDs were wired in parallel, increasing the chance of an LED burning out. The abort function ensured the LEDs were not left on too long, and lessened the possibility of any LEDs burning out.

All ferrets began with a single, red (635 nm) full intensity (relative intensity of 257) LED in all three (left, centre and right) positions (named Condition A). Once a ferret had met the pass criteria for Condition A, test stimuli were replaced with single IR (870 nm) full intensity (relative intensity of 212)

LEDs (Condition B). Depending on the results for each ferret after Condition B, the following conditions (named Conditions C to G) were presented. Table 11 describes the LED arrangement, number of LEDs used, and wavelength for each of condition A to G.

Once the IR (870 nm) LEDs had been introduced (Condition B), one of three scenarios could be observed: (1) the ferret's accuracy could drop to chance levels (falling into the fail criteria category), indicating it could not detect the IR light; (2) the ferret's accuracy could drop sufficient to indicate that it could detect the IR light most of the time (falling into the threshold criteria category), but with a low degree of accuracy (>50 but <75%); or (3) the ferret's accuracy could remain high (falling into the pass criteria category), indicating that it could detect the IR light most of the time with a high (>75%) degree of accuracy. These three scenarios, and the course of action that followed, are described below.

**Table 11.**  
Arrangement and description of each condition during operant conditioning sessions with IR lights.

Condition	LED arrangement (left; centre; right)	Description
A		All (left, right and centre) stimuli are single red (635 nm) LEDs.
B		Left and right stimuli are single IR (870 nm) LEDs; the centre stimulus is a single red (635 nm) LED.
C		Left and right stimuli are three IR (870 nm) LEDs; the centre stimulus is a single red (635 nm) LED.
D		Left and right stimuli are six IR (870 nm) LEDs; the centre stimulus is a single red (635 nm) LED.
E		All (left, right and centre) stimuli are six IR (870 nm) LEDs.
F		All (left, right and centre) stimuli are single IR (870 nm) LEDs.
G		Left and right stimuli are single IR (920 nm) LEDs; the centre stimulus is a single IR (870 nm) LED.

***No IR Detection***

If the ferret cannot detect the IR (870 nm) light, then a decrease in accuracy of its performance of the task to chance levels would be expected. If the ferret's accuracy remains at or close to chance levels for at least ten sessions, then either: (1) the ferret could not detect that wavelength; or (2) the ferret could potentially detect the wavelength but not at the current intensity. To test that second

possibility, intensity was systematically increased by introducing more IR LEDs. The conditions were changed in the sequence: B→A→C→A→D→A→E→A, as follows:

- If Condition B resulted in chance levels of performance, then the ferret was returned to Condition A (all red LEDs) to be sure it could still perform the task with visible lights before the IR spectrum could be re-explored.
- Once pass criteria were again met in Condition A, then the ferret progressed to Condition C (three times the IR intensity). If there was still no light detection after ten consecutive sessions, then the ferret was returned to Condition A again until pass criteria were met.
- Once pass criteria were once again met for condition A, then the ferret progressed to Condition D (six times the intensity). If there was still no IR light detection after ten consecutive sessions, then Condition A was returned to again until pass criteria were met.
- Finally, the ferret was tested under Condition E, to assess whether the bright red centre LED was affecting the brightness adjustment in the ferrets eyes. If there was still no IR detection, then it was concluded that that ferret probably could not detect the IR LED. Condition A was returned to once again to confirm that the ferret still had the ability to perform the task.

#### ***Threshold IR detection***

If performance in Condition B (single IR 870 nm test stimuli) did not meet the set pass criteria, but was still above chance levels (>50%) for at least ten sessions, then the ferret could still see the IR light some of the time. Such a result suggested that this light could have been near the ferret's threshold for IR light detection at the current wavelength and intensity. If so, an increase in intensity should result in an increase in accuracy. Conditions were changed to increase intensity as per the sequence for 'no light detection' above (B→A→C→A→D→A→E→A).

***Positive IR detection***

If the set pass criteria were met in Condition B (with the single IR 870 nm LEDs), then the ferret could already see the IR light at the current wavelength and intensity. To test whether it could possibly see IR light of a longer wavelength, or decreased intensity, the stimulus conditions were changed in the sequence: B→F→G, as follows:

- Condition B was first followed by Condition F (to decrease any glare from the relatively bright red light).
- If the set pass criteria were met during Condition F, then the ferret progressed to Condition G, which investigated whether an even higher IR (920 nm) wavelength could be detected.
- If an IR LED was detected, then the ferret could go onto experiment 2, the IR dimming experiment.

**Criteria for ‘Seeing’ the Light**

In this experiment there were three categories of criteria: (1) ‘pass’ (2) ‘fail’ and (3) ‘threshold’.

***Pass criteria***

The criteria used to define a ‘pass’ required an accuracy of at least 75% over four consecutive (or five out of six) sessions, with a minimum of 30 trials per session. Sessions in which ferrets completed fewer than 30 trials were generally not included, due to the risk of skewed data. However, if a subject repeatedly completed fewer than 30 trials per session, then allowances were made to pass that animal provided: (1) at least ten consecutive sessions were undertaken; and (2) an accuracy of at least 75% was still achieved.

This procedure accepts as almost certain that, if an animal can reach the 'pass criteria' for a given light wavelength, that animal can see the light. This is because it is highly unlikely that a subject could meet pass criteria by chance.

### ***Fail criteria***

A score centring around 50% during at least ten consecutive sessions was interpreted to indicate that a given animal may be unable to discriminate a lit-light from an unlit-light. If that animal showed signs of improving in accuracy, then more sessions were carried out until no trend was observed. No minimum number of trials per session could be enforced because the ferrets generally completed few trials when stimuli were difficult to see.

This procedure accepts the fact that an animal failing to demonstrate that it can see a given light wavelength has not proven beyond a doubt that it cannot see that wavelength. The animal may choose not to respond, or choose to respond incorrectly.

### ***Threshold criteria***

Occasionally subjects did not achieve an accuracy of 75%, but their accuracy was consistently above chance levels (more than 50%). This indicated that the light frequency was difficult to detect but was detected some of the time, perhaps because it was at or near the subject's threshold of light detection. If an animal met 'threshold criteria', but not 'pass criteria', then the animal was considered to be at or near its threshold for light detection, and was not advanced to the next wavelength of IR.

### **Data Collection and Analysis**

Data were collected automatically as in Chapter 2 and Three. Information recorded included: ferret identification; date; schedule of reinforcement; wavelength and number of LEDs, total trials per session; total number of left and right reinforcements over the session; number of correct left, correct right,

incorrect left and incorrect right lever presses; percent correct lever presses over the session; and total time of session. Data were analysed graphically and using the overall mean and 95% confidence intervals, as in Chapter 2 and Three.

## **Results**

The ferrets took between 46 and 92 sessions to complete the IR experiment. All ferrets completed at least 30 trials per session when all three LEDs were red (Condition A). However, Nero, Felix and Ajax often completed fewer than 30 trials per session in other Conditions (see Figure 8).

### *Nero and Felix – IR detection unlikely*

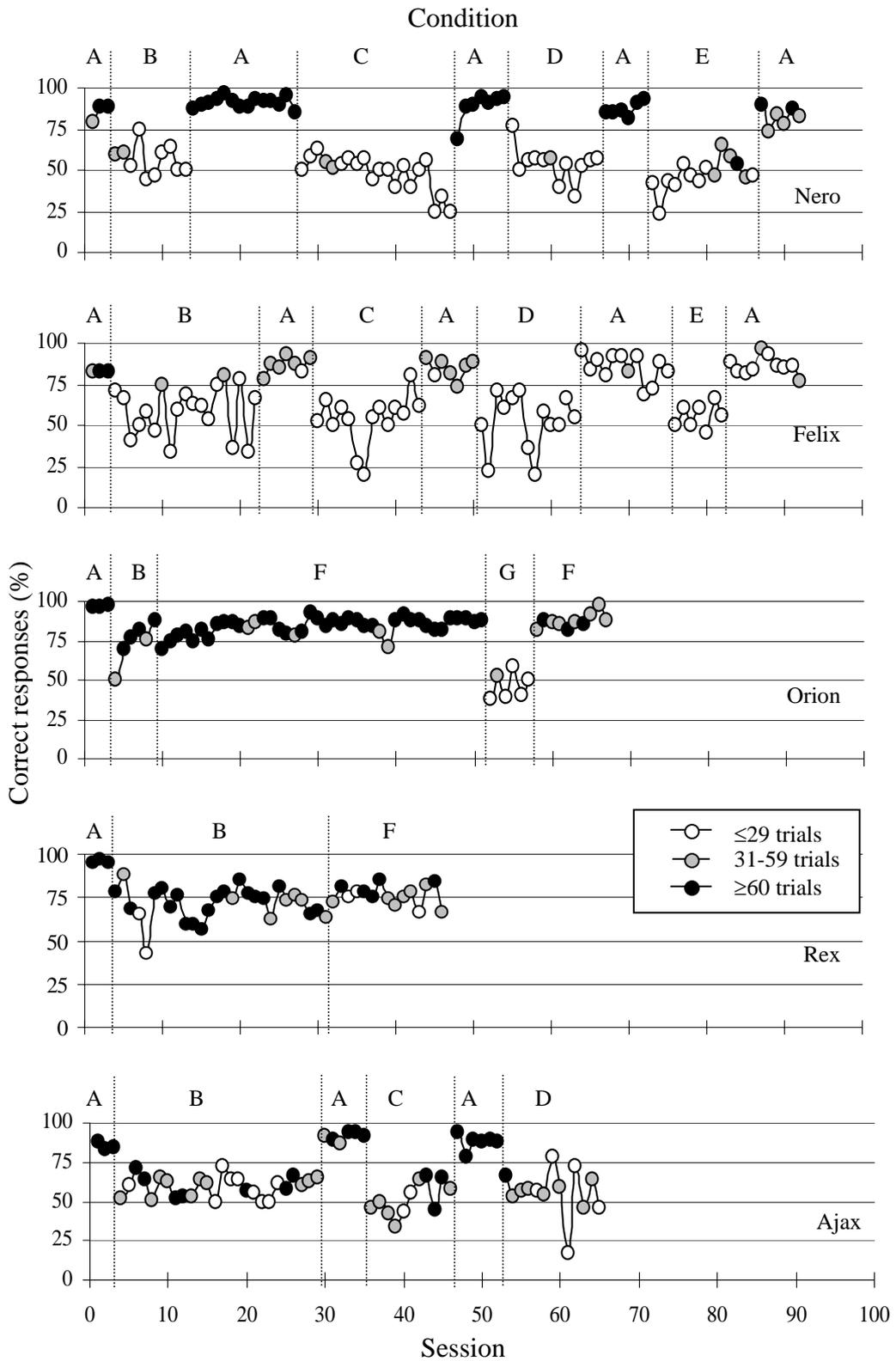
When the test LEDs were changed from red (a wavelength proved to be visible to all five ferrets in Chapter 2), to IR 870 nm (Condition B), Nero and Felix's accuracy fell to near chance levels ( $57\% \pm 7$  and  $63\% \pm 5$  respectively) (see Figure 8 and Table 12). When condition A was re-established, the pass criteria were met immediately, indicating that these two ferrets still had the ability to perform the task, and the single IR 870 nm LEDs were not visible to them.

The inability of these two animals to respond to the IR 870 nm LED could have been either because they could not detect that wavelength (870 nm) or because they could not detect 870 nm at that intensity (a relative intensity of 212). No IR LED intermediate between IR 870 nm and red (635 nm) was available, but the intensity of the 870 nm LED could be increased simply by adding more LEDs in clusters of three (Condition C) and six (Condition D). The red trial-starting LED remained because it was still the last LED proven to be visible to these ferrets. Increasing the intensity of the IR light had little effect on Nero and Felix's accuracy, which remained around chance levels ( $55\% \pm 7$  and  $51\% \pm 9$  respectively).

## *Chapter 4: Detecting Infrared*

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In case the red central trial-starting LED was causing glare and affecting the ferrets' ability to detect the IR LED, it too was changed to a cluster of six IR 870 nm LEDs (Condition E). Again, there was little to no change in Nero and Felix's accuracies, and their performance remained near chance levels (50%  $\pm$ 5 and 54%  $\pm$ 12 respectively) (see Table 12). Red (635 nm) was still the last light wavelength proven to be visible to Nero and Felix.



**Figure 8.** Mean percent correct responses plotted against session number (lower x-axis) for each stimulus condition, for each ferret. The key shows how many trials were completed per session.

### ***Orion and Rex – positive IR detection***

Orion maintained a high level of accuracy ( $77\% \pm 4$ ) after the test LEDs were changed to single IR 870 nm (Condition B). Orion met the set pass criteria most of the time, indicating he could see the single IR 870 nm LED (see Figure 8 and Table 12). To minimize any potential glare from the single red central trial-starting LED, it too was changed to a single IR 870 nm LED (Condition F). Accuracy remained high (mean  $84\% \pm 1$ ) and stable for the following 42 sessions.

For Condition G, the central trial-starting LED remained at 870 nm (the last known visible wavelength) and the side (test) LEDs were increased to 920 nm. Orion's accuracy dropped to near chance levels ( $48\% \pm 9$ ) indicating that the 920 nm LED was invisible to him.

No intermediate IR LED between 920 and 870 nm was available. Condition F was re-established to ensure Orion had not lost the task. Orion was now ready for 'IR-dimming' sessions to find the intensity threshold for the 870 nm wavelength (experiment 2).

Rex maintained a high accuracy when initially presented with the IR 870 nm LED, but subsequently decreased in accuracy after two sessions (see Figure 8). Accuracy met pass criteria ( $>75\%$ ) some of the time, and averaged  $72\% (\pm 2)$  over 29 sessions. After the single red central trial-starting LED also changed to a single IR 870 nm LED (Condition F), mean accuracy increased to  $78\% (\pm 3)$ .

Rex did not go on to Condition G (IR 920 nm) due to time restrictions, but he was declared ready for IR-dimming sessions to find the intensity threshold for the 870 nm wavelength (experiment 2).

### ***Ajax – threshold IR detection***

When the test LEDs were changed to IR 870 nm (Condition B), Ajax's accuracy decreased below the set pass criteria ( $<75\%$ ), but did not fall below chance levels ( $>50\%$ ) (see Figure 8 and Table 12). Average accuracy for Condition B over 26 sessions was  $60\% (\pm 3)$ , indicating that Ajax could see the IR 870 nm light most of the time. When the side (test) LEDs were returned to red (635 nm), his accuracy increased to meet the set pass criteria again, indicating that, given a red

light, Ajax could still perform the task with a high degree of accuracy, but not with the IR 870 nm stimuli.

If Ajax could see the IR 870 nm light some of the time, then an increase in intensity of the 870 nm stimuli should increase his accuracy in the follow-the-lit-light task. This was not the case, because when the intensity of the stimuli was increased (Condition C and D), Ajax's accuracy decreased to near chance levels ( $54\% \pm 5$  and  $57\% \pm 5$  respectively) and fewer trials were completed per session (see Table 8 and Figure 12).

**Table 12.**  
**Mean percent correct responses and confidence limits for each ferret under each condition.**

	<b>Mean correct responses (%) for each condition</b>						
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>
<b>Nero</b>	89 ( $\pm$ 1)	57 ( $\pm$ 7)	52 ( $\pm$ 1)	55 ( $\pm$ 7)	50 ( $\pm$ 5)	NA	NA
<b>Felix</b>	84 ( $\pm$ 2)	63 ( $\pm$ 5)	55 ( $\pm$ 1)	51 ( $\pm$ 9)	54 ( $\pm$ 12)	NA	NA
<b>Orion</b>	97 ( $\pm$ 2)	77 ( $\pm$ 4)	NA	NA	NA	84 ( $\pm$ 1)	48 ( $\pm$ 9)
<b>Rex</b>	96 ( $\pm$ 3)	72 ( $\pm$ 2)	NA	NA	NA	78 ( $\pm$ 3)	NA
<b>Ajax</b>	88 ( $\pm$ 2)	60 ( $\pm$ 3)	54 ( $\pm$ 5)	57 ( $\pm$ 5)	NA	NA	NA
<b>Overall Accuracy (%)</b>	91	66	54	54	52	81	48
<b>N (total no. trials)</b>	6220	4188	1698	786	542	2978	117

## **Experiment 2**

### **Infrared Dimming**

#### **Methods**

##### **Subjects and Husbandry**

The same husbandry was used as in Chapter 2 and Three. However, the only subjects used in this experiment were the two ferrets that had met the set pass criteria for seeing an IR (870 nm) light (Orion and Rex).

##### **Experimental Apparatus**

This experiment employed the same experimental apparatus (with the liquid food disperser) used in Chapters Two and Three. The difference here was that all three stimuli inserted into the operant chamber (one each for the left, right and centre position) were single IR (870 nm) LEDs. This wavelength was chosen because it was the highest wavelength that the ferrets could detect (see experiment 1 above).

The Red-dim program (described in Chapter 3) was re-activated for this experiment. Although the computer program worked in exactly the same way as in Chapter 3, it was renamed 'Ired-dim' because it was used in conjunction with IR LEDs instead of red LEDs.

##### ***Ired-dim program***

In the Ired-dim program, the 870 nm LED was systematically dimmed to reach an intensity threshold (as was done with the red (635 nm) LEDs in Chapter 3). Each Ired-dim session used a single IR LED in all three (left, centre and right) positions. All sessions began with the LEDs at full ('100% on') brightness. Once a ferret had completed ten trials at this brightness level and reached the set pass criteria, the intensity of the side LEDs was reduced. In the previous Red-dim experiment (with the red (635 nm) LEDs in Chapter 3), the intensity was halved

every dimming step. But because the IR LEDs are already at a lower intensity than the red LEDs (the relative intensity of IR 870 nm was 212 and the red was 257), the Ired-dim program dimmed the lights in much smaller steps. Systematic dimming by 30 'on' units for each dimming level produced a linear and more gradual decrease in intensity.

This experiment called for eight dimming levels to be tested: 255, 195, 165, 135, 105, 75, 45 and 15 'on' units. Each ferret was to be advanced to the next dimming level only when it had achieved an accuracy of 60% correct responses or better at the current dimming level. If a level was failed (a mean accuracy of less than 60% over at least 40 trials), the previous level was to be reintroduced and then intermediate levels would be trialled until a threshold was met. The final ten trials in each session were to return the brightness level to '100% on' (as in the Red-dim program) to re-establish the learned task and to check that the ferrets were still performing the task as accurately as at the beginning of the session. This would make a total of 90 trials per session per ferret.

No minimum number of trials per session was imposed, and if a ferret could not complete 90 trials per session, then the number of intermediate dimming levels was reduced, so that the ferret could complete all the trials to the end of the second 100% brightness level. Ired-dim trials did not stop until each subject had experienced at least 40 trials with every dimming level *and* had met the pass criteria (explained in experiment 1 above) with the first and last dimming level ('100% on'). Any dimming level less than '100% on' did not have to meet pass criteria because it was not known if this level was even possible for a ferret to see. The standard pass criteria, fail criteria and threshold criteria were explained in experiment 1 above.

## **Results**

No results could be obtained for this experiment. Both ferrets stopped lever pressing for food rewards as soon as the Ired-dim program was added to the procedure. The procedure included a check to ensure this result was not because they could not see the lights. The first and last ten trials of the dimming program presented IR (870 nm) lights at 100% brightness, which they could certainly see before, but the ferrets would no longer lever press at 100% brightness.

There is no obvious explanation why neither ferret would lever press for food rewards during the Ired-dim experiment. Because both ferrets had been achieving high accuracy for the 100% brightness level before the Ired-dim program was added (see experiment 1, Figure 8, condition B), a similar participation and accuracy level would be expected at least for the 100% brightness level. After 12 sessions with Orion and 34 sessions with Rex (yielding no results for either ferret), this experiment was cancelled.

## **Discussion**

### **The Problem of Dark Adaptation**

When presented with Condition B (left and right stimuli are single IR (870 nm) LEDs; centre stimulus is a single red (635 nm) LED), two out of the five ferrets (Orion and Rex) showed clear evidence of IR (870nm) light detection with a mean accuracy of 77% ( $\pm 4$ ) and 72% ( $\pm 2$ ). The other three ferrets' accuracies varied from 57% ( $\pm 7$ ) to 63% ( $\pm 5$ ) (Table 12).

The problem with Condition B was that, even with the added resistor, the central red trial-starting LED was probably too bright, inhibiting the ferrets' ability to see the relatively dimmer IR side lights. In all mammals, the process of dark adaptation dilates the pupil of the eye, improving night vision but opening the possibility of retinal damage if darkness is not maintained. The time for this process to be completed in ferrets' eyes is not known, but human eyes take about 40 minutes to become fully dark adapted. Once dark adapted, a single flash of a bright light can bleach the retinal pigments, preventing an immediate return to the fully dark-adapted state. In this experiment, the central trial-starting light had to remain red if it was the last wavelength proven to be detected by the ferret. The ferrets were required to press the lever under the red central trial-starting light, and then, while their pupils were still relatively dilated, immediately make a left or right choice.

The effect of this problem was demonstrated by Orion and Rex. Both had shown they could detect the IR (870 nm) LED under Condition B, so the central trial-starting LED was changed to an IR (870 nm) LED (Condition F). Orion's accuracy increased by seven percentage points to 84% ( $\pm 1$ ), and Rex's by six points to 78% ( $\pm 3$ ). This observation is consistent with the hypothesis that the brightness of the central red light was affecting their ability to detect the relatively dimmer side LEDs.

### **Experiment 1: Range of Detectible IR Wavelengths**

Only one of the ferrets that could give evidence of seeing the 870 nm light (Orion), progressed to Condition G (single IR 920 nm LEDs in all three (left, mid

and right) positions). Orion's accuracy dropped to chance levels, indicating that this light was invisible to him. It was later found that, although the manufacturer stated the LED emitted a peak wavelength of 920 nm, the actual peak wavelength was 956 nm (as measured by a spectrometer). This test therefore used a wavelength further into the IR spectrum than was intended, and which was, not surprisingly, invisible to the ferret.

Under Condition B (single test LEDs of 870 nm), Ajax scored a mean of 60% ( $\pm 3$ ) over 26 sessions, but every individual score was above chance levels. This was insufficient evidence to pass him on to the next level of IR light (920 nm). Ajax was instead tested with the same peak wavelength (IR 870 nm), but the intensity of the test LEDs was increased by adding more (three and then six) LEDs (Conditions C and D). Because the pass criterion (average of 75%) was not met, the central trial-starting LED remained red (635 nm). If Ajax could see IR light at all, an increase in IR intensity should have correlated with an increase in accuracy. However, the very opposite was observed: his mean accuracy actually decreased by three to six percentage points (see Table 12).

The most obvious explanation for this response is that the red central-trial-starting LED was causing a problem related to dark adaptation. The intention was to direct Ajax on to Condition E, in which all (left, right and centre) stimuli were six IR (870 nm) LEDs). Unfortunately Ajax developed leukemia (a cancer common in young adult ferrets) and retired from lever-pressing for food rewards. An alternative explanation could be some unidentified change in motivation. For example, if the IR lights at increased intensity actually hurt Ajax's eyes, or if their light was difficult for Ajax to see and the time-out for incorrect responses too frustrating, he might have decided that the reinforcement was not worth the effort of performing the task. There is no way to evaluate these possibilities from these data.

Nero and Felix did not show any signs of IR detection with single IR (870 nm) LEDs, as their accuracy remained near chance levels ( $57\% \pm 7$  and  $63\% \pm 5$  respectively) (see Table 12). Felix achieved a higher *mean* accuracy than Ajax, but many of the data points for Felix fell below chance levels (see Figure 8), indicating that if he had any ability to detect the IR light at all, it was marginal and inconsistent.

Increasing the number of IR LEDs to three and then six (Conditions C and D) made virtually no difference to Nero and Felix's discriminative performance. Neither did changing the central trial-starting stimulus from a single red (635 nm) LED to six IR (870 nm) LEDs (Condition E). Therefore, either they could not detect an IR 870 nm wavelength, or the intensity of the six IR LEDs continues to be too low. Because the tests were conducted within the confined space of the ferrets' home cages, there would be a risk of damaging the ferret's eyes with multiple full-intensity IR radiation presented at close range, so the effect of brightness was not explored further by adding more IR LEDs. It was concluded that Nero and Felix probably could not see the light emitted from the IR (870 nm) LEDs.

### **Experiment 2: IR Dimming**

After a wavelength threshold had been established for each ferret, the ferrets that could detect IR light (Orion and Rex) should have progressed to an IR dimming program to establish an intensity threshold for the IR (870 nm) LED (using the same procedure as in Chapter 3). Unfortunately, participation in a 'free' operant procedure is voluntary and the ferrets could not be forced to work if they chose not to. When Orion and Rex were moved on to the IR dimming program, they simply stopped participating. Even at the highest brightness level (100% on), they did not complete enough trials to determine a result, even though both ferrets had been readily working with this exact same stimulus prior to the addition of the IR dimming program.

It was unlikely that there was anything wrong with the dimming program itself, because the ferrets participated in the same task with the exact same program with a red LED (Chapter 3). The lack of responses was not necessarily due to the ferrets being unable to detect the IR (870 nm) light, because they were both detecting the light with high accuracy in experiment 1. One possible suggestion for their lack of participation could be that, as intelligent animals, they quickly learnt that the stimuli presented to them during dimming trials would become increasingly difficult or impossible to see. It would therefore take a lot more effort to obtain food reinforcements, and many more aversive consequences would be issued after mistakes. The ferrets could instead just wait for their daily

food ration and skip working for food altogether. Depriving them of food for longer periods did not improve their motivation to work for food rewards, and the experiment stopped for a two-week break. Subsequent attempts at IR dimming also failed. It became apparent that no intensity threshold data for IR light could be obtained using this method with these ferrets.

### **Implications for Conservation**

These experiments provide very strong evidence of individual variation in the range of visual perception among ferrets. In at least some individuals, light detection extended well over the threshold into IR wavelengths below 870 nm, which are usually assumed to be invisible to mammals. This means that at least some proportion of wild ferrets can see the light emitted from some IR equipment used for conservation in New Zealand. Equipment that uses relatively low peak IR wavelengths or that bleeds into the red or orange spectrum such as reported by S. Cockburn, (pers comm., September 2006), and Sanders & Maloney (2002); Brown et al. (1998); and Innes et al. (1994) will be at particular risk of being detected. Many other conservation projects besides those listed here probably use such equipment, but have not realised the importance of reporting this information.

When IR equipment is used in outdoor areas for conservational purposes, wild ferrets may be able to see, not only the LEDs themselves (usually arranged in multiple clusters), but also, the pool of light that they create (usually aimed at a nest). In this study, Orion and Rex were looking directly at single IR LEDs from a short distance (approximately 10 cm). The light emitted from the IR LEDs and reflected off natural surfaces is comprised of the same wavelengths as are emitted from the source of light. The only difference is that, as the light rays scatter away from the light source, the light intensity decreases. There is therefore no reason, (given a high enough intensity or a short enough distance), that at least some wild ferrets could not see the multiple patches of reflected IR light that may be present in New Zealand's conservation areas at night.

Since at least some captive ferrets and possums (Vanstone, 2006) could potentially see the light emitted from IR equipment at night time, and if this

ability is also present in wild ferrets and possums, we might expect some evidence of a reaction of wild animals towards the equipment. The lack of reported evidence of this effect may simply be that most experimenters do not notice or comment on it, or else they interpret their observations in other ways, on the assumption that animals cannot see IR light. For example, Sanders & Maloney (2002) maintained this view because predators usually approached the nests rather than the lights, even though some of their video footage showed predators looking towards the IR camera lights, and one possum investigated their video equipment.

On the other hand, Prout (2003) reported significantly fewer visits by ship rats to tunnels with IR light-gates compared with tunnels set in the same positions with no IR light-gates. Prout speculated that the rats may have been able to see the IR beams inside the tunnel and avoided entering. In response to direct questioning, S. Cockburn, (pers. comm., September 2006) quoted further anecdotal evidence implying the same conclusion. Cockburn has extensive experience of using and maintaining IR monitoring equipment for DOC. When flood-lights with IR filters were positioned above monitored nesting sites, possums and rats were reported to wait on the edge of the IR light field and to move quickly in and out of the lit area, suggesting they could detect it to some degree.

Few studies have measured the effect that IR monitoring might have on the behaviour of mammalian predators, and those that did so treated it as a secondary aim, not fully explored. For example, the primary aim of the five years' monitoring by Sanders and Maloney (2002) was to investigate the cause of mortality at nests of ground-nesting birds in the Upper Waitaki Basin in the South Island of New Zealand. They reported that, for the first two years, their equipment emitted IR light plus visible (to humans) red light, and for the final three years it did not. They named these two conditions 'visible IR' and 'invisible IR' respectively, and found no significant differences between predation rates under each condition ( $P = 0.64$ ). However, the two conditions were not tested simultaneously, so their results are not directly comparable. More importantly, they could not exclude the possibility that the predators (with the aid of their dark-adapted eyes and tapetum lucidum) could detect the lights under both conditions equally well, but chose to ignore them both, hence no difference in predation rates between the two conditions was found. Sanders and Maloney concluded that the

IR equipment had no effect on predation rates, although they did not rule out the possibility that the use of video cameras may have biased their results by influencing the behaviour of the birds, the predators or both.

The data reported in this chapter have important implications for conservation. When the data for all five ferrets were pooled, the response accuracy towards a single IR (870 nm) test LED (Condition B) averaged 66% correct responses from a total of 4,188 trials, which exceeded chance levels, but did not reach the set pass criterion of at least 75% (Table 12). This was because two ferrets (Orion and Rex) showed convincing evidence that they could detect a single IR (870 nm) light, but the other three ferrets did not. Vanstone's (2005) study on possum IR light detection yielded similar results; only three of six possums showed some detection of the IR (870 nm) light. However, Vanstone comments that even if only a small proportion of possums can detect IR light, then there are implications for the design of surveillance and detection systems used for conservation. The same goes for ferrets and other pest mammals that can potentially detect light wavelengths into the IR spectrum.

## **Conclusion**

Two ferrets (Orion and Rex) showed convincing evidence that at least some ferrets can see IR (870 nm) light. A third ferret (Ajax) showed weaker evidence, and two ferrets (Nero and Felix) showed no evidence of seeing IR light. This has important implications for the design of IR equipment for the use in conservation in New Zealand.

## **Chapter 5**

### **Extraneous Cues**

#### **Abstract**

The operant chamber and reinforcement schedule (used in Chapter 2, Three and Four) was used to check whether any of the test stimuli were accompanied by uncontrolled extraneous cues. First, an ultrasound detecting device (Batbox III) was used to test for ultrasound waves (between 20 and 120 kHz) emitted from the apparatus at the onset of a test light stimulus. No ultrasound was detected from any of the LEDs used in this thesis. Second, the same experiment described in Chapters Two, Three and Four was run with only the red trial-starting LED present and the visible side (test) LEDs removed. This procedure was designed to expose any sequential dependencies learned from a pseudorandom reinforcement schedule. The ferrets' accuracy decreased to chance levels (with an overall accuracy of 45%), indicating that no sequential dependencies were affecting the results. These experiments helped to confirm that the ferrets were using only the light stimuli to discriminate which side had the lit-light.

## **Introduction**

### **Extraneous Cues**

In a forced-choice discrimination test, an animal is strongly motivated by a potential reinforcement (and/or fear of punishment) to use any available cues to succeed in a discrimination task. Therefore, sensory data must not be confounded by other variables that could affect their choice, such as extraneous cues. These are cues that could make a subject develop response strategies different from those intended by the experimenter (Blough and Blough, 1977). Extraneous cues are commonly a result of a poorly designed apparatus, or of sequential dependencies developing from a predictable semi-random reinforcement schedule.

When designing a visual experiment testing responses to one specific cue, the apparatus must be free of unintended additional cues (such as extra light wavelengths or sounds) accompanying the onset and offset of the test stimulus. In particular, the apparatus must be checked for cues that may not be detectable by the human makers of the apparatus, such as sounds that are not audible to humans (e.g. ultrasound), but which may be audible to a non-human subject. In addition to this caution, the reinforcement schedules operating within the apparatus must not include a predictable sequence that the subjects might learn, and thereby take advantage of, in order to obtain more reinforcements. These extraneous cues are explained further below.

### **Ultrasound as an Extraneous Cue**

Human hearing is recognized as having an upper threshold for tone detection at around 17 to 20 kHz. Ultrasound is therefore defined as any sound frequency above 20 kHz, and is inaudible to adult humans. Some animals, including ferrets (Kelly et al., 1986) and weasels (Heffner and Heffner, 1985) have an upper sound frequency limit considerably higher than that detectable by the human ear. The upper limit of sound frequencies audible to ferrets is 44 kHz (Kelly et al., 1986), so they can hear at least the lower range of ultrasound. It was therefore important to check that the stimuli used in this study did not emit any ultrasound that could be used by the ferrets as a cue to determine the position (left or right) of the lit-light.

Electronic devices can be used to measure the presence of extraneous cues so that the source of the cue can be eliminated. One such device is the handheld Batbox III made by Stag Electronics ([www.batbox.com](http://www.batbox.com)), January 2007). This equipment is designed to detect the ultrasonic calls produced by many bat species. The Batbox III picks up bat calls between 20 and 120 kHz, and converts the ultrasonic sounds into audio signals that humans can hear. This same equipment can be used to detect any ultrasound produced from an operant chamber at the onset of a stimulus.

### **Sequential Dependency as an Extraneous Cue**

A reinforcement schedule must not have a predictable sequence of stimuli or reinforcement, because intelligent subjects may learn to take advantage of this to maximize their chances of receiving reinforcement. In Chapter 2, Three and Four, a pre-determined pseudo-random sequence of reinforcement was programmed: no more than three consecutive reinforcements could be delivered on one side before the next reinforcement was assigned to the next correct lever press on the other side (the procedure was described fully in Chapter 2). The advantage of this arrangement was that it could help eliminate side biases by forcing the subject to change sides or receive no more reinforcement. The disadvantage of this sequence was that it could become predictable, thereby changing the percentage of correct responses when the stimulus is invisible from chance levels (50%) to higher than chance levels (more than 50%). If the experiment is run with the test stimuli turned off, blocked from sight, or removed, and the responses then return to chance levels, this would prove that the discrimination was not influenced by sequential dependencies (Blough and Blough, 1977).

## **Experiment 1**

### **Ultrasound as an Extraneous Cue**

#### **Aims and Objectives**

The aim of this experiment was to test whether any ultrasound waves were produced at the onset of the lit-light stimulus. To investigate this aim, an ultrasound measuring device was obtained to measure any changes in detectible sound waves as the lit-light was turned on and off. Additionally, general sounds from the ferrets' home cages were measured for comparison.

#### **Methods**

##### **Equipment**

A Batbox III, with a tuneable frequency range of between 20 and 120 kHz was used to measure ultrasound waves. Any ultrasound picked up by this device would be converted into a sound-wave audible to adult humans.

##### **Procedure**

A quiet room (away from any movement or sound) was used to conduct the ultrasound experiment. A single red (635 nm) LED was placed close to a power source, but the wires leading to the power source were disconnected. Moving the wires to touch would turn on the LED, and moving them apart would turn off the LED. The Batbox III was placed within 10 centimetres of the red LED. The wires were touched together and then disconnected once every second to produce a flashing light effect. The frequency dial on the Batbox III was slowly turned clockwise to run systematically through all the ultrasound frequencies from 20 kHz to 120 kHz. If any ultrasound was picked up by the Batbox III, then it would emit a loud audible 'static' sound. After the red LED had been tested, other coloured LEDs used in this study: white (551 nm), blue (474 nm), green (521

nm), yellow (593 nm), orange (610 nm), IR (870 nm) and IR (920 nm) were also tested for ultrasound.

Next, the Batbox III device was taken into the room housing the ferrets and pointed at their home cages at a distance of 10 centimetres. The operant apparatus was turned off (no LEDs could be lit), but all three levers were inserted into their cages. Ferrets were free to move around in their cages, eat, drink and press levers. Again, the frequency dial on the Batbox III was slowly turned clockwise to systematically run through the ultrasound frequencies from 20 kHz to 120 kHz in order to determine if any general ultrasounds were produced within the room where the experiments were conducted.

## **Results**

No ultrasound between the frequencies of 20 kHz and 120 kHz was produced by the onset of the white, blue, green, yellow, orange, red, IR (870 nm) or IR (920 nm) LEDs.

A large amount of ultrasound was produced by the ferrets' activity in their cages. Movements by the ferrets, such as walking, eating, drinking, and lever-pressing, all produced ultrasound, especially between the frequencies of 20 to 60 kHz. Therefore, even if there had been any ultrasound produced by the onset of an LED, it would probably have been drowned out by the many other ultrasounds produced by the general activity of the ferrets.

## **Experiment 2**

### **Sequential Dependency as an Extraneous Cue**

#### **Aim and Objective**

The aim of this experiment was to determine whether the ferrets had developed sequential dependencies by learning that the reinforcement schedule was not completely random. The main objective was to run the experiment as usual, but with the test stimuli disconnected from the power supply. If the ferrets had learnt the sequence, then their responses would not be random. An accuracy of more than 50% correct responses over several consecutive sessions would be expected.

#### **Methods**

##### **Subjects and Husbandry**

This experiment used the same five subjects (Nero, Felix, Orion, Rex and Ajax) and husbandry as described in Chapter 2, Three and Four.

##### **Experimental Apparatus**

This experiment used the same experimental apparatus employed in training (with the liquid dispenser), except that all three LEDs were single red (635 nm) LEDs. Red LEDs were chosen because the experiments described in Chapter 3 showed that all five ferrets could detect this light with a high degree of accuracy.

##### **Procedure**

Experiments were conducted in a darkened room during the night-time cycle of the ferrets' reversed 12:12 hour night/day regime. To begin with, ferrets went through at least four sessions in which all three (left, centre and right) stimuli were single red LEDs. This was named a 'Yes Stimuli' (YS) condition. After the ferrets met the set pass criteria for the YS condition, the left and right stimuli were removed for the following five sessions, and only the centre trial-starter remained (named a 'No Stimuli' (NS) condition). At the onset of a NS trial, the trial-starter light was lit, and once the centre lever had been pressed, ferrets were free to make a left or right choice in the absence of test stimuli. If the response accuracy fell to chance levels, then ferrets were not able to predict the pre-determined pseudorandom schedule of reinforcement. If the percentage of correct responses was above chance levels (>50%), then the opposite could be true. In a final series of sessions the side stimuli were returned (YS condition), and accuracy was expected to return to its original high levels.

### **Criteria for 'Seeing' the Light**

In this experiment, light detection for the YS and NS condition could result in accuracy falling into a 'pass', 'fail', or 'possible sequential dependency' category, as defined below.

#### ***Pass criteria***

Pass criteria were the same as were used in training: an accuracy of at least 75% over four consecutive (or five out of six) sessions was required, with a minimum of 30 trials per session. Sessions in which ferrets completed fewer than 30 trials were generally not included because of the risk of skewed data. However, if a subject repeatedly completed fewer than 30 trials per session, then allowances were made to pass the animal provided more (at least ten) consecutive sessions were undertaken and an accuracy of at least 75% was still achieved.

Note: if an animal can reach the normal 'pass criteria' for stimulus detection, it is almost certain that the animal could see the light (or was using some extraneous cue to detect which side the light is presented on).

***Fail criteria***

Fail criteria were the same as in training (a score centring around 50%), except that only five sessions with the side (test) stimuli removed were required. If an animal showed signs of improving in accuracy, then more sessions were carried out until no trend was observed. No minimum amount of trials per session could be enforced, because a light that is impossible for the animal to detect naturally resulted in fewer responses.

Note: under this procedure, if an animal fails to achieve responses at better than chance levels (50%), that provides evidence confirming that no extraneous cues are present.

***Possible sequential dependency effect***

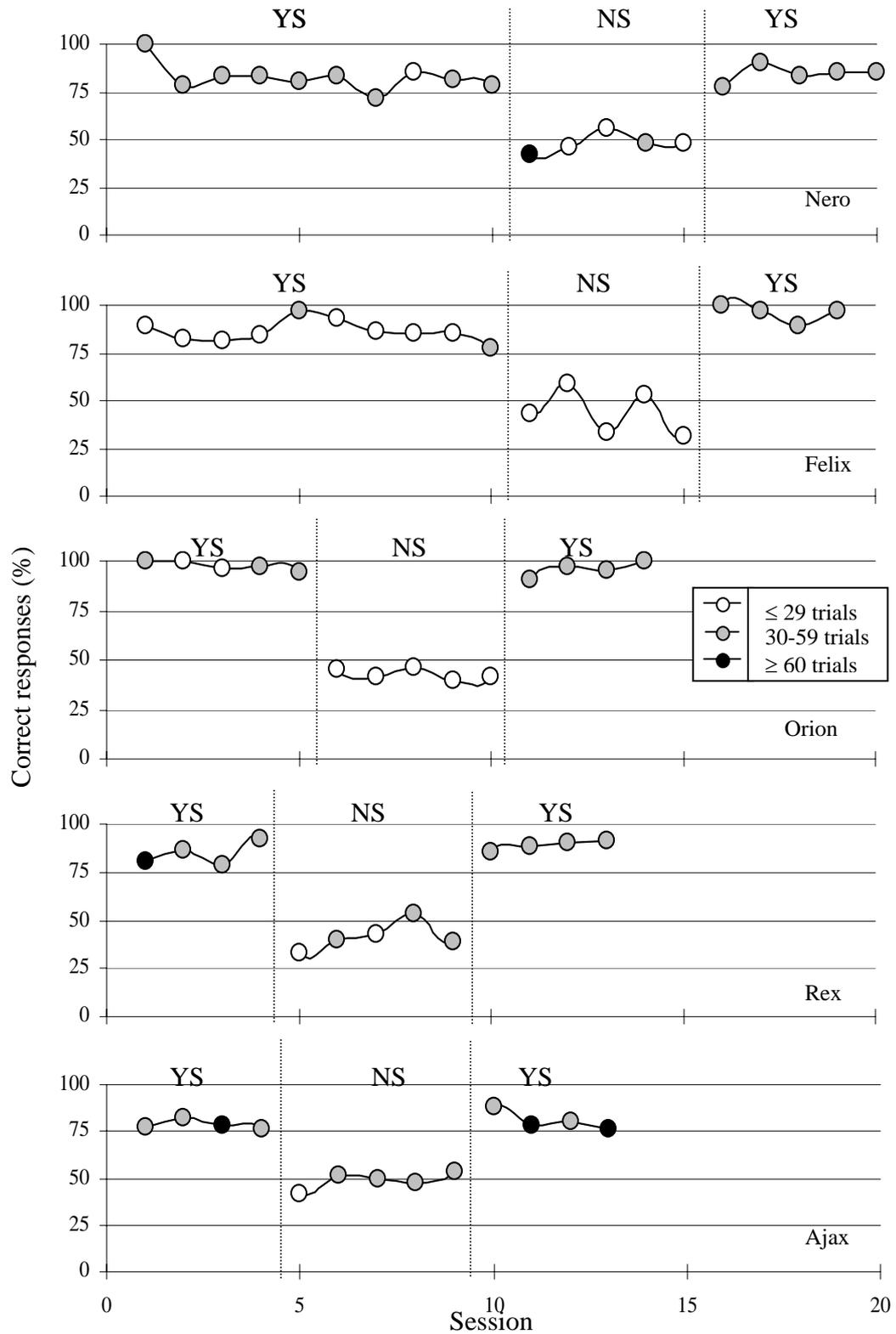
During the NS condition, if a subject could acquire an accuracy consistently above chance levels (more than 50%), this indicates that the moment at which the stimulus was expected to appear could be predicted even though no test stimuli were presented.

**Data Collection and Analysis**

Data were collected automatically as in the previous chapters. Information recorded included: ferret identification; date; schedule of reinforcement; NS or YS condition; total trials per session; total number of left and right reinforcements over the session; number of correct left, correct right, incorrect left and incorrect right lever presses; percent correct lever presses over the session; and total time of session. Data were analysed graphically and using the overall mean and 95% confidence intervals, as in the previous chapters.

## **Results**

The ferrets participated in fewer trials in the NS condition than in the YS condition. When visible side (test) stimuli were present, all ferrets had accuracy levels at or above 75% (see Figure 9). The overall mean accuracy for lever pressing under the lit-light was 85% (see Table 13). When the left and right LEDs were removed for five sessions, all ferrets' accuracies decreased to near chance levels, with overall mean accuracies of 45% (see Figure 9 and Table 13). This indicates that the ferrets could no longer detect which side stimuli were 'on'. To be sure this was not because the ferrets had lost the ability to perform the task, the left and right LEDs were replaced again. Accuracy immediately returned to high levels (with an overall mean accuracy of 89%, indicating that the ferrets had not lost the ability to perform the task. The accuracy for the second YS condition was generally higher than in the first YS condition.



**Figure 9.** Mean percentage of correct responses plotted against session number (lower x-axis) for the YS and NS conditions for each ferret. The key shows the number of trials completed per session.

**Table 13.**  
**Mean percentage of correct responses towards the lit-light when side (test) stimuli are present (YS) and removed (NS) conditions.**

	<b>Mean correct responses (%)</b>		
	<b>YS (first sessions)</b>	<b>NS (mid sessions)</b>	<b>YS (last sessions)</b>
Nero	82 ( $\pm 4$ )	45 ( $\pm 8$ )	84 ( $\pm 5$ )
Felix	86 ( $\pm 5$ )	45 ( $\pm 12$ )	96 ( $\pm 3$ )
Orion	97 ( $\pm 3$ )	44 ( $\pm 10$ )	96 ( $\pm 3$ )
Rex	84 ( $\pm 6$ )	42 ( $\pm 9$ )	89 ( $\pm 5$ )
Ajax	78 ( $\pm 6$ )	49 ( $\pm 7$ )	80 ( $\pm 5$ )
<b>Overall accuracy (%)</b>	85	45	89
<b>N (total no. trials)</b>	1237	642	885

## **Discussion**

### **Ultrasound Cues**

The main aim of this experiment was to eliminate the possibility that the ferrets' high accuracies at detecting lit-lights might have been assisted by extraneous cues (any cues other than the lit-light). Suspected extraneous cues were: (1) ultrasound signals produced at the onset of the lit-light, and (2) a predictable, pseudo-random reinforcement schedule.

As ferrets can detect ultrasound between 20 kHz and 44 kHz (Kelly et al., 1986), it was important to eliminate ultrasounds as an extraneous cue. The LEDs used in this study (white, blue, green, yellow, orange, red and IR) were tested for emissions of ultrasound frequencies between 20 kHz and 120 kHz correlated with the moment they were switched on.

No ultrasound waves were detected by the Batbox III ultrasound detecting device. Even if a small amount of ultrasound had been emitted, it probably could not have been heard by the ferrets over the large amount of background noise (audible sounds mixed with ultrasounds) produced by the general movement of the ferrets in their home-cages. This test confirms that ultrasound could not have been used by the ferrets as a cue to detect which test light was lit.

### **Learning**

As ferrets are intelligent and fast-learning predators, there was still a risk that, after many sessions with the same reinforcement schedule, they could learn that the reinforcements were not available completely at random. The reinforcement schedule had been programmed so that after three consecutive reinforcements on one side, the next reinforcement was assigned to the next correct lever press on the other side (described fully in Chapter 2: F-level-3). A ferret that had learnt this might be able to use this information to increase the chance of reinforcement when the light is difficult or impossible to see. To test this hypothesis, the test stimuli were removed for five sessions, and if the ferrets had learnt the reinforcement schedule, then their accuracy could have remained above chance levels (>50%). However, when the test stimuli were removed, the ferrets' response accuracies decreased to near chance levels (45%  $\pm$ 9). From these results

it can be concluded that the ferrets had not learnt the reinforcement schedule and were not using sequential dependencies to choose which side had the lit-light.

Detection and elimination of extraneous cues, involving equipment and insightful analysis, are important factors in the quality of animal psycho-physical studies (Blough and Blough, 1977). It can be difficult to pinpoint exactly which variables (other than those intended by the experimenter), an animal is responding to. For example, Terman (1970) used a modified random series of stimulus presentation to investigate auditory intensity detection in rats. He noticed that even at very low signal strengths, the rats' responses did not fall as low as chance levels of accuracy. He therefore suspected that sequential cues were influencing the rats' choices. In this study with ferrets, ultrasound and sequential dependencies were eliminated as possible extraneous cues. However, other extraneous variables (more difficult to measure) are almost always present, and there are occasions when even the most careful experimenter cannot be sure of achieving perfect control over all extraneous variables (Blough and Blough, 1977).

For example, even given a strong light signal (e.g. in the YS condition) ferrets did not often achieve 100% correct response accuracies. This is typical in animal psychophysical studies, as a perfect response on every trial is rarely achieved (Blough and Blough, 1977). There are many possible explanations for this error, including lack of motivation, attentiveness, and positional preferences (discussed in Chapter 2). This interpretation is further supported by the difference in overall mean accuracies obtained in the first YS (85%) and second YS (89 ±4) condition.

Stimulus preference may also confound psychophysical data. For example, Sadowski (1966) showed that rats discriminate more accurately when the positive stimulus is the more intense of two sounds. Blough and Blough (1977) reported that pigeons tend to respond more to some colours than others, and often have individual preferences. An experimenter may try to eliminate preferences by using a large number of subjects and a high training criterion. Alternatively, the use of one stimulus at a time with the 'follow the stimulus' method employed in this study should eliminate any potential to develop a preference for one stimulus over the other.

## **Conclusion**

No ultrasound waves were emitted at the onset of the light stimuli. There was no evidence that the ferrets had learnt any sequential dependencies from the pre-determined pseudo-random reinforcement schedule. These experiments helped to confirm that the ferrets were using only the light stimuli to discriminate which side had the lit-light.

## **Chapter 6**

### **General Discussion**

#### **Introduction**

Continuous monitoring with IR night-vision equipment is an important and frequently used technique exercised by organisations concerned with protecting native animals from nocturnal predators, in New Zealand (Brown et al., 1998; Innes et al., 1994; Sanders and Maloney, 2002), and elsewhere (Laurance and Grant, 1994; Savidge and Seibert, 1988). The information gained from monitoring can be used to learn more about the predators' nocturnal behaviour, in order to adapt management policy to better protect New Zealand's endangered birds. Ferrets are nocturnal predators of particular interest because, not only are they reported to kill threatened native animals (Department of Conservation, 1999; Graeme, 1996; Miller and Pierce, 1995), but they are also potential vectors of TB, a disease that puts New Zealand's cattle and deer herds at risk (Ragg and Walker, 1996). Once predator behaviour has been observed and better-understood, then new, safer, more cost effective and sustainable approaches to controlling them can be developed (Department of Conservation, 2000).

Very little is known about how ferrets perceive visual cues at night, or whether they can detect the presence of IR light-emitting equipment (although there are many speculations to this effect (Culter and Swann, 1999; Innes et al., 1994; Prout, 2003; Winder, 2003)). Controlled behavioural studies are required to determine whether IR light can be detected by a ferret's eyes, and if so, whether the ferret's brain can process these signals into meaningful information that the ferret can exploit. Such information could potentially be used by ferrets to find prey monitored under IR flood lights, or to avoid IR-emitting traps. Obviously, this information is important to the conservation of New Zealand's endemic wildlife and to halt the spread of TB. Better definition of the visual abilities of ferrets may contribute to identifying the most effective and cost efficient options for ferret control, and identify options that should be avoided because they introduce danger for protected species.

## **Primary Aim**

The purpose of this thesis was to provide baseline data for future conservation and animal behaviour studies by determining the range and sensitivity of light detection in the ferret, and the extent of individual variation in both. Behavioural experiments were conducted in logical order to: (1) assess whether ferrets are capable of working in an operant chamber with light stimuli; (2) test for ‘stimulus generalisation’ over light stimulus properties; (3) investigate whether ferrets can see IR light, and if so, establish the frequency range; (4) determine what brightness threshold is required for ferrets to detect IR light; and (5) eliminate the possibility of extraneous variables acting as response cues.

## **Summary of Results**

In this thesis I have presented data on training ferrets in an operant task, stimulus generalisation, IR wavelength and intensity thresholds, and extraneous cues. The results of my experiments are summarized below.

### ***Training (Chapter 2)***

Five male ferrets were trained in a two-alternative forced-choice visual discrimination procedure to perform a defined task: follow the lit-light for food rewards. This training was basic to all the following experiments because the task remained the same throughout the sequence of changes in the character of the lit-light stimuli. Four levels of training were required to induce the ferrets to perform the visual discrimination with a high level (>75% correct choices) of accuracy. The training phase was unexpectedly time-consuming (a total of up to 101 sessions were required from learning to eat from the reinforcement area until reaching acceptable accuracy criteria at the last training level (see Table 4), in part because the initial apparatus used an unattractive reinforcement, and in part because time was needed for changing programs and equipment between training levels.

### ***Stimulus generalisation (Chapter 3)***

When all five ferrets could reliably perform the learned visual discrimination task with a bright white light, further progress depended on a clear demonstration that they could generalise the task over different light wavelengths and intensities without disruption to the performance of the task. First, the wavelengths of the light stimuli were systematically increased from blue (474 nm) towards red (635 nm). Second, the wavelength closest to the human IR threshold (produced by the red LED) was halved in intensity nine times. These stimulus changes did not disrupt the ferret's performance of the learned task (see Figure 6 and Figure 7), indicating that they could easily generalise between visual stimuli of different spectral properties.

### ***Infrared detection (Chapter 4)***

When the visual stimuli were changed from wavelengths within the visible spectrum to potentially invisible IR (870nm) wavelengths, two of the five ferrets (Orion and Rex) continued the task with a high level of accuracy, indicating that they could see the IR light. Another ferret (Ajax) performed the task with lower accuracy, and did not meet the set pass criteria, but nevertheless, responded towards the single IR LED more than half of the time. The final two ferrets (Nero and Felix) did not show any signs of seeing the IR light (see Figure 8). Because Nero and Felix had both passed the stimulus generalisation tests, this result must have been due to failure in perception, not to failure in understanding the task. When the data for all five ferrets were pooled, the response accuracy towards a single IR (870 nm) test LED (Condition B) exceeded chance levels, but did not reach the set pass criteria of at least 75%: from a total of 4,188 trials, there was an average correct response rate of 66%. Increasing the intensity of the IR light did not increase the accuracy of Ajax, Nero or Felix in performing the task.

### ***Extraneous cues (Chapter 5)***

The possibility that the ferrets were responding to cues other than the lit-light had to be eliminated. Two possibilities (ultrasound and sequential dependencies) were considered. The results showed that: (1) No ultrasound was emitted when a test light was turned on; and (2) Although the schedule of reinforcement was not completely random, the ferrets did not learn to predict a pattern of responses

(sequential dependencies) that they could use to gain more reinforcement when visual discriminations were impossible (see Figure 9). These experiments confirmed that the ferrets were, as intended, using only the light stimulus to discriminate which side had the lit-light.

## **Discussion**

### **Contribution to Animal Behaviour Studies**

Most published studies on ferret vision are physiological. Of the very few behavioural experiments reviewed: Vargas and Anderson (1998) showed that young ferrets that witnessed a prey kill by their mother were more efficient at killing prey themselves; Apfelbach and Wester (1977) used prey-dummies to show that movement, size, orientation, brightness, and texture are all visual cues used by ferrets to find and kill prey; and Pollard et al. (1967) and Gewalt (1959) used operant conditioning to show that ferrets could discriminate between different visual properties including shape and colour. These studies all proved that ferrets readily use visual information when it is available in their environment, and that their vision is an important and possibly underestimated sense.

This thesis contributed some new and important details to our existing knowledge of ferret vision by providing evidence that at least some ferrets can respond towards IR (870 nm) light in a two-response forced-choice operant procedure. The implication is that the eyes of at least some ferrets could not only detect IR light, but also, their brains could process the stimulus into meaningful information, enabling them to respond to the light. No study has ever tested ferrets for detection of IR light before. A behavioural study of this nature was particularly important because this is the only technique (as opposed to a physiological study of the ferret retina) that can prove that an animal really can 'see' a visual stimulus.

The limited number of previous behavioural studies on ferrets, and on the visual thresholds of small mammals generally, meant that the literature offered very few guidelines on which physiological procedures would be most appropriate

to use in this study. A two-alternative, forced-choice operant method was originally chosen on the basis of Vanstone's (2005) study of visual thresholds in possums, and on the studies of Kelly et al. (1986) and Pollard et al. (1971) on ferrets on discrimination by ferrets between cues of different visual properties.

However, in this study, training the ferrets to perform an operant task was difficult and time consuming. Several different training and reinforcement schedules (described in Chapter 2) had to be especially developed to motivate the ferrets to choose the lit stimulus. Between 71 and 101 daily sessions were required before each ferret could press the lever under the lit-light with a high (>75%) level of accuracy. This initial failure was probably not because operant procedures are unsuitable in principle for use with ferrets, or because the ferrets were not intelligent enough to learn the task (pressing levers), but rather that either the task itself or the reinforcement used at first (dry kitten crunchies), or both, were too un-natural to ferrets to motivate them to work. When intelligent animals are constantly frustrated, they tend to find unexpected ways to get round the problem – such as, in this case, randomly pressing levers and receiving reinforcement some of the time rather than following a lit-light to get a reward every time. Ferrets do not naturally rear up to press their paws down on objects in the wild, and a more species specific behaviour, such as pushing their head through a door (Gewalt, 1959) or touching their nose to a spout (Kelly et al., 1986) might have been more appropriate for them.

Although it is seldom so difficult to motivate other species to work for food rewards, long training is still typical of operant procedures. Generally, it takes a large number of trials to be sure the animal has learnt the task before stimulus properties can be changed. For example, Neitz et al. (1989) used an operant procedure to test colour vision in dogs. Each subject had to receive extensive training of more than 4000 trials (about 13 sessions) before experiments could begin. The three cows that Entsu et al. (1992) put through an operant procedure required 22 to 40 daily sessions of 30-trials each to reach a pass criterion of only a 70% correct, and the entire experiment lasted seven months. Lengthy training and testing periods usually mean that sample sizes remain small, and experimenters must be patient for reliable results.

In this study, the five ferrets completed about 60 trials per session when the discrimination task was easy, but only about 15 trials when the discrimination

was more difficult. This is a small number of trials per session relative to the number recorded by experimenters using other animals for visual discrimination procedures. For example, the three dogs used by Neitz et al. (1989) for a colour vision experiment achieved between 200 and 400 trials per dog per session, and the eight cows tested by Gilbert and Arave (1986) in an experiment on bovine colour vision achieved 83 to 109 trials per cow per session. Here, attempts to increase the ferrets' motivation to work included trials of many different kinds of food (see Chapter 2), but nothing else could be done because there is no ethical option that could be more motivating than food (a primary resource important for survival). Fewer trials per session meant that more sessions were required than anticipated, which in turn extended the time needed to complete the experiments.

### **Captive Studies**

In captive experiments, an animal is studied in a controlled laboratory environment, as opposed to in the field. Small sample size is a common disadvantage of undertaking captive studies, because the number of animals that can be obtained, housed and cared for in the laboratory, and the availability of equipment, are all limited. A further disadvantage is that captive studies tell us little about what cues an animal in its normal environment would naturally pay attention to, habituate to or regard as novel.

However, captive studies are more ideal to answer a question concerning the sensory abilities of an organism. This is because they have the benefit of the organism being directly altered by the controlled conditions experienced in the lab, so the behaviour of the animal can be compared before and after a single variable is changed. Additionally, laboratory scenarios are highly controlled and simplified, and it is much easier to identify and remove any extraneous cues that may confound the results. For example, in this thesis, ultrasound cues and sequential dependencies could be tested for and eliminated as possible extraneous cues (Chapter 5). In the field, there would have been many more extraneous cues to contend with, including uncontrolled environmental conditions and subjects. Captive studies also allow better control over hunger or satiation, and were therefore an ideal scenario for the procedures carried out with domestic ferrets in this thesis. Domestic ferrets were chosen because they are easier to train and

handle than feral ferrets, and they can be screened to make sure they do not have TB.

### **Confounding Variables**

Although captive studies allow greater control over hunger and satiation than studies carried out in the wild, hunger was still a confounding variable in this thesis. Ideally, hunger levels should directly affect an animals motivation to work for food reinforcement; if an animal is deprived of food as opposed to satiated, it should work (e.g. lever press) for food at a greater rate and/or for a greater proportion of time (Baum, 1982).

Occasionally, ferrets in this study would not work for food rewards, such as during the IR dimming trials with Orion and Rex (Chapter 4, experiment 2). Depriving them of food for longer periods, or letting their weight drop to 80% of their free-feed weight, did not affect this apparent obstinacy. This is a known confounding variable of a free operant procedure – subjects are free to respond when, and as often as they like, as opposed to a discrete trial procedure used in a Y-maze, in which only one response can be recorded per trial (Lieberman, 1993). The ferrets were free to choose to participate in the operant procedure or be content with the small amount of base feed given daily after experiments. The alternative explanations, that Orion and Rex could not see the IR (870 nm) lights, or that there was a problem with the dimming program, were not accepted. Both ferrets could respond with high accuracy to the full intensity (100% on) IR (870 nm) lights prior to adding the dimming program, and both ferrets could respond with high accuracy to the dimming program when red (635 nm) LEDs were used.

Negative results (such as those recorded by Nero and Ajax when tested with IR (870 nm) lights) could have reflected no more than a failure on the part of the experimental design to cater for possible individual variation in the reactions of the five test subjects, discussed above. Alternatively, it could have reflected real genetic variation in their physiological capabilities (C. M. King, pers. comm. January, 2007<sup>3</sup>). The ferrets used in this study were domestic stock bought from a commercial breeding facility. The physical properties favoured by generations of

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<sup>3</sup> Dr Carolyn King is a senior lecturer in the Department of Biological Sciences, University of Waikato, Hamilton, New Zealand. Her expertise is with mammals introduced to New Zealand, particularly mustelids.

selective breeding in captivity (such as docility, high fertility, and high tolerance of confinement) (Diamond, 2002) are not the same as those favoured by natural selection in the wild (such as the physical and social skills needed for success in competition for prey and mates). Mutations affecting captive breeding stock in characters irrelevant to survival in a cage would not be eliminated unless linked to one of the characters relevant to that environment. Hence it is possible that the sensory capabilities of the five ferrets used in these trials are not quite the same as those of wild ferrets, so the conclusions of this work should be verified on ferrets of wild stock.

On the other hand, for the initial exploration of the subject reported here, there was a considerable advantage in using captive-bred ferrets, because (1) they were easier to obtain and handle than wild-caught ones would have been, (2) they were less likely to spend time attempting to escape rather than learning their tasks, and (3) they could be screened to make sure they did not have TB. For reasons related to the conditions specified in the Biosecurity Exemption permit required to authorize our purchase and holding of ferrets, and to the University's health and safety regulations, these advantages were considered to outweigh the acknowledged limitation in directly applying these results to feral ferrets in the wild.

### **IR Detection in Nocturnal Mammals**

The literature on human spectral light thresholds reports that wavelengths of up to about 700-750 nm (red) are visible (Kaiser and Boynton, 1996; Lomas et al., 1998), but any wavelengths above this are defined as IR wavelengths because they are invisible to humans. Night-vision equipment (including that routinely used by conservation agencies in New Zealand) is therefore considered safe if it emits light wavelengths that are above the reported human threshold for red. However, this does not necessarily mean that non-human species cannot see them. The spectral thresholds are unknown for many of the species that are being monitored or trapped using the IR equipment, and their visual systems are substantially different from that of humans. Furthermore, some of this equipment emits light wavelengths that are not far removed from the red spectrum ('very-near IR'), or light that includes wavelengths from within the red spectrum (Cockburn, pers. comm., September 2006).

At least two of five ferrets tested during this study could clearly detect a lit single IR (870 nm) LED with high accuracy (>75%) (Chapter 4), and another ferret could do so to some extent. The upper limit of ferret perception is unknown, but one ferret tested here could not see wavelengths of 920 nm (Table 12, condition G). The range of wavelengths emitted from the IR (870 nm) LEDs used in this study, as measured by a spectrometer, were 800 to 910 nm (Chapter 4, Table 10), so these IR LEDs did not bleed into the visible red spectrum. Since extraneous cues such as ultrasound and sequential dependencies were excluded, these data provide strong evidence that these ferrets were detecting the IR wavelengths alone. Three of six possums tested by Vanstone (2006) could also detect IR (870 nm) light to some extent. Together, these results have significant implications for conservation, because they mean that the standard night vision equipment used in conservation areas may be detected by ferrets and possums at night.

### **Implications for Conservation in New Zealand**

IR video surveillance (using active IR beams, large clusters of IR LEDs or large white spotlights with an IR filter) is an important part of conservation management of New Zealand's threatened native species today. These methods produce a field of IR light commonly reported to contain frequencies between 830 nm and 950 nm (Table 9), and have been widely used in the observation of predator/prey behaviour (Brown et al., 1998; Innes et al., 1994; Laurance and Grant, 1994; Sanders and Maloney, 2002; Savidge and Seibert, 1988). Information gained from these activities includes identification of the predator species visiting nests, and data on the rates, timing and sequence of predation events in complete darkness (Innes et al., 1994). Therefore, IR equipment has become the most frequently used type of surveillance for conservation (Culter and Swann, 1999).

It is extremely important that IR surveillance of conservation areas (especially those used for studies of the behaviour of prey or predators) remains inconspicuous. If IR lighting becomes detectable by the subject being observed, it may potentially influence the behaviour of interest, or attract or repel the animal. For example, the subject may show curiosity towards video equipment, or be wary of entering areas that have been disturbed by humans or flooded with IR light.

Worst of all, predators may learn to associate the lights with prey, and use the lights to guide them towards threatened native species.

### **Recommendations**

Even if only a small proportion of possums (Vanstone, 2006) and ferrets can detect IR (870 nm) LEDs, or detect it only some of the time, the implications for the design of surveillance and detection systems used for conservation are serious. Because the spectral thresholds of many species are yet unknown, and because IR equipment has become the most frequently used type of surveillance for conservation (Culter and Swann, 1999), I recommend that only IR equipment with long IR wavelengths (longer than 870 nm) should be used. This should minimize the risk of potential detection of the equipment by predators.

### **Evolution of IR perception**

There could be many adaptive advantages for nocturnal predators such as ferrets having a spectral threshold slightly above red (in the 'near IR' spectrum). Starlight and moonlight are significantly richer in the longer wavelengths than day-light (Lythgoe, 1979). Animals can be expected to be adapted to their natural light habitat (Burkhardt, 1983); ferrets are mainly nocturnal, so may be able to benefit from star and moonlight when hunting in open pastoral areas. Their ability to detect wavelengths into the IR spectrum, together with their tapetum lucidum, which enhances vision at very low light levels (Tjalve and Frank, 1984), could give ferrets very keen night vision.

On the other hand, there is no evidence that IR vision in mammals goes further into the IR spectrum than 870 nm. IR wavelengths longer than 8,000 nm (called 'far IR') become heat waves. Some nocturnal hunters such as the pit viper can detect heat waves given off by prey, and use them to direct accurate strikes in total darkness (Campbell et al., 2002). Ferrets hunting deep inside rabbit burrows would have a great advantage if they could do the same.

One theory on why mammals have no perception of long-wavelength (IR) vision is that red-sensitive rhodopsins (the pigment sensitive to red light in the retinal rods of the eyes) are chemically forbidden or highly unstable past a certain wavelength threshold (McFarland and Munz, 1979). Another theory is based on

the fact that mammals are homeothermic ('warm-blooded' animals that maintain a relatively stable body temperature that is independent of the temperature of the surrounding environment). If their eyes could detect wavelengths too far into the IR spectrum, then the heat emitted by the mammal's own body might be enough to trigger a visual signal, degrading the meaningful images of the outside world by heat noise. This effect would get much worse at night, when light levels are lower but the animal's body remains at the same temperature (Lythgoe, 1979). This problem is not relevant to poikilothermic ('cold blooded') animals such as the pit viper. It may therefore be an adaptive advantage to endothermic mammals to be able to see IR up to, but not beyond a certain point.

### **Directions for Future Research**

There is room for a great deal of further research on the visual perception of ferrets and other non-human mammals. This study has shown that at least some ferrets can perceive IR (870 nm) light. This discovery raises many more questions that need to be addressed in the future:

- What other pest mammals can detect the IR wavelengths emitted from night-surveillance equipment? To find out, an operant method similar to the one used in this thesis could be used to assess other pest mammals such as rats and stoats.
- Does detection of IR surveillance equipment or traps change the behaviour of pest mammals, and if so how? Does it attract or repel predators? To investigate these questions, a study directly aimed at determining the effect of infrared surveillance equipment on the behaviour of pest mammals' is needed. Two experiments must be conducted: one with equipment that emits IR light with relatively longer wavelengths (the 'invisible' treatment) and one that emits the standard (shorter-wavelengths of 830 nm to 850 nm) IR lights that are currently used for conservation (the 'potentially visible' treatment). These experiments must be conducted simultaneously (as opposed to Sanders & Maloney's (2002) study that used different IR treatments in different years). Hens' eggs could be placed in mock ground-nests under each treatment, and predator visitation rates could be recorded in different locations over a period of months or years.

- Can predators learn to associate the IR light over conservation areas with a food source? This question could be investigated with a mock-field trial in which subjects are released into a fenced area with an IR flood-light with food directly below, and the position of the IR light could be frequently moved. The animals' movements could be tracked with a GPS, and if over time they find the food under the floodlight more quickly than other food placed around the arena but not illuminated, then it is likely they have learnt to associate the light with food.
- Exactly how important is visual perception for nocturnal mammals compared with their other senses? This is a difficult question, and would involve complex, controlled behavioural studies in which the sensory cues associated with the distribution of prey could be manipulated independently somehow. An alternative approach could be a physiological study in which the olfactory or visual part of the animal's brain is altered.

## **Summary**

This study confirmed that the behaviour of ferrets can be studied under controlled experimental conditions. Five ferrets learned specified behavioural responses towards a visible lit-light of varying spectral properties. At least two of them could continue to detect the lit-light when the wavelength was changed to IR (870 nm), implying that at least some wild ferrets might be able to see the light wavelengths emitted from the standard equipment used in conservation projects.

I recommend that night-surveillance and trapping equipment with IR lights should be chosen carefully, and equipment that emits lights including red or very-near IR wavelengths should be avoided. Instead, equipment with a peak wavelength higher than 870 nm should be chosen to be sure that monitoring is unobtrusive and undetected by predators. This information could help to improve monitoring and trapping methods and protect New Zealand's threatened native species.

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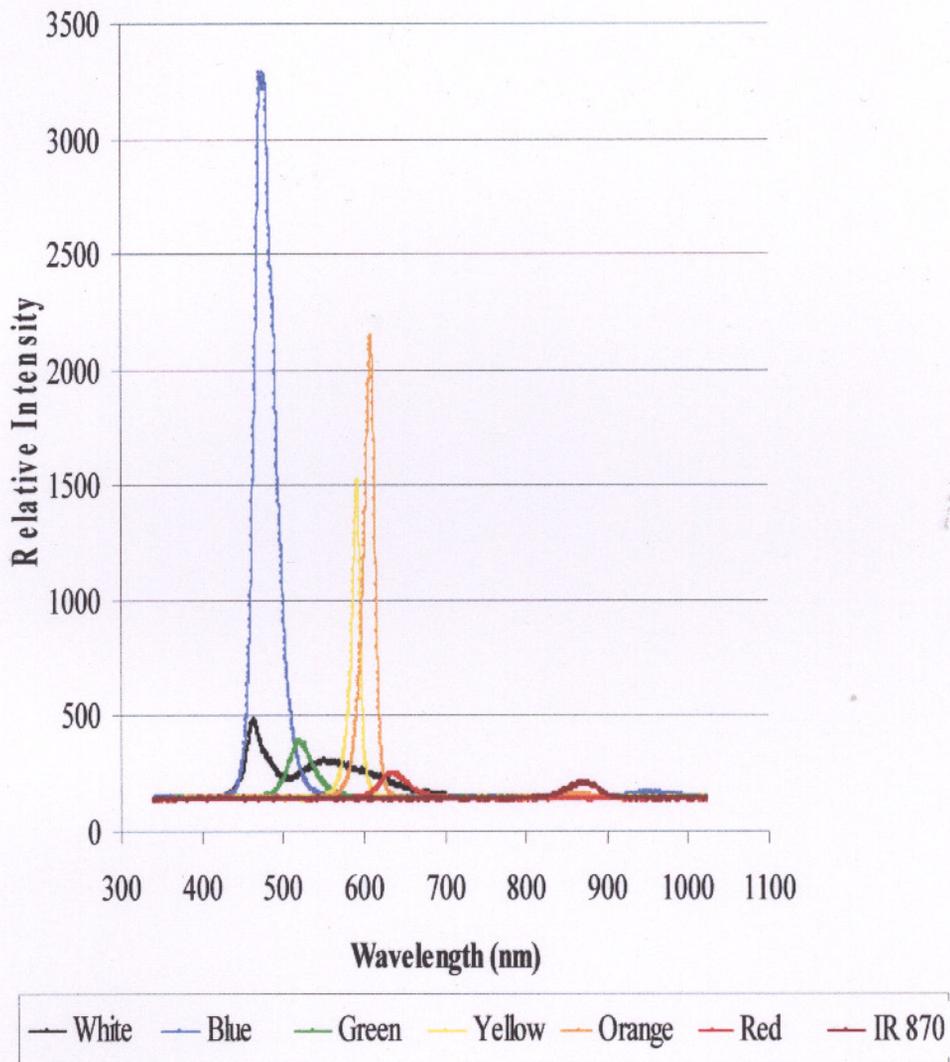
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### Appendix 1.

Relative intensity of each coloured LED as measured by a spectrometer against the blue LED. The blue LED was chosen as an intensity reference because it was the brightest LED and the first coloured LED tested. The Y-axis shows an arbitrary intensity scale and the X-axis shows the wavelength of each LED.