The role of steroid hormones in avian spatial learning and memory abilities

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Declaration

This thesis is the result of my own research and contains no work done in collaboration except where otherwise stated. The text does not exceed 70,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

Zoë G. Hodgson
Abstract

In mammals, both organisational and activational effects of steroid hormones influence spatial learning and memory abilities and hippocampal morphology. It is not clear whether such hormones play a similar role in birds, although the avian hippocampus is considered to be homologous to the mammalian hippocampus. The potential for steroid hormones to mediate avian spatial learning and memory and hippocampal physiology exists as the avian hippocampus, like the mammalian, contains androgen, oestrogen and corticosterone (CORT) receptors.

I used the great tit (Parus major) and zebra finch (Taeniopygia guttata) as model species to determine effects of steroid hormones on avian spatial learning and memory. To address this I took a four-pronged approach:

First, as spatial ability is to some extent reliant on appropriate cue use, I examined cue preference in the great tit. In a one-trial associative memory task birds were trained to a compound stimulus where both colour and location cues could be used to locate a reward. By dissociating the cues on probe trials I was able to determine which cues were controlling the birds' food-finding behaviour. The overall distribution of choices was significantly different from random but did not differ between the sexes. Both sexes exhibited a preference for the location cue over the colour cue.

Second, I exploited the existence of a well-characterised memory task that tests spatial and non-spatial memory. This was an operant conditioning delayed-non-matching-to-sample memory task, presented on computer-controlled touch screen. I tested for sex differences in performance in birds maintained under a breeding season (i.e., long-day) photoperiod and found no sex differences in performance on either a spatial or visual memory task.

Third, I experimentally manipulated hormone levels (testosterone (T), 5α-dihydrotestosterone and oestradiol, the latter two being T metabolites) and determined their effect on learning and memory using the same touch screen memory task. T improved spatial learning and memory abilities in females but not in males. T also increased response latencies (time taken to peck a touch screen image) in both sexes.
Finally, zebra finches selectively bred for differing peak (stress-induced) CORT levels were used to determine whether CORT affected avian spatial memory in a similar way to that seen in mammals. A one-trial associative memory task was used to assess the performance of birds bred for high peak CORT levels and controls. High CORT birds performed less well than controls on the spatial task but there was no difference in performance between lines on the visual task. Although High CORT birds had higher peak levels of CORT, there was no difference in CORT levels between the groups at test. This may mean that high peak levels of CORT, whether at an organisational or activational level, can have a detrimental effect on spatial ability even when circulating levels are normal.

In summary, spatial learning and memory abilities in birds appear to be sensitive to the effects of both gonadal and adrenal hormones as they are in mammals.
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Chapter 1. Introduction

Since the 1970s, the influence of steroid hormones on spatial learning and memory abilities has received much attention. This interest generally emerged in response to the frequently reported sex difference in spatial learning and memory abilities, where males tend to outperform females.

1.1 What is spatial ability?

Spatial ability consists of a number of different cognitive abilities all involved in navigation and the perception of three-dimensional objects. In humans, spatial ability has been defined as "the ability to imagine what an irregular figure would look like if it were rotated in space or the ability to discern the relationship among shapes and objects" (Halpern, 1991). In the context of everyday life, spatial ability would encompass an aptitude for navigational and map-reading tasks.

1.2 Ways of assessing spatial ability

Many different tasks have been used to assess mammalian spatial ability. Spatial ability tests in humans usually involve map reading and navigation, mental rotation (see figure 1) and judgements about moving objects. Spatial ability tests in rodents usually involve mazes, where different mazes can be used to measure different aspects of spatial memory. For example, the memory involved may be short-term working or long-term reference memory. Working memory is defined as memory for events on a specific trial whereas reference memory is memory for the unchanging characteristics of a task (Honig, 1978). Different mazes may also require different cognitive strategies: a Morris water maze (MWM; see figure 2) requires the use of distal cues such as the geometry and features in the room housing the maze, whereas a radial arm maze (RAM; see figure 3) with all arms rewarded may be effectively solved with an adjacent arm strategy.
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Figure 1: Mental rotation test: an example of a task used to assess spatial ability in humans. A participant would be asked, "Which of the 3 figures on the right is the same as the figure on the left, except for orientation?" (e.g., Tan et al., 2003)

Figure 2. The Morris water maze (Morris, 1981) is frequently used to assess cognitive ability in rodents. In this place-learning task, animals search for a hidden platform in a pool of opaque water. Latency to reach the platform is used as the performance measure (e.g., Galea et al., 1995).
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1.2 Assessing avian spatial ability

A number of techniques have been developed to assess avian spatial ability in the laboratory. These spatial ability tasks are usually appetitive tests where the birds’ ability to locate a hidden reward is measured. For example, birds may have to locate a feeder containing food in an aviary. The bird’s ability to relocate the reward after a retention interval is then assessed (see figures 4a & 4b). Alternatively, food may be hidden in a tray. After finding the food initially, the bird’s ability to relocate it, after a retention interval, is monitored (see figures 5a & 5b). Spatial memory can also be measured using an operant touch screen task, where the automated delivery of a reward is dependent on the bird pecking the correct image on a computer touch screen (see figures 6a & 6b). All of these tasks can be used to assess spatial ability and “visual” memory (processing...
information about the non-spatial characteristics of a visual stimulus, such as colour or form; Colombo & Broadbent, 2000).

Figure 4a: Uniquely decorated feeders are positioned in an aviary. The location of the reward is indicated by a compound stimulus (i.e. colour and location).

Figure 4b: After a retention interval, the cues are dissociated. The reward is now either in the feeder of the correct colour (assessing visual memory) or in the feeder in the correct location (assessing spatial memory). The reward is hidden by a piece of string attached to each feeder.

(e.g., Brodbeck, 1994)

Coloured flaps covering wells

Perspex tray containing wells

Figure 5a: Spatial task. The reward is found in a unique location (R.I. = retention interval)

Figure 5b: Visual task. The location of the reward is indicated by a uniquely coloured flap.

(e.g., Patel et al., 1997)
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1.3 The hippocampus

The hippocampus is the brain region most frequently implicated in spatial learning and memory (e.g., Morris et al., 1982; Sherry et al., 1992; Poucet & Benhamou, 1997; Pearce et al., 1998; Jarrard, 1983; Jarrard et al., 1984; Morris, 1983; O'Keefe, 1976; O'Keefe & Nadel, 1978; Olton & Samuelson, 1976; Olton et al., 1978; Olton et al., (e.g., Biegler et al., 2001))
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1979). It is a discrete, evolutionarily ancient structure found in both hemispheres of the brain in birds and mammals (see figure 7).

![Figure 7: Cross-section of the hippocampus of a rat. Labelled areas are sensitive to the action of steroid hormones (redrawn from Amaral & Witter, 1989).](image)

Data supporting hippocampal involvement in spatial learning and memory in mammals have come from two main sources: 1) lesion studies; 2) correlational studies demonstrating a positive relationship between hippocampal size and spatial ability.

First, maze-learning ability is impaired in rats with hippocampal lesions (Save & Poucet, 2000; Morris et al., 1982; Bannerman et al., 1995; Gallagher & Holland, 1992; O'Keefe & Nadel, 1978; Olton, 1983). The cognitive impairment is specific to the spatial domain as rats with hippocampal lesions perform like controls in learning and handling non-spatial information (Jarrard, 1995).

Second, spatial ability is correlated with hippocampal parameters, with a larger hippocampus generally being associated with a superior spatial ability. For example, male polygynous meadow voles have a relatively larger hippocampus than their female counterparts and also outperform females on tests of spatial ability. Monogamous species in the same genera show neither hippocampal nor spatial ability sexual dimorphism (Galea et al., 1994a; Galea et al., 1996; Gaulin et al., 1990).
1.3.1 The avian hippocampus

The avian hippocampal formation consists of a medially-situated hippocampus and a dorsomedially situated area parahippocampalis. There are a number of structural differences between the avian and mammalian hippocampal formations. The avian hippocampus lacks a number of structures found in the mammalian hippocampus, such as a distinct Ammon’s horn, dentate gyrus, hilar regions and subiculum (Macphail, 2002; Szekely, 1999). However, analogous structures in the avian hippocampus have been suggested (Montagnese et al., 1996; Erichsen et al., 1991; Krebs et al., 1991; Szekely, 1999) and a number of other lines of evidence support the view that the avian hippocampus is an anatomical homologue of the mammalian hippocampus (Szekely, 1999; Craigie, 1935; Bingman et al., 1989; Casini et al., 1986; for review see Bingman, 1993). For example, the avian hippocampus emerges from the same portion of developing telencephalon as does the mammalian hippocampus (Kuhlenbeck, 1938), with the afferent and efferent connections being homologous (Trottier et al., 1995; Benowitz & Karten, 1976; Krayniak & Siegel, 1978; Casini et al., 1986; Bouille et al., 1977; Bons et al., 1976; Bingman et al., 1989; Szekely, 1999). In addition, parallels can be drawn between the morphology of neurons and types of neurotransmitters and neuropeptides in the avian and mammalian hippocampus (Molla et al., 1986).

Lesion studies and correlational studies supporting avian hippocampal involvement in spatial learning and memory have also been performed. Hippocampal lesions impair the development of navigational abilities in naïve homing pigeons (Bingman et al., 1990) and disrupt navigation on novel trajectories using familiar landmarks in experienced pigeons (Bingman & Mench, 1990). Again, the induced cognitive impairment is specific to the spatial domain, with there being no performance deficit on non-spatial tasks (Hampton & Shettleworth, 1996).

Secondly, there are differences in hippocampal size between food-storing and non-storing birds. Within food-storing species, birds that rely more on hoarded food have a larger relative hippocampus than those that do less so (Healy & Krebs, 1992; Healy et al., 1994; Healy & Krebs, 1996; Hampton et al., 1995; Basil et al., 1996; Sherry et al.,
1989; Clayton, 1995; Clayton, 1998; Krebs et al., 1989; Smulders & DeVoogd, 2000). Variation in neuroanatomy is correlated with changes in hoarding intensity, with the increase in hippocampal volume in the autumn, at the time of peak food hoarding and recovery, being due to neurogenesis (Barnea & Nottebohm, 1994; Smulders et al., 1995). Another example of avian hippocampal plasticity that is also associated with seasonal differences in spatial memory demand, is seen in parasitic cowbird species (Molothrus sp.). Parasitic cowbirds lay their eggs in the nests of other species, the hosts, which incubate and rear their young (Rothstein, 1990). Successful parasitism only occurs when parasitism coincides with the host’s laying period. In shiny cowbirds (M. bonariensis), females search for host nests without the assistance of the male, whereas in screaming cowbirds (M. rufoaxillaris) males and females inspect hosts’ nests together. Both parasitic species have a relatively larger hippocampus than the non-parasitic species (e.g., bay-winged cowbird (Agelaioides badius)). There are no sex differences in relative hippocampus size in screaming or bay-winged cowbirds, but female shiny cowbirds have a larger hippocampus than males during the breeding season (Astié et al., 1998; Sherry et al., 1993; Reboreda et al., 1996).

1.4 Sex differences in spatial ability

A sex difference, favouring males, in mammalian spatial cognition is often reported. For example, male humans outperform females on spatial tasks requiring mental rotation, judgements about moving objects and geographical knowledge (e.g. Beatty, 1979, 1984; Voyet al., 1995; Silverman & Eals, 1992). Similarly, the majority of maze-learning studies with rodents yield a male-advantage (e.g., Gaulin & FitzGerald, 1986; Gaulin et al., 1990; Barrett & Ray, 1970; Davenport et al., 1970; Krasnoff & Weston, 1976; McNemar & Stone, 1932). However, to my knowledge, avian sex differences in spatial ability have only been investigated in two cases and neither study found sex differences (Astié et al, 1998; Petersen & Sherry, 1996).
1.5 What causes the sex difference in spatial ability?

Mechanistic explanations for the sex difference in spatial learning and memory abilities focus on the influence of gonadal hormones on hippocampal anatomy and spatial ability. Recent research has shown that gonadal hormone levels, both at an organisational level, during development, and activational level, cyclic and seasonal variation, can affect spatial ability. The influence of gonadal steroids on avian spatial learning and memory abilities has received little attention. The purpose of this thesis is to determine whether steroid hormones affect avian learning and memory in a similar way to how they do in mammals.

The underlying mechanisms for the sex difference in spatial ability are not yet fully understood. Since the 1970s, evidence that gonadal steroids may modulate sexually dimorphic spatial ability, through their effects on the nervous system, has accumulated (Dohanich, 2002). The mammalian hippocampal formation appears to be sensitive to a variety of hormones. Steroid hormones released by the gonads and adrenal gland are thought to be transported intact across the cell membrane and attach to intracellular receptors that directly act on the DNA (Thompson, 1993). However, nongenomic mechanisms, whereby steroids interact with receptors on the surface of the cells have recently been suggested (Losel et al., 2003; Losel & Wehling, 2003; Davis et al., 2002; Falkenstein & Wehling, 2000; Falkenstein et al., 2000; Gerdes et al., 2000; Schmidt et al., 2000; Wehling, 1997; Wehling, 1996; Wehling, 1995; Wehling, 1994; Breuner et al., 1998).

The mammalian hippocampus expresses receptors for gonadal steroids (Parsons et al., 1982; Loy et al., 1988) as well as thyroid hormone (Dratman et al., 1982), glucocorticoids (McEwen et al., 1986), mineralocorticoids (Arizza et al., 1987). Changes in the circulating levels of these hormones result in dramatic alterations in hippocampal physiology (Terasawa & Timiras, 1968; Vicedomini et al., 1985; Diamond et al., 1989; Sloviter et al., 1989). Therefore, it is possible that sexual dimorphisms in rodent hippocampal size (Pfaff, 1966), weight (Madeira et al., 1993) and neuronal morphology (Loy, 1986; Diamond, 1987; Juraska, 1991; Madeira et al., 1991; Roof &
Havens, 1992; Tabibnia et al., 1999; Kolb & Stewart, 1991; Kavaliars et al., 1998; Juraska et al., 1989) result from sex differences in gonadal hormone concentrations.

While the number and range of hormones produced by each sex are virtually the same, females usually produce a preponderance of oestrogen and progesterone, secreted by the ovaries, while males produce more androgens (e.g. testosterone), secreted by the testes (Gross, 1992). Spatial ability is influenced by both organisational and activational actions of sex hormones. Organisational effects occur during early development (before or soon after birth) and result in 'permanent designation of sex or of a sexual characteristic' (Norris, 1997). Activational hormones refer to those circulating in the subject at the time of testing; these tend to have smaller, transient effects, which fluctuate with hormone concentration. For example, sex differences in spatial abilities have been found in rats gonadectomised as adults even though this manipulation removed the major activational source of sex hormones (Williams et al., 1990; Luine & Rodriguez, 1994). In addition, sex differences can also be found in prepubertal rats, which have low levels of activational sex hormones (Roof, 1993a; Kanit et al., 1998).

In this thesis, I concentrate on the influence of androgens on spatial learning and memory abilities since there is a more limited literature on the role of androgen in learning and memory than there is for oestrogen. In the following review, however, I not only address the influence of testosterone (T) on cognitive abilities but also refer to studies that have examined the relationship between oestradiol (E₂) and spatial learning and memory abilities because of the relationship between the two hormones. Circulating T can be converted to active androgenic and oestrogenic metabolites in the brain. The enzyme 5α-reductase converts T into 5α-dihydrotestosterone (DHT), and aromatase converts T into E₂ (see figure 8). Therefore, any effects of the steroid on spatial learning and memory may be mediated through activation of not only androgen, but also oestrogen receptors in the hippocampus.
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TESTOSTERONE

5α-Reductase

5α-DHT

Nucleus

Androgen Receptor

DNA

Aromatase

Oestrogen Receptor

Oestradiol

Redrawn from Brown (1994)

Figure 8: The metabolism of testosterone (T) into 5α-dihydrotestosterone (DHT) and oestradiol (E₂)

1.5.1 Organisational effects

Studies of the intrauterine environment in mammals facilitate the examination of organisational steroid effects on spatial learning and memory. Foetuses are exposed to testosterone from up to three sources – from the mother, from their own developing gonads (if they are male), and from male littermates. The position of a foetus relative to its intrauterine neighbours affects its physiological, morphological and behavioural characteristics (Vom Saal & Bronson, 1980; vom Saal & Dhar, 1992; Clark et al., 1991; Clark & Galef, 1998b; Clark & Galef, 1998a; Clark et al., 1998; Jones et al., 1997; Sherry et al., 1996; Forger et al., 1996; Clark & Galef, 1995; Clark & Galef, 1994; Clark et al., 1993; Clark et al., 1992b; Clark et al., 1992a; Vomachka & Lisk, 1986). Individuals from litters with a high male to female ratio will be exposed to more prenatal T than individuals from female-biased litters. T diffuses from the blood of male foetuses into the amniotic fluid and, from there, into the circulation of the adjacent foetuses, resulting in an increase in the concentration of T in their blood (Vom Saal & Bronson,
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1980; vom Saal & Dhar, 1992; Even et al., 1992). For example, female mice located between two males *in utero* have higher amniotic fluid and blood concentrations of T, and lower concentrations of E2, than females with two female neighbours (Vom Saal & Bronson, 1980). Similarly, male and female Mongolian gerbil (*Meriones ungingulatus*) foetuses that occupy an intrauterine position between two males have greater blood concentrations of T than do their same-sex siblings between two females (Clark et al., 1992b).

The difference in exposure to sex hormones due to intrauterine position has been shown to influence postnatal spatial ability. Williams and Meck (1991) found that female rats from litters with a high proportion of male foetuses performed more accurately during the acquisition of a 12-arm radial maze than did females from litters with a low proportion of male foetuses. Similarly, adult meadow voles of both sexes from a male-biased litter performed significantly better on a Morris water maze task than voles from a female-biased litter (Galea et al., 1994b; Galea et al., 1996).

As well as producing differences in tests of spatial ability (Galea et al., 1994b; Williams & Meck, 1991), perinatal exposure of foetuses to androgens can produce sex differences in hippocampal anatomy (Roof & Havens, 1992; Roof, 1993b; Sherry et al., 1996a). For instance, male Mongolian gerbils have a larger hippocampus relative to the rest of their telencephalon than do their female counterparts. Intrauterine position and associated differences in exposure to steroid hormones can affect this relationship (Sherry et al., 1996). Males flanked by two females *in utero* have a relative hippocampal size that does not differ from that of females positioned between two males *in utero*. Male rats have larger CA1 and CA3 pyramidal cell volumes (see figure 7) than females. These sex differences are steroid-sensitive as prenatal administration of T to females and flutamide (an anti-androgen) to males reverses the sex difference in CA1 and CA3 pyramidal cell morphology (Isgor & Sengelaub, 1998). Similarly, the positive correlation between the size of the dentate gyrus granule cell layer (DG-GCL; see figure 7) of the hippocampus and rodent maze-learning ability is mediated by T. Male rats have a larger and thicker DG-GCL than females and also outperform females on tests of spatial ability. However, administration of T propionate (an oil-based injectable T) to
neonatal female rats improved their performance as adults in the MWM and caused the sexually dimorphic DG-GCL of the hippocampus to more closely resemble that of a male's (Roof, 1993b; Roof & Havens, 1992; Frye, 1994; Williams et al., 1990; Stewart et al., 1975; Joseph et al., 1978; Dawson et al., 1975).

Increased exposure to androgens does not necessarily have further masculinising effects on spatial ability and the hippocampus of males. For example, the administration of T to newborn male rats decreases adult male spatial ability (Roof 1993b, Dawson, 1993). T level frequently shows an inverse-U shaped relationship to spatial ability (Gouchie & Kimura, 1991; Kimura & Toussaint 1991; Nyborg 1983, 1984), suggesting that there is an optimal level of organisational T for maximum adult spatial ability (see figure 9). There may also be an optimal level of female-typical sex hormones for peak performance on spatial tasks. E2-administration often has the same effect as T, suggesting that it is not T itself, but its metabolite, that causes the effect. Hull et al. (1980) gave female rats progesterone implants during pregnancy and lactation, and found that the male offspring had impaired spatial performance on a maze task when tested as adults, compared to the offspring of dams given placebo implants. The spatial ability of female offspring was unaffected by progesterone treatment, presumably because the treatment did not elevate levels beyond the optimal for spatial task performance (Hull et al., 1980).

![Figure 9: The bi-phasic relationship between T-level and spatial ability](image-url)
Organisational levels of sex hormones can also indirectly affect spatial learning and memory abilities through influencing cue use. Two categories of spatial cues have been described: geometric (i.e., where information about distance and angle relationships of the environment, relative to an important target, may be encoded) and landmark (i.e., encoding the location of important targets in reference to salient objects in the environment) (Williams & Meck, 1991). The act of recalling the location of an object is often more accurate if the former strategy is used, especially if a navigational error is made as landmark cues are more susceptible to change and can therefore become misleading (Saucier et al., 2002). The sexes differ in the extent of reliance on each cue. Male rats predominantly rely on geometric cues when navigating in the RAM. Females, on the other hand, use a combination of both landmark and geometric cues to solve the spatial task. This sex-specific reliance on cues seems to be influenced by sex hormones. Adult female rats injected with E_2_ neonatally (which has masculinising effects) employ a male-type strategy to solve the maze: they rely on geometric cues. Conversely, adult male rats, castrated at birth, behave like normal females: they use both landmark and geometric cues (Williams & Meck, 1991).

### 1.5.2 Activational effects

The natural variation in levels of sex hormones enables correlations to be drawn between activational hormone levels and spatial learning and memory abilities. Natural seasonal fluctuations in T and E_2_ are also correlated with spatial ability. Sex differences are often only reported during the breeding season, when sex hormone levels are elevated. For example, the sexual discrepancy in maze-learning ability in meadow voles is only found during the breeding season (Gaulin & FitzGerald, 1986; Galea et al., 1995). Male deer mice (*Microtus sp.*) in photoperiodically induced breeding season (reproductive) condition (associated with high T levels) outperform males in non-breeding (non-reproductive) condition (associated with low T levels) when tested in the MWM (Galea et al., 1994a; Galea et al., 1995). The positive relationship between hippocampal size and activational T may, indeed, underlie this performance discrepancy.
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The study of the activational effects of E2 is also facilitated by the examination of spatial learning and memory abilities across the fertility cycle in females. However, such studies have yielded inconsistent results. Studies usually find a negative relationship between E2 level (with high levels occurring during the pro-oestrus or luteal phase) and spatial ability (Hampson & Kimura, 1988; Frye, 1994; Moody, 1997; Hausmann et al., 2000; Lacreuse et al., 2001; McCormick & Teillon, 2001; Phillips & Silverman, 1997; Warren & Juraska, 1997). For example, female meadow voles with naturally high E2 levels exhibit longer latencies to find the hidden platform in a MWM than females with low E2 levels (Galea et al., 1995; Galea et al., 1996). However, the opposite result has also been reported frequently: spatial ability is often found to be enhanced during high E2 cycle phases (Healy et al., 1999; Postma et al., 1999; Frick & Berger-Sweeney, 2001). For example, Frye (1994) gave hormone implants that mimicked hormone levels of the oestrus (low E2) and di-oestrus (intermediate E2) phases of the rat oestrous cycle to ovariectomised female rats. The 'oestrus' subjects were impaired on a water maze task compared to the 'di-oestrus' subjects (Frye, 1994).

Studies of changes in hippocampal physiology across the fertility cycle show that the density of dendritic spines on hippocampal CA1 pyramidal cells (see figure 7) fluctuate with hormone concentration. The lowest density is found in ovariectomised rats, and rats in the low E2 and progesterone phase of their cycle (Woolley & McEwen, 1992; Gould et al., 1990). Furthermore, the modifications occur quickly; spine density declined by as much as 30% over the 24 hour period between the natural late pro-oestrus and late-oestrus phases of the cycle (Woolley et al., 1990). Through experimental manipulation, even shorter-term (5 hours) in vivo morphological sensitivity of hippocampal neurons to fluctuations in ovarian steroid levels have been noted (Gould et al., 1990).

There are also a number of studies that have found no relationship between fertility cycle and spatial ability, including studies that assayed hormone levels to determine cycle stage. Therefore, the lack of a relationship between spatial ability and stage of fertility cycle cannot be attributed solely to inaccurate methods of cycle phase determination (Gordon & Lee, 1993; Berry & McMahan, 1997; Stackman et al., 1997;
1. Introduction

Epting & Overman, 1998; Halpern & Tan, 2001; Mumenthaler et al., 2001). It is not clear whether the inconsistency in observing the spatial ability fluctuations is due to variation in experimental testing conditions, or because the effect is small and easy to miss.

In summary, the activational effects of steroid hormones on spatial learning and memory abilities in mammals are complex and although they may only be moderate in magnitude there is much evidence to show that they can modulate performance on spatial tasks (Dohanich, 2002).

1.6 The effect of sex hormones on avian spatial learning and memory abilities

In birds, both food storing and brood parasitism have been useful for relating neuroanatomy to the spatial abilities required for various behaviours. The hoarding studies have made use of the fact that some species of birds store food items in a large number of separate caches, and retrieve them accurately hours to months later. In these species, the hippocampus is enlarged relative to the rest of the telencephalon as compared to non-storing relatives (Krebs et al., 1989; Brodbeck, 1994; Biegler et al., 2001; Healy & Krebs, 1992, 1996; Sherry et al., 1989). Lesions have shown that the hippocampus must be intact for successful retrieval performance, but not for active storage activity or for performance of non-spatial tasks (Sherry et al., 1989). Storing species have a better performance in spatial memory tasks than closely related non-storing species (see Clayton & Krebs 1995 for a review), supporting the hypothesis that the larger hippocampus in food-storing birds is associated with an enhancement of either ability or reliance on spatial memory.

Despite the vast number of studies that have looked at the influence of gonadal hormones in mammals, the influence of steroid hormones on spatial learning and memory abilities in these birds is yet to be addressed explicitly. Only two studies have tested for sex differences in avian spatial ability. In the first, spatial memory for cache sites was compared in male and female black-capped chickadees (Parus atricapillus). No sex differences were found in the relative size of the hippocampus, in food-caching
behaviour or memory for cache location (Petersen & Sherry, 1996). In a subsequent study, the ability of male and female parasitic shiny cowbirds (Molothrus bonariensis) to complete a food-finding task was compared. Parasitic cowbirds have a larger hippocampus than nonparasitising species with this volume being larger in the sex (females) involved in nest searching and recovery (Astié et al., 1998; Clayton et al., 1997; Sherry et al., 1993; Reboreda et al., 1996). Given the nature of the differences in neuroanatomy and behaviour, females were expected to have a better memory performance than males, particularly in tasks requiring the use of spatial information. There were, however, no differences in performance between the two sexes on the spatial task (Astié et al., 1998). However, the timing of this study may have resulted in the failure to find a sex difference in spatial ability. Although the birds were maintained under a breeding season photoperiod, levels of sex hormones may not have reflected the natural breeding season elevation and hippocampal dimorphisms may not have developed in captivity to the same extent as in nature.

There are instances, however, where the influence of gonadal hormones has been studied in birds. Plasticity in the avian brain is found in the avian song control system, which has become a leading model of morphological and functional plasticity in the adult central nervous system (CNS). The volumes of entire brain regions that control song increase dramatically in anticipation of the breeding season. These volumetric changes are induced primarily by increases in circulating sex steroids and are accompanied by increases in neuronal size, number and spacing (Smulders, 2002; DeVoogd, 1991; DeVoogd et al., 1991; DeVoogd, 1990; Kirn et al., 1989; DeVoogd, 1986; Tramontin & Brenowitz, 2000; Smith et al., 1997a; Smith et al., 1997b; Brenowitz, 1992; Deviche & Gulledge, 2000; Gulledge & Deviche, 1998; Soma et al., 2002). In several species, these structural changes in the song control circuitry are associated with seasonal changes in song production and song learning (Tramontin & Brenowitz, 2000).

Whether the avian hippocampus is as sensitive to fluctuations in gonadal hormone levels as the song control circuitry remains to be seen. The potential for gonadal hormones to mediate hippocampal physiology exists as the avian hippocampus contains
both androgen and oestrogen receptors (Gahr & Metzdorf, 1997; Gahr, 2001).
Suggestive evidence for a role of gonadal hormones in influencing hippocampal anatomy comes from a finding of a sex differences in hippocampal size in two species of European corvid, magpies (*Pica pica*) and jackdaws (*Corvus monedula*), where males have a slightly larger relative hippocampus than females (Healy & Krebs, 1992).

1.7 The influence of adrenal steroids on spatial learning and memory

It is not only gonadal steroids that affect mammalian spatial learning and memory abilities in a concentration-dependent fashion. The glucocorticoid hormone corticosterone (CORT), commonly termed the stress hormone, also has a biphasic relationship with spatial learning and memory abilities (Schantz & Widholm, 2001; Shors et al., 1992). An elevation in CORT level, often achieved through exogenous administration of glucocorticoids or through stressing protocols (e.g., restraint), is detrimental to maze-learning ability in rats (Arbel et al., 1994; Bodnoff et al., 1995; Bohus, 1994; De Kloet et al., 1988; Kerr et al., 1991; Endo et al., 1996). For example, 21 days of daily restraint stress is associated impaired RAM performance in male rats (Luine et al., 1994). Similarly, spatial orientation learning is impaired in rats with abnormally low levels of CORT (usually achieved through removal of endogenous glucocorticoids by adrenalectomy) (Oitzl & De Kloet, 1992). Conversely, intermediate levels of stress can have a facilitative effect on spatial learning and memory abilities. For example, daily restraint stress for 13 days caused an enhancement RAM performance in rats (Luine et al., 1996).

Stress effects on spatial learning and memory abilities are mediated through the binding of glucocorticoids to receptors in the hippocampus. Two types of adrenal hormone receptor have been identified in the mammalian hippocampus; Type I, mineralocorticoid receptors (MR) and Type II, glucocorticoid receptors (GR). At basal levels, Type I receptors are activated and the survival of neurons in the dentate gyrus (see figure 7) is enhanced (Woolley et al., 1991), optimising performance on hippocampal-dependent tasks. When Type I receptor antagonists are administered, or
endogenous glucocorticoids are removed, spatial orientation in rats is impaired (Douma et al., 1998; Oitzl & De Kloet, 1992). It is only through an increase in endogenous glucocorticoids that Type II receptors become activated. The occupation of Type II receptors exacerbates the destructive effects of certain neurotransmitters (particularly excitatory amino acids) on hippocampal neuronal survival (Sapolsky, 1990; Lawrence & Sapolsky, 1994; McEwen & Sapolsky, 1995), and have a similar detrimental effect on spatial learning and memory to hippocampal lesions (Vidal et al., 1986). The activation of Type II receptors has an adverse effect on spatial learning and memory abilities through a resultant decrease in long-term potentiation (LTP; an increase in synaptic efficacy involved in the regulation of learning and memory formation) (Diamond et al., 1992; Foy et al., 1987; Shors et al., 1992).

The influence of CORT on spatial learning and memory abilities in birds has been addressed in studies of food-storing species, where an elevated level of CORT has been found to improve memory retrieval. For example, Pravosudov and Clayton (2001) experimentally demonstrated that a long-term limited and unpredictable food supply resulted in moderately elevated CORT levels (lower than standardised stress response) in mountain chickadees. Such small but chronic elevations in CORT levels triggered by an unpredictable food supply were also correlated with enhanced cache retrieval efficiency and spatial memory performance (Pravosudov & Clayton, 2001). However, the effects of CORT on avian learning and memory abilities are not unanimous as the ability of black-legged kittiwakes (Rissa tridactyla) to solve both a visual food-finding task and a test of spatial ability was compromised by experimental elevation of CORT during early development (Kitaysky et al., 2003), possibly suggesting different effects of organisational and activational levels of CORT.

To summarise, the influence of steroid hormones on spatial learning and memory abilities in mammals is well documented. However, although a number of studies have examined interspecific variation in avian spatial learning and memory ability, the influence of steroid hormones on such capacities has received little attention.
In this thesis I will address some of the major questions raised by this review, namely:

1. Are there sex differences in avian cue preference? The way in which an animal solves a spatial problem has received much attention. Through research with humans and rodents the terms 'male-typical' (i.e., reliance on geometric cues) and 'female-typical' (i.e., reliance on geometric and landmark cues) cognitive style have been coined. I aimed to determine whether this sexual dimorphism in cue preference occurs in birds in a one-trial associative food-finding task.

2. Are there sex differences in spatial ability? By maintaining birds under a breeding season (long-day) photoperiod, I hoped to increase endogenous T levels in males significantly above those in females, to levels comparable with the breeding season maxima. I predicted that higher T levels in males would lead to a sex difference, favouring males, in performance on a one-trial associative spatial memory task.

3. Does testosterone have activational effects on spatial learning and memory abilities? Compared to oestrogen (E$_2$), there is a more limited scientific literature on the role of androgen in learning and memory. By manipulating levels of T, E$_2$ and DHT, I hoped to determine not only if, but also how, androgen influences performance on a spatial memory task.

4. Does corticosterone interfere with the relationship between androgens and spatial ability? Corticosterone is a steroid hormone that, like testosterone, has a biphasic relationship with spatial learning and memory abilities. Through taking advantage of the production of zebra finches selectively bred for peak corticosterone level, I aimed to investigate the relationship between these two steroids and spatial ability by presenting birds with a one-trial associative food-finding task.
Chapter 2. Cue preference in great tits

2.1 Abstract

In rodents, gonadal hormones influence the choice of cue used to solve spatial tasks. A sex difference in cue preference could underlie the sex difference in spatial learning and memory abilities since spatial ability is, to some extent, reliant on appropriate cue use. In this study, I used a one-trial associative food-finding task to test for sex differences in cue use in the great tit (Parus major). Birds were trained to a compound stimulus where both colour and spatial cues could be used to locate the food reward. When the cues were dissociated in probe trials, I did not find a sex difference in cue use in Experiment 1. The distribution of choices of location and colour cues were equal and did not differ between the sexes. A short-coming in the experimental design meant that it was impossible to determine whether the birds performed at random or actually had no cue preference. In Experiment 2 a distractor (an unrewarded well) was added to the probe trial to determine whether the association between sample attributes and reward had been learnt. With this modified experimental design, the overall distribution of responses of birds was significantly different from random but did not differ between the sexes. Both sexes exhibited a preference for the location cue over the colour cue and distractor.

2.2 Introduction

The difference between the sexes in spatial learning and memory abilities is mediated through the action of gonadal hormones. Androgens and oestrogens influence spatial ability in two ways. Firstly, gonadal steroids alter hippocampal morphology; the dentate gyrus-granule cell layer (DG-GCL) is larger and thicker in male rodents than female conspecifics (Roof, 1993a; Roof & Havens, 1992). This sexual dimorphism is influenced by testosterone (T), as giving neonatal female rats T propionate increases the size of their DG-GCL, such that it more closely resembles that of a male, and also improves their adult performance on the MWM (Roof &
Chapter 2. Cue preference in great tits

Havens, 1992; Roof, 1993b). Secondly, gonadal steroids influence how a spatial task is solved (i.e., cue use) (Williams & Meck, 1991; Williams et al., 1990).

2.2.1 Cue use

To some extent, spatial ability is reliant on appropriate cue use. Two general categories of spatial cues have been described: geometric and landmark (Williams & Meck, 1991). Geometric cues refer to information about distances and angles between landmarks in the environment, relative to a goal, while landmark cues are those features of salient objects in the environment close to the goal (Williams & Meck, 1991). Recalling a goal’s location is often more accurate if the former strategy is used, especially if a navigational error is made, as landmark cues are more susceptible to change and can therefore become misleading (Saucier et al., 2002).

Williams et al. (1990) hypothesised that the sexual discrepancy in rodent maze-learning ability may be due to a sex difference in cue use. Male rats performed less well on a maze task when geometric cues had been manipulated (through changes in the position of a curtain surrounding the maze), appearing largely to ignore manipulations of landmarks. Females, on the other hand, attended to both landmark and geometric cues. Thus, males may outperform females on the RAM when geometric cues remain reliable as females learn about more cue types and thus take longer to learn appropriate associations (Williams et al., 1990).

Subsequently, it was shown that this sex-specific reliance on cues was under the influence of gonadal hormones. Adult female rats injected with oestrogen neonatally (which has masculinising effects) employed a male-type strategy to solve a radial arm maze: they relied primarily on geometric cues. Conversely, adult male rats that had been castrated at birth behaved like normal females: they used both landmark and geometric cues (Williams & Meck, 1991). The results of this study suggest that rodent sex differences in cue use are determined by early exposure to sex hormones.

Studies of the intrauterine environment have provided further support for this argument. Rats exposed to high levels of androgens in utero (i.e., males and offspring of both sexes from a male-biased litter) solve spatial tasks by attending to a single cue type (Williams et al., 1991). Like the males in the Williams et al. (1990)
study, these rats make errors when the geometric cues are altered regardless of the arrangement of landmark cues. Exposure to low levels of androgens in utero (i.e., females and offspring of both sexes from a female-biased litter) does not result in cue preference and both geometric and landmark cues are used to solve spatial tasks (Williams & Meek, 1991). Therefore, early hormone exposure may modify the acquisition of the radial arm maze task by altering associational/perceptual biases which influence the number or types of cues used.

Single unit recording techniques demonstrate that there are at least two types of cells in the hippocampus that are sensitive to different stimuli: G cells are sensitive to geometry and L cells are sensitive to landmarks (O'Keefe & Nadel, 1978). At birth males and females have both G and L cells. If the hippocampus is exposed to androgen, G cells grow and make more synaptic connections. Without androgen, G and L cells make equal connections. This could explain why geometric cues are chosen by males and why there is no cue preference in females.

Lesions of the entorhinal complex, a brain area with both afferent and efferent connections with the hippocampus (Burgess et al., 1999), also had a more detrimental effect on the ability of male rats to solve a spatial task than on that of females. There were no sex differences in performance in sham-operated controls (Roof, 1993b). Lesions to the frontal cortex, on the other hand, specifically affected the maze-learning ability of female rats (Kolb & Cioe, 1996). It is possible, then, that the information regarding the different cues may be processed in different brain areas.

2.2.2 Cue preference in birds

Most studies of cue preference in birds have been carried out to examine whether the memory ability of food-storing species differs from that of nonstorers. Food-storing species appear to rely more heavily on spatial cues than colour and/or pattern cues to locate food whereas both cues are equally relevant to non-storing species. The cues examined in avian studies (i.e., location vs. colour/pattern cues) parallel those examined in the rodent studies described above (i.e., geometric vs. landmark cues) but are not entirely analogous. Although colour/pattern cues are relevant to
landmark cue use because the ability to use colour and pattern will affect the ability to identify landmarks, colour/pattern cues may also be used to solve non-spatial tasks (described below).

Brodbeck (1994) investigated what cues were controlling food-finding behaviour in black-capped chickadees (*Parus atricapillus*, a food-storing bird) and dark-eyed juncos (*Junco hyemalis*, a non-storing bird). Each bird was allowed to explore an aviary containing a number of uniquely decorated feeders, one of which was baited. After a retention interval, the bird was allowed to relocate the reward, which was now hidden (see figure 4, chapter 1). The birds could potentially use three different types of cue: global spatial information, the spatial relationships among the feeders themselves, and, because each feeder was uniquely decorated, the colours and patterns on the feeders. Transformations of the feeder array made it possible to determine which cues controlled the food-finding behaviour. Chickadees responded to spatial cues preferentially over colour and pattern cues whereas juncos responded to all types of information equally. The same pattern of species differences have been found in an operant setting (see Brodbeck & Shettleworth, 1995; Shettleworth & Westwood, 2002). Food-storing marsh tits (*Parus palustris*) and jays (*Garrulus glandarius*) also have a stronger preference for spatial cues over featural cues than do non-storing blue tits (*Parus caeruleus*) and jackdaws (*Corvus monedula*) (Clayton & Krebs, 1994a).

Despite these repeated demonstrations of species differences in cue preference, only two studies have examined differences in cue preference within a species. Astié et al. (1998) examined the whether the shiny cowbird (*Molothrus bonariensis*), a brood parasite, displayed sexual differences in a laboratory memory task. During the breeding season, female shiny cowbirds not only have a larger relative hippocampal volume than non-parasitic species, but have a larger relative hippocampal volume than male conspecifics. Owing to these behavioural and neuroanatomical dimorphisms, females were expected to perform better than males, especially at tasks requiring the use of spatial information. In the experiment, birds had to locate the food reward by attending to the appearance or location of a covering disk. Contrary to prediction, females did not perform better than males when food was associated with a specific location but did retrieve food faster than males when food was
associated with appearance cues. Although females were not better than males at using spatial information, in nature their superior ability to use appearance cues could lead to superior performance at spatial tasks (e.g., relocating a tree containing a host nest) (Astié et al., 1998). Female superiority in using appearance cues was also noted by Vallortigara (1996) in a study of cue learning in chicks. Chicks were trained to a compound stimulus where both appearance and location cues could be used to locate a food reward. When the cues were dissociated chicks were trained either to discriminate on the basis of appearance (irrespective of location) or on the basis of location (irrespective of appearance). Trials to criterion (10 consecutive correct choices) were used as the performance measure. Females performed better (took fewer trials to reach criterion) than males on the appearance task whereas males performed better than females when the food reward was associated with a location cue.

The design of the current study is rather different to that of Astié et al. (1998) and Vallortigara (1996). Rather than assess the ability of the subject to remember location versus appearance cues, I aimed to see whether the sexes showed a preference for using a particular cue type (i.e., location versus colour). My experimental design was therefore more comparable to the comparative studies of food-storing and non-storing birds, mentioned above. I examined sex differences in cue preference in a non-storing species, the great tit (Parus major). The advantage of studying a non-storing species is that cue preference is less likely to be influenced by prior experience than it would be in a food-storing species, as it is not clear when their preference for spatial cues develops. A one-trial associative memory task permitted the assessment of which cues were governing the birds' food-finding behaviour. Initially, the reward could be found by attending to both location and colour cues (i.e., a compound stimulus), with colour perception in birds being well-documented (e.g., Finger & Burkhardt, 1994). Through altering the task on probe trials (dissociating the cues) it was possible to examine cue preference. In other words, I was able to find out what each sex remembered.

The birds were maintained on a breeding season (long day) photoperiod. Therefore, males were assumed to have higher levels of circulating T than females,
as photostimulation causes an increase in endogenous T (Silverin & Sharp, 1996; Silverin & Goldsmith, 1997).

2.3 Experiment 1

2.3.1 Introduction

This experiment was carried out as a pilot study. I initially wanted to see whether the great tits could be trained to complete the task. Secondly, I wanted to assess how well the design of the experiment satisfied a test of cue preference.

In Experiment 1, on the basis of rodent study results that show that a sex difference in cue preference, I predicted that males would preferentially use the location cue over the colour cue, whereas females would show no cue preference.

2.3.2 Materials and methods.

Animals. The subjects were two male and two female great tits (all wild-caught in deciduous woodland in Edinburgh, mid Lothian; females in March 1999; males in April 2000). Birds were housed individually in wire-mesh cages (77cm long x 44cm wide x 44cm high) and were fed daily with ad libitum water and an insectivorous bird food mixture (Orlux, Sunring Cooke, Greasbrough, Rotherham, U.K.), supplemented by peanuts, sunflower seeds and wax moth larvae. They were maintained on a 13.5:10.5 h light: dark cycle (6.30am lights on 8pm lights off) and under a temperature range of 16-19°C. For both training and experiments, birds were deprived of food at 8am each morning and provided with fresh food when their session was complete. Training and testing began in April 2001, at 10am. Birds tested later in the day were provided with nuts through the day.

Apparatus. The experimental tray consisted of a 29cm x 22cm Perspex board containing 48 circular wells (1cm diameter; 1cm deep) arranged in an eight by six array. The wells were surrounded by Velcro, to which square pieces of felt, measuring 2.5cm x 2.5cm were attached (see Figure 1).
Chapter 2. Cue preference in great tits

Training. To begin, the experimental tray was placed into the centre of the birds’ home cage for twenty minutes with half of the wells containing reward (a small piece of pine nut) to allow the birds to familiarise themselves with feeding from the tray. Gradually, flaps were introduced to cover the rewarded wells, at first partially, and later fully (see Figure one). The number of rewarded wells was gradually reduced to one, as was the time the tray remained in the cage. Training was complete when the bird removed at least three flaps to obtain a reward within five minutes. After 15 days of training, all birds were lifting the flaps to obtain the reward, within 5 minutes with an observer present.

![Figure 1: A schematic of the experimental tray used in training](image)

Testing. Each day, the tray was placed into the bird’s cage with two fully covered wells. The wells were covered by different coloured felt and only one was rewarded (with two pieces of pine nut; Figure 2a). The bird was allowed to find and eat part of the reward (birds took one piece of pine nut back to their perch to consume), completing the sample phase of the experiment. The tray was then removed and after a 5 minute retention interval, the tray was placed back into the bird’s cage. This second presentation of the tray represented the choice phase of the experiment. The wells were covered as before, again with only one containing a reward (fig. 2a). However, in the choice phase, the bird was allowed to eat the reward only if it went to the rewarded well first; if not, the tray was removed. The sample and choice
phase constituted one “trial”. Each bird received two trials every day, separated by two hours.

Each bird received a probe trial (Figure 2b) once it had chosen the rewarded well in the choice phase on eight occasions in ten consecutive trials with a given combination of coloured flaps and locations (i.e., an “array”). The minimum number of trials with each array was ten (i.e., 5 days of testing). In the probe trial the cues were dissociated and the bird had to choose between correct colour and correct location (both colour and location were rewarded). Choosing the previously rewarded well would indicate a preference for spatial location, whereas choosing the previously rewarded colour would signify a preference for the appearance cue. After making a choice in the probe trial, the bird was presented with a different array and tested in the same way. Each bird received eight different arrays in total. The arrays differed not only in the colour of felt flaps used but also in position of rewarded and distractor wells.

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**Figure 2a. Sample & Choice**

**Figure 2b. Probe**
2.3.3 Results

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Table 1: Distribution of responses across eight probe trials

The number of times the birds chose the correct location or correct colour in the probe trials was analysed. As there were eight trials in total, a uniform distribution of choices (i.e., choice of colour four times and choice of location four times) would indicate no cue preference. A chi-squared analysis was used to see if there was any sexual discrepancy in cue preference. There were no differences in cue use between the sexes ($\chi^2 = 0.533$, $p = 0.47$; see figure 3). The sexes were pooled to determine whether there was an overall cue preference. Even though birds tended to choose the colour cue more frequently than the location, there was no overall cue preference ($\chi^2 = 2.0$, $p = 0.16$).
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Figure 3: Average (mean ± s.e.) proportion of total choices of location and colour cues on eight probe trials (males n = 2, females n = 2). No cue preference was found in males or females.

2.3.4 Discussion

No sex difference in cue preference was found in Experiment 1. Neither sex had a cue preference. The results of this pilot study, however, must be interpreted with caution as there was a potential short-coming in the methodology. Although the birds were trained to a criterion, the lack of a “distractor” (i.e., an unrewarded well) in the probe trial may have meant that the choice of a particular cue did not reflect cue preference (or lack thereof). The probe trial itself did not demonstrate that the birds had made any association between the reward and colour and/or location cue. Birds could have reached criterion by using either cue or both cues together. By dissociating cues on probe trials I may have made the task too difficult, i.e., birds may have looked for both cues together (i.e., the compound stimulus) and, when they failed to find them, may have chosen one or other cue randomly.
2.4 Experiment 2

2.4.1 Introduction

To test whether birds were using colour and/or location cues in the probe trial (i.e., not lifting flaps at random), a third, unrewarded well, a "distractor", was included in the probe trial of Experiment 2. If the bird lifted the cover of the distractor well as frequently as those of the rewarded wells, it would indicate that it was not using colour or location cues in the probe trial.

The sample size of birds was increased to improve the power to detect cue preferences.

In Experiment 2, I made the same predictions that I had made in Experiment 1, that males would show a preference for the location cue and females would show no evidence of cue preference.

2.4.2 Materials and methods.

Animals. The subjects were six female and six male great tits (all wild-caught in deciduous woodland in Edinburgh, mid Lothian; Three females caught in March 1999 and three between January and April 2001. Three males were caught in April 2000 and three in January 2001). All birds received the same training (described previously). None of the birds were subjects in Experiment 1. Training and testing began in May 2001, at 10am. Birds tested later in the day were provided with nuts through the day.

Apparatus. As in Experiment 1.

Testing. Each bird received ten days of testing. The location of the rewarded well and colour of felt flaps were changed everyday (i.e., a different array was presented each day). The tray in which one well was covered with felt and contained a reward (a small piece of pine nut) was placed into a bird's home cage. The bird was allowed to find and eat the reward and the tray was removed. After three minutes the tray was placed back into the bird's cage with the reward in the same location and covered by the same coloured flap. This procedure was repeated until the bird had
received ten presentations of the tray (fig. 4a). On the eleventh presentation of the tray, the probe trial, a further two wells were covered. The previously rewarded well was covered with a novel-coloured felt and contained a reward (i.e., correct location), the coloured felt from the initial ten sample presentations covered a different well which was rewarded (i.e., correct colour), and a third, novel-coloured, piece of felt covered an empty well not used before (fig. 4b).

The choice made by a bird could be used to determine its cue preference. Choosing the previously rewarded well would signify a preference for spatial location. Choosing the felt used in the initial ten sample presentations of the tray could be suggestive of a colour cue preference. Finally, choosing to lift the novel-coloured piece of felt covering a well not used before would suggest that the bird had not learnt the association between reward and either cue.

**Data Analysis.** A uniform distribution of responses among all three stimuli would indicate a complete lack of control by either location or colour (i.e., random choice). A distribution skewed toward either location or colour would indicate control by that stimulus. Finally, a distribution with high frequencies in both location and colour relative to the distractor would show control by both features. Probe trial response distributions were analysed using the $G$ statistic (Sokol and Rohlf, 1981; as used by Brodbeck and Shettleworth, 1995).
2.4.3 Results.

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<td>Grand total</td>
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Table 2: Distribution of responses across ten probe trials.

On probe trials, both sexes chose the stimulus in the location of the sample most often. The individual distributions of choices are shown in Table 2. The overall distribution of probe choices differed significantly from random ($G(2) = 15.20$, $p<0.001$) and the distributions did not differ between the sexes ($G(2) = 1.94$, $p>0.1$). A distribution that is different from random could still, in effect, indicate a lack of cue preference, as high frequencies of choices of both location and colour cues relative to choice of the distractor would show control by both cues. If the responses to the distractor stimulus are removed from the table, it is possible to see whether the sexes differed in their distribution of responses when they chose a well with one of the sample’s attributes. Such an analysis tests for cue preference, i.e., when birds picked a rewarded well, were they more likely to pick it based on colour or location? The overall distribution of correct choices, consistent with one of the sample attributes, differed significantly from uniform ($G(1) = 13.82$, $p<0.001$) and there was no difference in distribution between the sexes ($G(1) = 1.28$, $p>0.1$). Birds chose the well in the correct location significantly more often than they chose the well bearing the correctly coloured flap (see figure 5).
2.4.4 Discussion

Overall, the responses of the birds were significantly different from random. Both sexes showed a preference for the location cue, and picked the correct colour with approximately the same frequency as the distractor. This differs from the trend in Experiment 1, where both sexes tended to pick the colour cue more frequently than the location cue. Differences in the experimental design may have led to the dichotomy in results.

2.5 General Discussion

In Experiment 1, I did not find any evidence of cue preference in the great tit. A problem with the experimental design did not allow me to distinguish between equal choice of a stimuli and random performance (i.e., no cue preference). Although the birds were trained to a criterion, without the inclusion of a distractor (an unrewarded well) in the probe trial it was not possible to ascertain whether the birds had learnt the association between attributes of the sample and reward. By incorporating a
distractor in the design of Experiment 2, I was able to make this distinction. A uniform distribution of choices of colour and location cues and the distractor would have indicated random performance. Preferential choice of cues consistent with one of the features of the sample, on the other hand, would suggest learning. In Experiment 2, the birds performed better than would be expected by chance overall. In other words, their distribution of responses to the three stimuli was significantly different from uniform. Both sexes overwhelmingly chose the location cue over the colour cue and distractor; there was no overall sex difference in choice of cue.

It is possible that birds showed a preference for the location cue as this was deemed more reliable than colour. A colour used to cover a distractor well in one trial may have been used to cover a rewarded well in a subsequent trial, thus if a bird had picked a distractor in one trial, it may have avoided that colour on subsequent trials. However, the same argument could be applied to use of the location cues although unique locations were used on every trial, a correct location may have been close to a distractor on a previous trial. Therefore, it was unlikely that birds considered the location cue to be more reliable but, to avoid this problem in future experiments, a unique combination of coloured flaps could be used in each trial. In addition, through incorporating a training criterion, such as having a distractor well in the sample phase, as in Experiment 1, one would increases the chances of the birds learning the task before being given the probe. Alternatively, the birds may showed a preference for the location cue as blue tits, a non-storing species, have been found to visit previously visited sites, independent of whether they were rewarded or not, whereas food-storing species only revisit previously rewarded sites (Clayton and Krebs, 1993). During training in Experiment 1, the birds could chose between 2 wells, rather than just 1 as in Experiment 2, which may explain why a preference for the location cue did not emerge in the former experiment.

The differences in experimental design between Experiments 1 and 2, may account for the dichotomy in results in the following ways. In Experiment 1, birds were trained to a criterion and were more experienced with the protocol when given the probe trial. Birds in Experiment 1 tended to opt for the colour cue whereas birds in Experiment 2 preferred the location cue. It is possible then, that location is used preferentially if birds are less familiar with the task. Birds may be quicker to learn
the association between the reward and the location cue than the reward and the
colour cue. Although speculative, this hypothesis could account for why the results
of Experiment 1 were not replicated in Experiment 2. By comparing the number of
trials required to learn the association between colour and reward and location and
reward, differences in rate of cue-learning could be tested.

The results from Experiment 2 contrast with the findings from rodent studies
where males prefer to use geometric cues while females use both geometric and
landmark cues (e.g., Williams et al, 1990; Williams & Meck, 1991). Discrepancies
also exist between my results and the results of comparative studies of food-storing
and non-storing birds. Surprisingly, the great tits in this study behaved rather like
food-storers, despite them being a non-storing species. Food-storing birds respond
preferentially to location cues whereas non-storing species respond equally to colour
and location cues (Clayton, 1995; Brodbeck & Shettleworth, 1995; Shettleworth &
Westwood, 2002; Clayton & Krebs, 1994b). However, reliance on location cues by a
non-storing species, the rufous hummingbird (*Selasphorus rufus*) has been found.
Male hummingbirds primarily used spatial cues to return to previously visited
flowers that contained a reward (Hurly & Healy, 1996).

The finding of a location cue preference in both sexes of a non-storing species
was unexpected. Most comparative studies of food-storing and non-storing species
are carried on birds maintained under a short-day photoperiod or in the autumn, at
the time of peak food hoarding and recovery, and hippocampal enlargement (Clayton
& Krebs, 1994a; Clayton, 1998; Clayton, 1995; Basil et al., 1996; Hampton et al.,
Krebs et al., 1989; Sherry et al., 1989; Shettleworth et al., 1990; Smulders et al.,
2000; Smulders et al., 1995), in the storing species. The birds in this study, on the
other hand, were maintained under a long-day photoperiod, corresponding to a
breeding season, springtime day length. It is therefore possible that an elevation in
sex hormones, resulting from photostimulation, resulted in a location cue preference,
although this cannot be confirmed without plasma radioimmunoassay. Whether sex
hormones influence avian cue preference as they do in mammals (Williams et al.,
1990; Williams & Meck, 1991) remains to be tested. The demonstration that
exogenous androgen administration in females and in males maintained under short-
day photoperiod leads to a location cue preference would give my hypothesis more weight and clearly opens up an avenue for future research.
Chapter 3. Sex differences in spatial ability

Chapter 3. Sex differences in spatial ability in great tits?

3.1 Abstract

In mammals, both organisational and activational effects of sex hormones influence spatial learning and memory and hippocampal anatomy. It is not clear whether sex steroids play a similar role in birds although the avian hippocampus, like the mammalian, contains both androgen and oestrogen receptors. Here, I used the great tit (Parus major) as a model to determine whether there were sex differences in spatial learning and memory abilities, which might be caused by gonadal steroids. Birds were tested on two versions (spatial and visual) of a DNMTS task, presented on computer-controlled touch screen. I predicted that higher testosterone levels in males would result in this sex showing superior performance on the hippocampal-dependent, spatial task. However, I found no sex differences in performance on either task.

3.2 Introduction

Male mammals are better than females at solving spatial problems. For example, male humans outperform females on spatial tasks such as map reading and navigation, tasks requiring mental rotation and judgements about moving objects (Beatty, 1979; Beatty, 1984; Voyer et al., 1995; Silverman & Eals, 1992). Similarly, the majority of maze-learning studies with rodents yield a male-advantage (e.g., Hubbert 1915; Gaulin & FitzGerald, 1986; Gaulin & FitzGerald, 1989; Gaulin et al., 1990; Barrett & Ray, 1970; Dawson, 1972; Stewart et al., 1975; Joseph et al., 1978; Krasnoff & Weston, 1976; McNemar & Stone, 1932; Sadownikova-Koltzova, 1926; Davenport et al., 1970). Sex differences in spatial learning and memory abilities have been related to dimorphisms in hippocampal morphology. Hippocampal size is usually positively correlated with a superior spatial ability and, in species exhibiting a sex difference in spatial ability, males usually have a larger relative hippocampal volume. For example, during the breeding season male meadow voles (Microtus pennsylvanicus) have a
superior spatial ability and a relatively larger hippocampus than females (Jacobs et al., 1990; Gaulin & FitzGerald, 1986; Gaulin & Fitzgerald, 1989; Gaulin et al., 1990).

Dimorphisms in the structure of the hippocampus (Juraska, 1991; Loy, 1986; Madeira et al., 1991; Roof & Havens, 1992; Roof, 1993a; Roof, 1993b) may be related to gonadal hormone levels. For example, the sex difference in hippocampal size and spatial ability in meadow voles is only witnessed during the breeding season, when testosterone (T) levels are elevated in males. In addition, the width and thickness of the dentate gyrus granule cell layer (DG-GCL) of the hippocampus is larger in male than in female rats. The sex differences in water maze navigation and DG-GCL morphology are both reversible by neonatal T manipulations (Roof & Havens, 1992).

Aside from seasonal studies and hormone manipulation experiments, supporting evidence for the role of T in mammalian spatial learning and memory abilities has come from in utero studies. Intrauterine position, and associated differences in exposure to sex hormones, influences spatial ability. Female rats from litters with a high proportion of male foetuses perform more accurately during the acquisition of a 12-arm radial maze than females from litters with a low proportion of male foetuses (Williams & Meck, 1991). Similarly, male and female meadow voles from a male-biased litter perform significantly better on a Morris water maze task in adulthood than voles from a female-biased litter (Galea et al., 1994).

To my knowledge, avian sex differences in spatial ability have only been investigated in two cases and no sex differences were found (Astié et al., 1998; Petersen & Sherry, 1996). In the first, spatial memory for cache sites was compared in male and female black-capped chickadees (Parus atricapillus). No sex differences were found in the relative size of the hippocampus, in food-caching behaviour or memory for cache location (Petersen & Sherry, 1996). In a subsequent study, sex differences in the ability of a brood parasite, the shiny cowbird (Molothrus bonariensis), to complete a food-finding task were examined (Astié et al., 1998). Parasitic cowbirds have a larger hippocampus than non-parasitising species, with this volume being larger in the sex (females) involved in nest searching and recovery. Given the nature of the differences in neuroanatomy and behaviour, females were expected to have a better memory.
performance than males, particularly in tasks requiring the use of spatial information. The task involved searching for food in a restricted space using either location or appearance cues. Females learnt to retrieve food faster than males when the location of food was indicated by the appearance of a covering disc but there was no sex difference in performance when food was associated with a specific location (Astié et al., 1998).

The influence of gonadal hormones on avian hippocampal morphology and spatial learning and memory abilities has received little attention. Nevertheless, the relationship between spatial ability and hippocampal size is well documented in birds, with relative hippocampal volume increasing with the amount of spatial information processed (Rehkamper et al., 1988; Sherry et al., 1993; Sherry et al., 1992; Sherry et al., 1989; Hampton & Shettleworth, 1996; Healy et al., 1994; Healy & Krebs, 1996; Smulders et al., 2000; Smulders et al., 1995). In addition, like the mammalian hippocampus, the avian hippocampus contains a number of steroid hormone receptors (Gahr & Metzdorf, 1997; Gahr, 2001). Therefore, gonadal hormones have the potential to affect hippocampal morphology and spatial ability, as they do in mammals.

The influence of T on the song control circuitry in the avian brain is well established. Seasonal plasticity and changes in morphology, in addition to changes in response to hormone levels, have been documented (e.g., Nottebohm, 1980). Several lines of evidence strongly suggest that T (or its active metabolites) is the primary physiological cue that mediates the cyclical anatomical changes in song circuitry (e.g., Smith et al., 1997; Tramontin & Benowitz, 2000). For example, T implants increase the size of several song nuclei (e.g., RA, Area X, nXIIIts), regardless of photoperiod, in castrated male Gambel’s white-crowned sparrows (Zonotrichia leucophrys gambelli) (Smith et al., 1997). Therefore, it is plausible that this seasonal variation in T could also affect other brain areas, such as the hippocampus.

This study examines the ability of the great tit, Parus major, to perform two versions (spatial and visual) of a delayed-non-matching-to-sample (DNMTS) task. Although the great tit has been used in studies that have examined memory differences between food-storing and non-storing passerines (e.g., Biegler et al., 2001), intraspecific differences (e.g., sex differences) have not been explored. In this experiment, the ability of birds to
perform a spatial memory task was assessed. Due to the proposed effect of T on spatial learning and memory a sex difference in spatial task performance, favouring males, was expected.

Volumetric studies have shown that T can influence mammalian spatial ability, probably through its influence on hippocampal morphology (Roof & Havens, 1992; Roof, 1993a), but no study has yet determined whether T is associated with a specific aspect of memory. Memory may be broken down into at least three elements: capacity (the number of locations to be remembered), persistence (the duration over which a location is remembered), and resolution (the least distance over which remembered locations can be discriminated or the similarity at which differences in features can be distinguished). In this experiment, the task, presented on a computer touch screen, allowed two of these aspects to be assessed: memory persistence and resolution. Memory persistence was tested by manipulating the retention interval between the sample and choice phase, using a titration procedure. The resolution of memory was tested by varying the similarity or proximity of the images. McGregor and Healy (1999) found the performance of three species of tit (coal, great and blue tits) to be affected by image proximity. In their spatial delayed-matching-to-sample touch screen task, all birds performed less well when the distractors were close to the target. The performance of food-storing coal tits was less affected by the proximity of the distractors than was that of non-storing great tits and blue tits. However, Biegler et al. (2001) found coal tits to outperform great tits on a task that assessed memory persistence but did not find there to be differences in performance between the species on a task that assessed memory resolution or one that tested memory capacity.

In this experiment, all birds were presented with two tasks, with two squares appearing on the touch screen in each. In the sample phase of the spatial task, the squares were white and differed only in location. Squares disappeared once pecked and, after a retention interval, birds were presented with the choice phase: a square in the one of the earlier locations and a second square in a new location. Birds were rewarded for pecking the square in the new location (the “target”) following a DNMTS design. Spatial resolution was tested by presenting the target close to the location of the sample.
in half the trials, but farther away on others. In the visual task, the two squares were different pictures that, as before, disappeared once they had been pecked. This time, one of the pictures reappeared but the other one was replaced with a new picture in the choice phase. Birds were rewarded for pecking the new picture. Memory resolution was tested by varying the similarity of the pictures. On half of the trials the pictures were similar, but dissimilar on others. Memory persistence on both versions of the task was assessed by titrating retention intervals. After a correct response in the choice phase, the retention interval in the following trial increased, whereas after an error the retention interval was decreased.

I predicted that any sex difference in performance would be specific to the spatial domain. If the sexes differed only in the resolution of spatial memory, they should achieve similar (high) performance levels when items are far apart, but the males should perform better when the items are close together. If males have a longer-lasting spatial memory, then they should achieve longer retention intervals than females.

3.3 Materials and methods

**Animals.** The subjects were ten male and seven female great tits (seven males and five females wild-caught in deciduous woodland in Edinburgh, mid Lothian in January and February 2000 and 2001, and two females and three males caught in Northumberland in March 1999). All birds were housed individually in wire-mesh cages (77cm long x 44cm wide x 44cm high) in a windowless room. Each cage had a removable sliding door (33.5cm x 27.5cm) at the front. Three dowel perches, 1cm in diameter, were attached across the doorway, at 9 cm intervals. Another two perches were fitted across either end of the cage, from front to back, approximately 10cm from the sides of the cage and at least 20cm above the cage floor (see figure 1). Birds were fed daily with ad libitum water and an insectivorous bird food mixture (Orlux, Sunring Cooke, Greasbrough, Rotherham, U.K.), supplemented by peanuts, sunflower seeds and wax moth larvae. They were maintained on a 13.5:10.5 h light:dark cycle and under a temperature range of 16-19°C. During training and experiments, birds were deprived of
food at 8am each morning and provided with fresh food when their session was complete. Training and testing began in October 2001, at 10am each day. Subjects received the equivalent of approximately one peanut in rewards during a typical experimental session. Birds tested later in the day were provided with nuts through the day.

Figure 1: The experimental set-up

Apparatus. The apparatus consisted of Acorn A7000+ processors and monitors (21cm x 28cm). The touch screens (21cm x 28cm) were calibrated to match the area of the monitor screen to which they were attached. Computers were placed on adjustable shelving units that could be aligned with the height of the home cages. This allowed subjects to be tested without the need to remove them from their cages. A standard rat-pellet dispenser (Campden Instruments 442 Pellet Dispenser) was used to deliver food rewards. When a reward (a ca. 20mg piece of peanut) was dispensed, it fell down a plastic tube onto a tray on the cage wall beside the touch screen (see figure 1).

The computer program allowed the persistence and resolution of working memory to be tested in a DNMTS task. All subjects had been involved in a previous study but their initial training in these tasks had been carried out several months prior to the commencement of this study. To re-familiarise birds with the touch screens, all birds were retrained. Subjects carried
out one session of 20 trials each day, 5 days a week, until they reached criterion (defined below).

Each bird completed two tasks, in different domains. In the first, *spatial*-only domain, two white squares appeared on the touch screen. Once the bird aimed a peck at each image, they disappeared and were replaced, after a short retention interval (R.I.), by a square in one of the original locations, the "distractor", and another square in a new location (see figures 2a and 2b). The bird was rewarded for pecking the square in the new location, the "target", which could only be identified by its location.

![Figure 2a: Sample phase](image)

![Figure 2b: Choice phase](image)

*The one-trial associative spatial memory task*

In the second, *visual*-only domain, two different pictures appeared on the touch screen. Once pecked, the pictures disappeared and were replaced by one of the original pictures and a new picture after the R.I. In this task, the distractor and target were in the same places in the sample and choice phase, but the target had changed its appearance (see figures 3a & 3b). As in the *spatial*-only task, the only change was in the task-relevant domain.
The computer program allowed the resolution of working memory to be tested by presenting images that differed in similarity/proximity on the touch screen. In the spatial task, the distractor and target could either be close together or far apart. When “near”, the distractor was always within the eight positions next to the target (fewer at edges and corners; fig. 4a). In “far” conditions the distractor was never adjacent to the target (fig. 4b). In the visual-only domain, there were 40 pictures, paired so that the pictures within a pair were more similar to each other than the remaining 38 pictures. In “different” trials the target and distractor were never both from such a matched pair of pictures. In the visual task, distractor and target were never adjacent to each other (fig. 4b).
The retention interval between sample and choice was held constant at one second throughout training. Birds received 20 trials each day and were trained to a criterion of 70% correct choices across a minimum of three days. Birds were initially trained on the task type which they had most recently experienced in the previous study. For the majority of birds (n = 11; 4 females, 7 males), this was the spatial task; the remaining six birds (3 females, 3 males) began with the visual task. Once a bird had reached criterion it started the titration phase of the experiment, where memory persistence was tested by titrating retention intervals (R.I.). In the first trial of the first test session, the R.I. between the sample and choice array was one second. Subsequent to this, every time a bird made a correct choice the R.I. was increased by 0.3 seconds. Every time the bird made an error, the R.I. was decreased by 0.7 seconds, to a minimum of one second. At the beginning of a day of testing a bird started on the R.I. it had reached on the final trial of the preceding day. Ideally the titration phase would have ended when all birds had reached a stable level of performance. In practice, titration took so long that testing was terminated after 25 days. The final R.I.s (i.e., R.I.s achieved on the 500th trial) were used as a measure of memory persistence for each bird. Upon completion of titration on one task type, a bird was trained on the alternative task to the same criterion as before (i.e., an average of 70% correct across a minimum of three days), followed by a further 25 days of titration. Again, the final R.I.s were used as the performance measure for each individual.

An unforeseen error in the computer program altered the nature of the spatial task. In a choice trial, 50% of images should have been designated by “near” presentations of images on the touch screen (see figure 4a), and the remainder by “far” image presentations (see figure 4b). Therefore, in 20 trials, a bird received 10 near image presentations and 10 far. When a bird correctly chose the target image in a “near” trial, the retention interval on a subsequent “near” trial should have been increased by 0.3 seconds. Similarly, when a bird correctly chose the target in a “far” trial, the retention interval in the subsequent “far” trial should have been 0.3 seconds longer. However, the error in the program meant that the majority of “far” image presentations were interpreted as “near”. This meant that the “far” retention intervals were not altered by
3. Sex differences in spatial ability

the birds’ decision in the majority of “far” trials. Instead, the retention interval for “near” image presentations was altered. More specifically, over the 25 days of titration birds received 250 near presentations and 250 far presentations but the retention interval in “far” trials was altered on 126 trials, whereas the “near” retention interval was altered on 374 trials.

The 126th final R.I. for “far” accurately reflects how well the bird did when presented with “far” images because those “far” presentations were correctly titrated. However, the 374th R.I. for “near” presentations does not give a true impression of how well the bird did when presented with “near” images because some of those presentations will have been “far”. As “far” presentations are easier then “near” presentations (Biegler et al., 2001; McGregor & Healy, 1999), the inclusion of “far” image positions in “near” trials is likely to overestimate how well the bird does on “near” trials.

3.4 Results

Data analyses were performed using SAS (SAS Institute, 1989). The assumptions of normality of error (Kolmogorov-Smirnov normality test of the residuals) and homogeneity of residuals (plot of fitted values against residuals) were tested using Minitab and appropriate transformations applied to the data where necessary.

3.4.1. Test sequence

The data were initially analysed to determine if test sequence (i.e., whether the bird completed the visual task or the spatial task first) had an effect on performance. The retention intervals achieved on the 126th trials of the spatial task (average of near/far) were compared to those achieved on the visual task (average of similar/different)(n = 15; two birds died after being tested on the initial task). The following general linear model (GLM) was fitted to the square-root transformed data using SAS:
Chapter 3. Sex differences in spatial ability

\[ R.I. = \text{sex} + \text{bird (sex sequence)} + \text{sequence} + \text{task} + \text{sex} \times \text{sequence} + \text{sex} \times \text{task} + \text{sequence} \times \text{task} + \text{sex} \times \text{task} \times \text{sequence} \]

When the non-significant interaction terms (\( p > 0.05 \)) were removed from the model, test sequence (whether birds were tested on the spatial or visual task first) did not affect R.I. (Effect of test sequence: \( F(1,12) < 0.001, p = 1.00 \)).

3.4.2. Spatial versus Visual task

To compare the overall performance on the two task types, the 26th retention intervals on the visual task (average of similar/different) was compared with that from the spatial task (average of near/far). The error in the computer program meant that the retention interval achieved for “near” presentations in the spatial task was likely to be an overestimation of the birds’ ability. The following model was fitted to the square-root transformed data:

\[ R.I. = \text{sex} + \text{bird (sex)} + \text{task} + \text{sex} \times \text{task} \]

When the non-significant (\( p > 0.05 \)) interaction term was removed from the model, there was no significant sex difference in performance (\( F(1,14) = 0.38, p = 0.55 \)). All birds performed significantly better on the spatial than the visual task (\( F(1,15) = 9.02, p = 0.009 \); see figure 5). There was a significant effect of bird (\( F(14,15) = 2.43, p = 0.05 \)), i.e., there was significant variation in performance between individuals.
3.4.3. Spatial task

To examine the effect of image proximity on the birds' performance in the spatial task, the R.I.s achieved on the 126th "near" and "far" presentations were compared. As mentioned above, the R.I. for "near" actually reflects the retention interval for a mixture of near and far image presentations. The following model was fitted to the square-root transformed data:

\[ R.I. = \text{sex} + \text{bird (sex)} + \text{proximity} + \text{sex} \times \text{proximity} \]

When the non-significant interaction term (\( p > 0.05 \)) was removed from the model, as predicted, both sexes performed significantly better when the images were presented in far positions (\( F_{(1,15)} = 9.02, p = 0.009 \); see figure 6). There was no significant sex difference in performance (\( F_{(1,14)} = 0.38, p = 0.55 \)). Again, there was significant variation in performance between birds (\( F_{(14,15)} = 2.43, p = 0.05 \)).

Figure 5: Mean (± s.e.) retention intervals (sec) achieved on the spatial and visual tasks after 126 trials (\( n = 15 \)). All birds performed better on the spatial task than the visual task.
3.4.4. Visual task

To examine the effect of image similarity and sex on the birds' performance in the visual task, the retention intervals achieved on the 500th trial, for similar and different image presentations, were compared. The following model was fitted to the square-root transformed data:

\[ \text{R.I.} = \text{sex} + \text{bird (sex)} + \text{similarity} + \text{sex} \times \text{similarity} \]

When the non-significant interaction term (p > 0.05) was removed from the model, there was no difference between the sexes in performance (F(1,14) < 0.001, p = 0.98) and no effect of image similarity (i.e., similar versus different; F(1,15) = 0.61, p = 0.45; see figure 7). There was a significant effect of bird (F(14,15) = 4.43, p = 0.004).
2.5

Figure 7: Mean (± s. e.) retention intervals (sec) achieved on the visual task (n = 16). Retention intervals are not affected by image similarity and do not differ between the sexes.

3.5 Discussion

I found no evidence of a sex difference in the ability of great tits to perform two versions (spatial and visual) of a DNMTS task. Contrary to prediction, males did not outperform females on memory resolution nor persistence measures on either task. I predicted that males would do better than females when images were close together/similar but that sexes would do equally well when the images were far apart/different. However, both sexes achieved longer retention intervals when the images on the touch screen were far apart on the spatial task. Image similarity did not affect the performance of either sex on the visual memory task. The retention intervals achieved by males were not significantly different to those of females. Overall, males
and females achieved longer retention intervals on the spatial memory task compared to visual memory task.

I predicted that there would be a sex difference, favouring males, on the spatial task. There are three possible reasons why a sex difference was not found: 1) I failed to detect an existing sex difference; 2) Elevations in sex hormones levels were not sufficient to produce a sex difference; 3) A sex difference does not exist.

Firstly, I may have been unable to detect an existing sex difference. Whether or not sex differences appear often depends on the type of task. It is therefore possible that the tasks in this study were not of the sort to reveal sex differences. However, the spatial task has previously been used to reveal performance differences in species which differ in hippocampal size (e.g., food-storing versus non-storing songbird species; see Biegler et al., 2001). In rodents, the same task (RAM) used to reveal a species difference in rodents also uncovers a sex difference in performance (Jacobs et al., 1990; Gaulin & FitzGerald, 1986; Gaulin & Fitzgerald, 1989). However, a task that is sensitive to species differences should only be expected to reveal sex differences if one expects similar effect sizes and, even then, sample sizes need to be comparable. A similar touch screen memory task revealed a species difference in memory persistence using a smaller sample size than that in the current study (see Biegler et al., 2001). Therefore, if this task was indeed sensitive to sex effects then my failure to detect them was not due to a lack of power.

Secondly, a sex difference in spatial ability may only be found during the breeding season. It is possible that the differences in T levels between males and females were not of a sufficient magnitude to produce a sex difference in performance. Although birds were maintained under a long-day (breeding season) photoperiod, sex hormone levels may not have paralleled natural breeding season elevations. Astié et al. (1998) failed to find a sex difference in spatial ability in the shiny cowbird. They suggested that the possibility that the sexual dimorphism in hippocampus volume had not developed in captivity to the same extent as in nature may explain their null result (Astié et al., 1998). All birds in the current study had been kept under the same light cycle for a number of months it is possible that they were photorefractory. Continued exposure (7-10 weeks
Chapter 3. Sex differences in spatial ability

(Meddle et al., 1999; Bentley et al., 1998) to long days eventually leads to a centrally mediated desensitisation of the hypothalamo-pituitary-gonadal axis to the stimulating effects of long days and birds become photorefractory (Nicholls et al., 1988; Juss, 1993). A sex difference in spatial learning and memory abilities may therefore not have been found because the sexes did not differ in levels in circulating T.

A third possibility is that a sex difference in spatial learning and memory abilities in the great tit may not actually exist. Although sex differences in spatial learning and memory abilities are often reported, there have been a number of studies that have failed to find any sex difference. Any sex difference in spatial ability is thought to be mediated, in part, by the organisational effects of gonadal hormones (Beatty, 1979; Williams & Meck, 1991). For example, litter-sex ratio studies show how differential exposure to androgens in utero can affect adult spatial performance (Williams & Meck, 1991; Williams et al., 1990; Galea et al., 1994). In birds, organisational effects of steroids on behaviour are explored through the examination of variation in the level of yolk androgens. Chicks from eggs with higher levels of T grow faster, are more competitive (Eising et al., 2001; Schwabl, 1993, 1996), beg for food more intensively and are more likely to become dominant once they fledge (Schwabl, 1993, 1996). Levels of yolk androgens could also affect adult cognitive abilities. However, it is difficult to assert that males will necessarily have had higher organisational levels of T. The level of androgen found in the egg correlates with that found in the mother (Eising et al., 2001) and does not depend on offspring sex. In fact, allocation of androgens to eggs may increase or decrease with laying order, depending on the species (Schwabl, 1993; Schwabl, 1996; Schwabl et al., 1997; Lipar et al., 1999; Gil et al., 1999). Therefore, the lack of a sex difference in spatial learning and memory abilities in the great tit may result from the lack of a sex difference in not only activational but also organisational levels of androgens.

In summary, the failure to find differences in performance between the sexes on the spatial task is only weakly indicative of the possibility that the null hypothesis of lack of sexual differences in avian spatial learning and memory abilities may be true. The sex difference in spatial learning and memory abilities in mammals can be attributed, in part,
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to sex differences in T levels. Although I failed to find a sex difference in the spatial
learning and memory ability of the great tit, without plasma radioimmunoassay, I was
unable to determine if T levels differed between the sexes. Therefore, it is difficult to
assert that variation in T, either at an organisational or activational level, does not lead to
sex differences in avian spatial learning and memory abilities.
4.1 Abstract

Experimental elevation of testosterone (T) levels in mammals generally improves spatial learning and memory abilities. Although the behavioural effects of experimentally elevated levels of T have been monitored in a number of ways, studies addressing the effects of T on avian spatial learning and memory abilities are scarce. In this study, the great tit (*Parus major*) was used as a model to explore the effect of experimentally manipulated T levels on ability to perform a spatial memory task. In Experiment 1A, females, but not males, performed better when fed T immediately prior to testing than on control days. In Experiment 1B, no effect of T was found in either sex. In Experiment 2, levels of T, oestradiol (E₂) and 5α-dihydrotestosterone (DHT) (T metabolites) were manipulated. Although overall performance of both sexes was not affected by the experimental manipulation of hormones, I found an effect of treatment on response latencies: T-treatment lengthened response latencies in the sample phase of the experiment in both males and females. T-treated birds were able to take longer before responding to the touch screen images than when they had been given the vehicle or E₂ and perform at the same level.

4.2 Introduction

Through the experimental manipulation of testosterone (T) levels, it is possible to explore the relationship between the level of circulating T and spatial learning and memory abilities. The primary aim of an earlier experiment (see Chapter 3) was to determine the influence of T on spatial learning and memory abilities in the great tit (*Parus major*). I attempted to manipulate T levels by photostimulating the birds with the aim of elevating endogenous sex hormone levels. However, as plasma was not assayed for T, I could not be sure that T levels were higher in males compared to females. The
potential lack of a difference in T levels could explain the lack of a sex difference in performance on the spatial task. In this experiment I therefore manipulated T levels in the birds directly.

A number of studies have looked at the effect of experimental T-elevation in wild birds. For example, increased T led to an expansion in territory size in dark-eyed juncos (Junco hyemalis) and song sparrows (Melospiza melodia morp翰a) (Chandler et al., 1994; Chandler et al., 1997; Wingfield, 1984; Smulders et al., 2000). During the breeding season T-treated male dark-eyed juncos also sang more often, increased mate-guarding activity and fed young less often than sham-treated controls (Ketterson & Nolan, 1992; Chandler et al., 1997; Enstrom et al., 1997). The majority of these behavioural experiments were accomplished using T implants, which raised circulating levels of T to a constant level over a period of days to weeks. Using implants to modulate circulating T levels is convenient in that implants can be given to free living animals and the procedure is repeatable (the same animal can be repeatedly implanted) with no lasting effect. There are, however, several drawbacks. First, implantation requires surgery which, in itself, is invasive and increases endogenous levels of other steroids, such as corticosterone (CORT: the glucocorticoid in most nonmammalian tetrapods (Breuner et al., 1998)) which co-varies with T (see Chapter 5), and also often necessitates a period of recovery. Second, there is a lag period of a number of hours before a steroid implant yields maximum hormone levels in the circulation, so immediate effects of the hormone cannot be assessed. Finally, the implant brings circulating steroid hormone levels up to a sustained high level which does not mimic normal dynamic changes (see Smulders, 2002) and can result in effects opposite to those that result from natural seasonal variation in T levels (Smulders, 2002). An alternative is to administer exogenous hormones through injection, but an injection can also be stressful and increase endogenous levels of CORT in treatment and control groups (Evans et al., 2000; Ketterson et al., 1991; Schoech et al., 1999; Klukowski et al., 1997). Breuner et al. (1998) devised a nonstressful method of steroid hormone delivery to investigate the immediate behavioural effects of elevated CORT in Gambel's white-crowned sparrows (Zonotrichia leucophrys gambelii): CORT solution was injected into
mealworms (*Tenebrio molitor*) that were fed whole to sparrows. Sperry (2001, *pers. comm.*) suggested that I could evaluate the effect of T on spatial learning and memory abilities in birds by adopting the same method of hormone manipulation.

While very few studies have addressed the effect of T on avian cognitive abilities, experimental elevation of T and related effects on cognitive ability have been examined in mammals. The relationship between circulating levels of T and spatial ability in mammals is biphasic, with intermediate levels of T being optimal for spatial learning and memory performance. For example, treatment of neonatal female rats with T abolishes the often-reported sex difference, favouring males, in the ability to learn a maze. When tested in the MWM as adults, T-treated females perform better than female controls and at a level comparable to that of control males. Moreover, the biphasic relationship between T level and spatial learning and memory abilities can also be demonstrated through experimental elevation of T as T-treatment of males is detrimental to their performance (Roof & Havens, 1992; Roof, 1993b; Joseph et al., 1978).

Although a number of T-manipulation studies have been carried out with birds (see above), manipulation studies examining the effect of elevated T on cognitive abilities are scarce. In one of the few studies to have examined the effect of T on learning, T had a detrimental effect. The test involved an aversive stimulus whereby day-old domestic chicks were allowed to peck a number of coloured beads before experiencing a single unpleasant-tasting bead. After a retention period they were unlikely to peck a bead of the same colour as the previously aversive stimulus. Conversely, T-treated birds were much more likely to peck the previously aversive bead, showing greatly reduced overt avoidance compared to controls (Andrew et al., 1981; Clifton et al., 1982). In another study, T-treatment interfered with vocal learning in songbirds. T-treated birds produced abnormal songs that resembled those of males raised in acoustic isolation (Whaling et al., 1995). Castration and antiandrogen treatment delayed song stereotypy in zebra finches (Bottjer & Hewer, 1992) whereas exogenous T treatments caused premature song stereotypy in white-crowned sparrows (Whaling et al., 1995).

Although these experiments examined the influence of T on cognitive abilities, to my knowledge, the influence of elevated T on avian spatial learning and memory is yet
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities to be addressed. I predicted that T would influence avian spatial learning and memory abilities in a similar way as in mammals. The mammalian hippocampus, the brain area sub-serving spatial ability, contains both androgen receptors (AR) (Kerr et al., 1995) and oestrogen receptors (ER) (Weiland et al., 1997), although the expression is strongly AR biased. Correspondingly, both androgens (Pouliot et al., 1996) and oestrogens (Woolley et al., 1997) modulate excitatory signalling in the rodent hippocampus. Little is known about AR expression in the avian hippocampus but ER- and AR- expressing neurons are found therein and the avian hippocampus is a site of high aromatase (Gahr et al., 1993; Gahr & Metzdorf, 1997; Gahr, 2001; Fusani et al., 2000; Saldanha et al., 1998; Schlinger, 1997; Shen et al., 1995), suggesting that both androgens and oestrogens may affect avian spatial learning and memory abilities. I experimentally manipulated levels of sex steroids in birds immediately before assessing their ability to perform two versions (spatial and visual) of a DNMTS task.

4.3 Experiment 1A

In this first experiment I investigated whether elevated T influenced performance on two versions (spatial and visual) of a DNMTS task. The visual memory task served as a control as I predicted that any effect of T would be specific to the spatial domain. As the spatial task was hippocampal-dependent, I predicted that performance would be mediated through the activation of sex hormone receptors in the hippocampus. T was manipulated though oral administration of the steroid (see Breuner et al. 1998). Sperry (2002; pers. comm.) injected 10μl of 8mg/ml T in peanut oil into mealworms and fed them to Gambel’s white-crowned sparrows maintained under a short-day photoperiod in which levels of T were non-detectable. The manipulation produced a rapid peak of 10ng/ml T at 7 minutes (comparable to breeding season hormone levels in the wild), which then dropped to 4ng/ml at 15 minutes where it remained at 30 minutes. Although the white-crowned sparrow is not closely related to the great tit phylogenetically, it is similar in weight (17-20g) so these measurements provided a rough idea of the effect of the same treatment in the great tit.
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On the basis of the results of an earlier experiment (see Chapter 3), I predicted that performance on the spatial task would be better than that on the visual task. I also predicted that image proximity would affect performance on the spatial task but that image similarity would not affect performance on the visual memory task, as no effect of similarity was found in an earlier study (see Chapter 3). I expected the highest scores to be achieved on the spatial task when the images on the touch screen were far apart. I expected birds that received T immediately prior to testing to perform better than they did on control days. The benefit of increased T was expected to be specific to the spatial domain and not to affect performance on the visual memory task.

4.3.1 Materials and methods.

Animals. The subjects were 11 male and 5 female great tits (4 males and 2 females caught in deciduous woodland, Northumberland, in March 1999, and 7 males, 3 females in Edinburgh, mid Lothian in April 2000, January and February 2001). One female had died since completion of the previous experiment (see Chapter 3). All birds had previous experience with the touch screen set-up and had completed 25 days of testing during October-November 2001 (see Chapter 3). Birds were housed individually in wire-mesh cages (77cm long x 44cm wide x 44cm high) and were fed daily with ad libitum water and an insectivorous bird food mixture (Orlux, Sunning Cooke, Greasbrough, Rotherham, U. K.), supplemented by peanuts, sunflower seeds and wax moth larvae. They were maintained on a 13.5:10.5 h light:dark cycle with ambient temperature in the range of 16-19°C. Testing commenced immediately after the 25 days of titration (December 2001; see Chapter 3). Birds were deprived of food at 8am each morning and provided with fresh food once their session was complete. Testing began at 10am. Birds tested later in the day were provided with nuts through the day.

Apparatus. See Chapter 3.

Protocol. The retention intervals achieved by each bird over the last 5 days of titration (see Chapter 3) were averaged for each task type (spatial and visual). Therefore, each bird had a pair of retention intervals for the spatial task (near and far) and a second set for the visual task.
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities (similar and different). The retention intervals were fixed at these averages throughout the experiment (each bird having its own retention intervals), with hormone treatment being the only thing that differed across trials. Through keeping the retention intervals fixed, I was better able to compare the effect of treatment on performance (i.e., percentage correct choices). All birds were tested on both the spatial and visual tasks. Birds were initially tested on the task type which they had experienced most recently. For the majority of birds (n=11), this was the feature task. The remaining five birds began with the spatial task. All birds completed 20 trials per day, 5 days a week.

To examine the effect of T on spatial learning and memory, birds were fed a single wax moth larva injected with 10μl of 8mg/ml T (Testosterone (4-Androsten-17β-ol-3-one (Sigma)) in peanut oil immediately before testing. The order of T treatment was varied such that 10 birds completed five days of testing with T followed by five days without whereas 6 birds were tested on the task for five days before they received the hormone treatment. Birds were tested with a given task (visual versus spatial) and treatment (T versus control), receiving 20 trials a day for five consecutive days, termed a “block”. Each bird was tested with every combination of task and treatment, thus was tested for four blocks (i.e., spatial + T, spatial + control, visual + T, visual + control). The order in which the tasks were presented varied between birds, although if a bird received T before control on the visual task, it also received T before control on the spatial task and vice versa. The effect of T-treatment was explored by comparing scores (percentage correct choices in a block) achieved on days of T-manipulation with those achieved on control days.

4.3.2 Results.

The data were analysed using SAS (SAS Institute, 1989). The assumptions of normality of error (Kolmogorov-Smirnov normality test of the residuals) and homogeneity of residuals (plot of fitted values against residuals) were tested using Minitab. To determine if T-treatment had an effect on performance, the following model (proc GLM) was fitted to the arcsine square-root transformed data. This model was the
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equivalent of a univariate Analysis of Variance (ANOVA), making the same
assumptions about correlation structure within subjects, and yielded identical results:

Model score = sex + bird (sex) + block + proximity + task + treatment + sex *
treatment + sex * task + treatment * task + proximity * task + block*treatment +
block*task

The interaction between treatment and task was not significant (F(1,98) = 0.57, p =
0.45) nor was the interaction between sex and task (F(1,98) = 1.58, p = 0.21). The effect
of T and performance of males and females did not differ between the spatial and visual
tasks. There was also no interaction between block and treatment (F(2,98) = 0.76, p =
0.47), nor block and task (F(2,98) = 1.12, p = 0.33), suggesting that order of T-treatment
(control followed by T, or vice versa) and test sequence (spatial followed by visual, or
vice versa) had no effect on performance. Once these non-significant interaction terms
were excluded from the analysis, there was no significant effect of treatment (F(1,104) =
2.05, p = 0.15). T-treatment scores did not differ from scores achieved on control days.
There was also no significant effect of sex on performance (F(1,14) = 0.78, p = 0.39).
Overall, there was no difference in scores achieved by males and females (see figure 1).
However, the interaction between treatment and sex was significant (F(1,104) = 5.22, p =
0.02; see figure 2). When the sexes were analysed separately, T-treatment improved
performance in females (F(1,27) = 6.44, p = 0.02; see figure 2) but not males (F(1,69) =
0.53, p = 0.47). There was a marginally non-significant relationship between task and
performance (F(1,104) = 3.11, p = 0.08; see figure 1) such that all birds tended to perform
better on the spatial than the visual task. The interaction between image
proximity/similarity and task was significant (F(1,104) = 10.66, p = 0.002; see figure 1),
such that there was a significant effect of image proximity in the spatial task (F(1,43) =
15.46, p = 0.0003) but no effect of image similarity in the visual task (F(1,43) = 0.4, p =
0.53; see figure 1). Birds achieved higher scores in the spatial task when the images
were presented in far positions. There was significant variation in performance between
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birds ($F_{(14,104)} = 5.20, p < 0.0001$) but no effect of block ($F_{(3,104)} = 1.82, p = 0.15$).

Performance on days 1-5 did not differ from performance on days 6-10 and so on.

Figure 1: Mean percentage correct (± s.e.) choices made by birds in the spatial and visual tasks. The number of correct choices did not depend on sex. All birds tended to perform better on the spatial task than the visual task. Birds achieved higher scores on the spatial task when images were presented in far positions but image similarity had no effect on performance in the visual task.

Figure 2: Mean (± s.e.) percentage correct choices made by males and females. The number of correct choices depended on the interaction between treatment and sex. T-treatment had a significant effect on performance in females but not males.
I predicted that T-treatment would have a positive effect on performance and that the benefit would be specific to the spatial domain. Consistent with my prediction, the T-manipulation appeared to affect performance although differentially in the two sexes. T seemed to have a beneficial effect on performance in females on both tasks. Counter to my predictions, however, males tended to achieve lower scores on T-treatment compared to control days, although this tendency was weak. On the basis of the results of an earlier study (see Chapter 3), I predicted that performance would vary between task types, with birds attaining higher scores on the spatial memory than visual task. I also predicted that image similarity would have an effect on scores on the spatial but not visual task. In line with my predictions, birds did tend to achieve higher scores on the spatial compared to visual task although this difference was not significant. As expected, image proximity affected score on the spatial task, with birds achieving higher scores when images on the touch screen were far apart, whereas there was no effect of image similarity on the visual task.

The effect of T in females on the hippocampal-independent visual task was unexpected and implies that androgens must be acting in a brain region other than the hippocampus. A recent review of hippocampal involvement in non-spatial tasks (Day, 2003) showed that many vertebrate species share non-spatial functions of the hippocampus (Sutherland & Rudy, 1989; Wan et al., 1994; Winocur, 1990; Winocur & Olds, 1978). For example, hippocampal lesions impaired reversal learning, increased resistance to extinction, and reduced responding to contextual cues in several taxa. Reversal learning requires an animal to learn a discrimination task in which a response to one stimulus is rewarded whereas a response to another is not and then to learn the same discrimination with the reward contingencies reversed (Day, 2003). Pigeons with hippocampal damage were impaired on a reversal learning task as they responded persistently to the originally correct choice (Good, 1987). T-treatment may have affected performance on the visual task in the same way that it was hypothesised to do so on the spatial task: through activating androgen receptors in the hippocampus. However,
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this possibility is unlikely as no other data have shown a hippocampal dependence on performance levels on tasks like the visual DNMTS task (Hampton & Shettleworth, 1996b; Shettleworth & Westwood, 2002). For example, chickadees with hippocampal lesions performed poorly on a spatial memory task that required return to the same baited locations trial after trial, but performed as well as did controls on a task that required approaching colour cues consistently associated with reward (Sherry et al., 1989). Both chickadees and juncos were impaired in spatial matching-to-sample, but not colour matching following hippocampal lesions (Hampton & Shettleworth, 1996b). Lesioned pigeons were impaired at spatial delayed-matching-to-sample whereas retention of non-spatial information was unaffected by the lesion (Reilly & Good, 1989; Reilly & Good, 1987).

As I only fed the birds a larva on days when they received T before testing, it is possible that the lack of an overall treatment effect was due to motivational differences, although this explanation would only explain the lack of a treatment effect in males. During the manipulation period, males may not have been as motivated to make the correct choice (and hence be rewarded with a piece of peanut) as they were during the control period. In rodent studies a sex difference in motivation may mediate the often-reported sex difference in spatial learning and memory abilities (Seymoure et al., 1996; Luine & Rodriguez, 1994; Roof, 1993; Williams et al., 1990; Einon, 1980). Female rats are smaller and lighter than males and are thus more likely to be affected by food-deprivation (Jones, 2003; Weinstock, 1972). However, as there is no size dimorphism in the great tit (both sexes weigh between 17-20g) the sexes are unlikely to be differentially affected by food deprivation. Moreover, Healy and Cleland (submitted) gave coal and great tits a delayed-matching-to-sample (DMTS) task after 0, 2 or 4 hours of food deprivation and found no variation in spatial performance (Healy & Cleland, submitted), i.e., there was no evidence of variation in motivation.
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4.4 Experiment 1B

To examine the effect of T without the possible confound of differences in motivation between control and hormone treatment days, in Experiment 1B I fed all birds a wax moth larva before testing, regardless of treatment. As the order of treatment had no effect in Experiment 1A, in this experiment, I decided to use a between subjects design and to feed some birds T and others the vehicle. Through using this design, I was able to investigate the effects of the T-manipulation while eliminating the possibility of carry-over effects of the steroid. It is possible that I did not find an overall treatment effect in Experiment 1A because T levels were still elevated during testing on control days. Although this possibility cannot be investigated without plasma radioimmunoassay, by using a between-subjects design I prevented this possibility.

In Experiment 1B, I made three predictions:

1. Birds fed T before testing would outperform controls.
2. The effect of T would be greater on the spatial task when images were in far locations.
3. The effect of T would be greater in females than males.

4.4.1 Materials and Methods.

Animals. The subjects were 8 male and 5 female great tits (all wild-caught in deciduous woodland in Edinburgh, mid Lothian). Three of the males tested previously were not available as they were involved in another experiment. All birds had the same experience with the touch screen set-up, having completed Experiment 1A. Birds and were housed and fed as before.

Apparatus. See Chapter 3.

Protocol. To control for differences in motivation that may arise when some birds eat a wax moth larva before testing and others do not, all birds in this experiment received a wax moth larva immediately before testing. Using a between-subjects design, a wax moth larva injected with 10μl of 8mg/ml T in peanut oil was fed to seven birds (3
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities females and 4 males). The controls (4 males and 2 females) received a wax moth larva injected with 10μl of the vehicle, peanut oil. Treatment was assigned randomly. All birds completed five days of testing on the spatial task. The retention intervals for each bird were the same as they were in Experiment 1A (ranging from 1.1-20.5 seconds for near image presentations and 1.3-16.6 seconds for far presentations). Again, the number of correct choices made by each bird over 100 trials (i.e., percentage correct) was used as the performance measure.

4.4.2 Results.

To determine if T-treatment had an effect on performance, the following model (proc GLM) was fitted to the arcsine square-root transformed data:

\[
\text{Model score} = \text{sex} + \text{bird (sex treatment)} + \text{treatment} + \text{proximity} + \text{proximity } \ast \text{sex} + \text{proximity } \ast \text{treatment} + \text{sex } \ast \text{treatment}
\]

There was no significant interaction between sex and image proximity \((F(1,9) = 0.38, p = 0.55)\), treatment and image proximity \((F(1,9) = 2.45, p = 0.15)\), nor treatment and sex \((F(1,9) = 1.16, p = 0.31)\). Once these non-significant interaction terms were removed from the model, the effect of treatment was non-significant \((F(1,10) = 3.34, p = 0.10)\); see figure 3): birds fed testosterone immediately before testing performed at a comparable level to controls. There was also no difference between the sexes in performance \((F(1,10) = 0.01, p = 0.94)\) and no effect of image proximity \((F(1,12) = 1.28, p = 0.28)\) such that birds achieved similar scores when the images were presented in near and far positions. There was, however, significant variation among the birds in performance \((F(10,12) = 4.07, p = 0.01)\).
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Figure 3: Overall mean scores (±s.e.) achieved by males and females. Scores did not differ between T-treated and control birds, nor males and females.

4.4.3 Discussion

I predicted that birds fed T immediately before testing would outperform controls. However, scores achieved by T-treated birds and controls did not differ. I predicted that the effect of T would be most obvious with far image presentations. However, there was no effect of image proximity on performance, nor an interaction between treatment and image proximity. I predicted that the T-manipulation would have the greatest effect on performance in females. However, the interaction between treatment and sex was non-significant such that the effect of the manipulation did not differ between the sexes.

The results of Experiment 1A were not corroborated by those of Experiment 1B as I did not find a treatment effect in either sex. The failure to find a treatment effect in Experiment 1B may have resulted from the experimental design. A between-subjects design may have meant that I did not have enough power to detect a treatment effect. In line with my prediction, however, T-treated birds tended to achieve higher scores than controls. This pattern was seen in both males and females suggesting that the tendency for males to achieve lower scores on T-treatment days than on control days in
Experiment 1A may have been due to lower motivation on days when they received the manipulation.

4.5 Experiment 2A

In Experiment 1A, the T-manipulation appeared to improve the ability of females to perform a one-trial associative spatial memory task. In Experiment 1A, females achieved higher scores when they had received the T-treatment immediately before testing than they did on control days. In Experiment 1B, although T-treated birds tended to outperform controls, there was no significant treatment effect. I may not have found an effect of the manipulation on performance because the birds in Experiment 1 had been maintained under a breeding season photoperiod for a long time and thus it was unclear what state they were in (i.e., photosensitive / photorefractory). I also did not run hormone assays on their plasma to confirm T levels. Therefore, I released the birds and caught more birds for Experiment 2, where I not only manipulated levels of T but also manipulated levels of T metabolites as the effect of T on spatial learning and memory abilities may be mediated through its conversion to one or more of its metabolites.

In rodents, increased memory performance is not only associated with T (Gouchie & Kimura, 1991; Roof & Havens, 1992) but a corresponding enhancement is seen in response to oestradiol (E2) administration (Singh et al., 1994; O'Neal et al., 1996). These data suggest the involvement of T or its aromatised metabolite E2 in aspects of spatial learning and memory. The enzyme 5α-reductase converts T into 5α-dihydrotestosterone (DHT), and aromatase converts T into E2. Both of these enzymes are widely distributed in the songbird brain (Schlinger et al., 1995; Schlinger, 1997). The presence and interaction among these enzymes may represent a mechanism whereby neural sites, including the hippocampus, are provided with the appropriate steroid product (Saldanha et al., 1999). High amounts of aromatase in the songbird hippocampus (Shen et al., 1995; Saldanha & Schlinger, 1997; Saldanha et al., 1998) suggest that these enzymes may modulate the concentrations of steroids required for hippocampal function within the hippocampus itself (Saldanha et al., 1999). Thus, in
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Experiment 2, in addition to manipulating T levels, I also fed the birds E2 and DHT to address how T exerted its effect on spatial ability in Experiment 1A (i.e., whether T acts directly or indirectly via one of its metabolites). Experiment 2A addressed the influence of E2 and T on task performance, as DHT was not available at the time of testing.

In Experiment 2A, I made three predictions:

1. **Titration experiment.** On the basis of the results of an earlier experiment (see Experiment 1A and Chapter 3), I predicted that performance (retention intervals) would be affected by image proximity, with birds achieving longer retention intervals when the images were presented in far, compared to near, locations.

2. **Hormone manipulation experiment.** I expected that the T-manipulation would have a beneficial effect on performance (score) in both sexes.

3. I predicted that if T acted at the neural level by aromatisation into E2, then E2 would have the same effect as T. Conversely, if T influenced spatial learning and memory abilities directly or after being metabolised into DHT by the reductase enzyme, I would expect E2 to have no effect on spatial task performance.

4.5.1 Materials and methods.

**Animals.** The subjects were 8 male and 16 female great tits (all wild-caught in deciduous woodland, between December 2002 and February 2003, in Edinburgh, mid Lothian). All birds were naïve to the touch screen set-up. Birds were housed and fed as before. They were maintained on a 9:15 h light:dark cycle (i.e., winter photoperiod) and under a temperature range of 14-16°C. Birds were deprived of food at 8am each morning and provided with fresh food once their session was complete. Testing began at 10am, 7 days a week. Birds tested later in the day were provided with nuts through the day.

**Apparatus.** See Chapter 3.

**Protocol.** To initiate a peck response, a wax moth larva was attached to an image on the touch screen. Initially, any peck directed at the image led to a positive reinforcement
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities (i.e., the automated delivery of a piece of peanut). Through pecking the larva, birds learnt to associate the action of pecking with the reward. After several exposures to such trials, birds pecked the image even when the larva was removed. Once a bird had completed 40 trials (responded to the presentation of images both in the sample and choice phases) over two days, the “pretraining” phase of the experiment began. Birds were trained to a criterion of an average of 70% correct choices (i.e., choice of the novel stimulus in the choice phase) across three consecutive days. Once the criterion was satisfied, the “titration” phase of the experiment commenced. The program tested memory persistence by titrating retention intervals (see Chapter 3). Birds received 20 days of testing on the spatial task and received 20 trials each day, with both near and far image presentations, referred to as the titration experiment. Retention intervals were titrated so that I could get a measure of each bird’s ability and set the retention intervals for the hormone manipulation phase of the experiment, as before. The retention intervals achieved on the last 5 days of titration with far image presentations were averaged and fixed as the retention interval for the hormone manipulation phase of the experiment. Touch screen images were only presented in far positions during the hormone manipulation phase of the experiment to simplify and increase the power of the test.

During the hormone manipulation phase of the experiment, all birds were tested on consecutive days, receiving 20 trials per day. The testing regime was as follows: 5 days of vehicle (vehicle 1), 5 days of either T or E2, 5 days of vehicle (vehicle 2), 5 days of either T or E2, and 5 days of vehicle (vehicle 3). The vehicle consisted of a larva injected with peanut oil fed to the bird immediately before testing each day. T and E2 treatments consisted of a larva injected with 10μl of 8mg/ml T in peanut oil or 10μl of 8mg/ml E2 (β-Estradiol (1, 3, 5 (10)-Estratriene-3, 17β-diol (Sigma)) in peanut oil fed to the bird immediately before testing). The order of hormone treatment was counterbalanced such that 12 birds received the T-treatment first and the E2-treatment second, whereas the order of hormone treatment was reversed for the remaining 12 birds.

Birds were maintained under a winter (short-day) photoperiod. In winter, T levels in males are basal and it is at the onset of territorial behaviour, which coincides with the
breeding season, that endogenous T levels rise. The relationship between T level and spatial ability is biphasic, therefore by maintaining the birds on a short-day (non-breeding season) photoperiod the T-manipulation was unlikely to elevate circulating levels to a level beyond the optimal for performance on the spatial task.

**Bleeding protocol.** Before commencing the titration experiment, all birds were bled. The blood (approximately 100μL) was collected from the wing vein using heparinised capillary tubes. Plasma T concentrations are affected beginning 10 minutes after disturbance. Therefore, nobody entered the laboratory where the birds were housed prior to bleeding. All plasma samples were taken within 10 minutes so as to reflect basal T levels. Blood samples were stored in the refrigerator until they were centrifuged (14000 rpm for 10 minutes) in the laboratory, within one hour of collection. Plasma was collected and stored at -20°C until assayed for T.

### 4.5.2 Results

Initially, the data were analysed to determine if the sexes differed in retention intervals (i.e., the measure of memory persistence). The retention intervals achieved over the last 5 days of titration (the last 100 trials) were averaged for near and far image presentations (i.e., each individual had an average retention interval for near and an average retention interval for far presentations). To determine whether the retention intervals differed between the sexes and image proximities, the following model (proc GLM) was fitted to the square-root transformed data:

\[ \text{R.I.} = \text{sex} + \text{bird (sex)} + \text{proximity} + \text{sex} \times \text{proximity} \]

Once the non-significant (p > 0.05) interaction term was removed, there was no difference between the sexes in retention interval reached (\( F_{(1,22)} = 0.76, p = 0.40 \); see figure 4). The lack of a sex difference in performance may be explained by the lack of a sex difference in plasma T levels (\( t = 0.138, p = 0.89 \); see figure 5). There was a significant effect of proximity on performance (\( F_{(1,23)} = 7.34, p = 0.01 \)). Higher retention
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities intervals were achieved when the images on the touch screen were presented in far, as opposed to near, positions (see figure 4).

![Figure 4: Mean retention interval (±s.e.) achieved with near and far image presentations in the spatial task. Retention interval achieved is correlated with image proximity but not sex. Both sexes achieved longer retention intervals when the images were presented in far positions.](image)

![Figure 5: Mean (±s.e.) plasma T levels (ng/ml). The sexes did not differ in measurements of circulating T.](image)

The retention intervals achieved for far image presentations over the last 5 days of the titration experiment were averaged and set as the retention intervals for the hormone
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manipulation phase of the experiment. Performance was assessed by looking at the total number of correct choices (i.e., “score”) made when the images were consistently presented in far positions on the touch screen. As birds received 5 days of testing with each treatment, receiving 20 trials per day, the maximum score for any given treatment was 100. As I had rectified the error in the computer program (see Chapter 3), I was able to include day as a variable, increasing the power of the test, rather than examine the average of each 5 day block. The following model (proc GLM) was fitted to the data with day entered as a linear covariate. Treatment was E<sub>2</sub>, T or vehicle:

\[
\text{Score} = \text{bird (sex)} + \text{treatment} + \text{sex} + \text{day} + \text{sex * treatment}
\]

There was no interaction between sex and treatment (F(2,497) = 0.13, p = 0.88). Hormone treatment affected the performance of males and females in the same way. Once this non-significant interaction was removed from the model, hormone manipulations had no effect on performance (F(2,499) = 0.73, p = 0.48; see figure 6). There was no significant effect of sex (F(1,20) = 2.73, p = 0.11), although there was a trend for females to achieve higher scores than males (see figure 7). There was a significant effect of bird (F(20,499) = 7.44, p < 0.0001), with large variation in performance between individuals. There was a significant effect of day (F(1,499) = 10.27, p < 0.001), with performance improving across the course of the experiment (slope = 0.4 ± 0.01/day).
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Figure 6: Mean (±s.e.) scores achieved on the spatial task. Scores did not vary with hormone treatment.

Figure 7: Mean (±s.e.) scores achieved on the spatial task. Males and females achieved comparable scores.

The failure to find an effect of treatment on score may have occurred because the hormones did not exert an effect immediately due to a time-lag in steroid action. To examine this possibility I analysed the data from within each day. Because some birds may have completed 20 trials after 40 minutes when others took much longer, I calculated the time, in seconds that had elapsed between the feeding of the larva and the beginning of a given trial, referred to simply as “time”. An interaction between time and
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treatment may indicate that the effect of the treatment changed throughout the course of
a day's testing. The following model was fitted to the data using the proc LOGISTIC
command, which fits logistic regression models for binary response data by the method
of maximum likelihood (SAS Institute, 1989).

Model score = bird + day + trial + time + treatment + time*treatment

I could not run a nested model within the proc LOGISTIC command and therefore did
not include "sex" as a class variable because it would have been confounded by "bird". The interaction between time and treatment was non-significant ($\chi^2 = 3.55, p = 0.17$), indicating that any possible effect of the hormone on performance did not vary across the test session.

Although I did not find a significant effect of treatment on score (i.e., number of
correct choices), I performed further analyses on the raw data to determine whether
hormone treatment affected other aspects of cognition, such as time to respond to (peck)
the images on the touch screen. Although the inter-trial interval (time period between
one trial and the next) and retention interval were fixed between and within trials for
each individual, during the sample and choice phases the birds were free to choose how
long they took before responding to the stimuli. The bird was first presented with two
images, simultaneously, in the sample phase, before being presented with a further two
images, after the retention interval, in the choice phase. Each image disappears
immediately after it is pecked. In this model, "latency" refers to the time (in seconds) to
peck the image. "Sample 1" is the time taken to peck the first image in the sample phase
and "Sample 2" is the time taken to peck the second image in the sample phase.
"Choice" refers to the time taken to peck the image in the choice phase of any given
trial. Latency to respond to the sample 1 image does not affect the difficulty of the task,
whereas a delayed response to sample 2 or choice images would make the task more
difficult, as the memory of image position would have to be retained for longer.
Therefore, sample 1 response latencies were not examined. To determine whether
latencies (time to respond to sample 2 and choice images) varied in response to the
hormone treatments and sex the following model (proc GLM) was fitted to the log transformed data:

\[
\text{Latencies} = \text{bird (sex)} + \text{sex} + \text{treatment} + \text{day} + \text{time} + \text{sex \times treatment} + \text{time \times treatment}
\]

When the non-significant (p > 0.05) interaction terms were removed from the model, hormone treatment had a significant effect on the response latencies for sample 2 \(F(2,10181) = 8.75, p = 0.0002\) but not the choice latencies \(F(2,10181) = 2.20, p = 0.11\); see figure 8). Post-hoc pairwise comparisons were made to determine where the differences between treatments occurred. There was no difference between control latencies and \(\text{E}_2\) latencies \(p = 0.83\) whereas the difference between T latencies and control latencies was significant \(p < 0.0001\), as was the difference between \(\text{E}_2\) latencies and T latencies \(p < 0.0001\); the Bonferroni adjusted threshold for these comparisons was 0.05/3 = 0.017.

Birds took significantly longer to respond to sample 2 images after having received the T-treatment than they did after having received the \(\text{E}_2\) treatment or vehicle (see figure 8).

Latencies to respond to the images did not differ between the sexes (sample 2: \(F(1,20) = 0.07, p = 0.80\), choice: \(F(1,20) = 0.48, p = 0.50\); see figure 9). There was a significant effect of bird (sample 2: \(F(20,10481) = 43.21, p < 0.0001\), choice: \(F(20,10481) = 33.76, p < 0.0001\), with significant variation in response latencies between birds. There was no significant effect of day (sample 2: \(F(1,10481) = 0.47, p = 0.50\), choice: \(F(1,10481) = 1.96, p = 0.16\), i.e., individuals’ response latencies did not differ between days. There was a significant positive effect of time on response to sample 2 images \(F(1,10481) = 22.59, p < 0.0001\), with response latencies increasing across the course of a given day’s testing but no such effect on choice latencies \(F(1,10481) = 3.30, p = 0.07\).
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Figure 8: Average (± s.e.) response latencies in the sample and choice phase of the spatial task. Sample 2 response latencies (median = 2.1 seconds) were affected by hormone treatment. The T-manipulation increased sample 2 latencies compared to the E2-treatment and vehicle. Hormone treatment did not affect choice response latencies (median = 1.4 seconds).

Figure 9: Average (± s.e.) response latencies to the touch screen images in the spatial task. Time taken to respond did not differ between the sexes.
4.5.3 Discussion

In Experiment 2A, birds achieved longer retention intervals when the images on the touch screen were presented in far, compared to near, positions. There was no sex difference in performance in the titration experiment. In the hormone manipulation experiment, there was no effect of treatment on score. Whether birds received T, E$_2$ or the vehicle before testing had no bearing on the score achieved. The lack of an effect of hormone treatment on score was unexpected, especially considering the results of Experiment 1A. As the birds were maintained on a winter (short-day) photoperiod, it is unlikely that the experimental manipulation elevated the steroid hormones to detrimental levels. However, I cannot conclusively state this until plasma samples are analysed. A more likely explanation is that the putative elevation in hormone levels was not sufficient to affect cognitive performance or that the 20 trials were completed before the hormone had exerted its effect. That there was some small effect on cognition can be seen in the results of the analyses of time taken to respond to the images. Treatment affected response latencies, with T-treatment leading to longer response latencies to the second sample image. The effect of T on response latency was significantly different to the effect of the vehicle and E$_2$. McGregor and Healy (1999) found response latencies correlated with performance. They compared the ability of coal tits (Parus ater) great tits and blue tits (P. caeruleus) to remember spatial locations in a spatial delayed-matching-to-sample (DMTS) task and found all birds made correct decisions sooner than errors. Shorter response times to the initial, sample image (which corresponds to sample 2 as there was only one sample phase image in their experiment) were associated with subsequent correct choices. In the current experiment, T-treated birds took longer before responding and yet still performed at the same level as when they had been given either no hormone or E$_2$. The difference in behaviour following T and E$_2$ ingestion implies that T did not exert its effect through being aromatised into E$_2$. However, the effects of the other T metabolite, DHT, on performance needs to be tested before this conclusion can be accepted. The effect of treatment on response
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latencies suggests that steroid levels were sufficiently elevated to affect behaviour at least to some degree.

In the titration experiment, the effect of image proximity on memory retention was in the expected direction, corroborating the results of Experiment 1B and an earlier study (see chapter 3). The presence of a near distractor had a significant effect on the ability of birds to make a correct choice when compared with that when distractors were in far positions. Birds were more likely to make a correct choice when the distractor was far from the target. This supports the finding of McGregor and Healy (1999) who found that the proximity of a distractor in a DMTS task affected the performance of both food-storing and non-storing tits: the closer a distractor, the more incorrect choices made (McGregor & Healy, 1999). The lack of a sex difference in performance in the titration experiment is consistent with the lack of a sex difference in plasma T levels. It is only during the breeding season, when T levels are elevated in males, that a sex difference in rodent spatial ability is found. For example, the sexual discrepancy in maze-learning ability in meadow voles (Microtus pennsylvanicus) and deer mice (Peromyscus maniculatus) is only found if the voles are in breeding season condition (Galea et al., 1996).

4.6 Experiment 2B

The results of Experiment 2A suggest that T exerted its effect on spatial learning and memory abilities (Experiment 1A) and response latencies (Experiment 2A) by acting as T or through being aromatised to DHT as E2 was not found to have any effects. However, as stated previously, the effect of the other T metabolite, DHT, on performance needs to be tested before this hypothesis can be accepted. In Experiment 2B I therefore manipulated levels of DHT (5α-androstan-17β-ol-3-one (Sigma)) as well as T and E2. All birds had the same experience with the touch screen set-up. The retention interval of each bird was increased by 50% (e.g., a R.I. of 2 seconds was increased to 3 seconds) to make the task more difficult. Birds were tested on each hormone treatment for two days.
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In Experiment 2B, I made 3 predictions:

1. On the basis of Experiment 2A results, I predicted that there would be no sex difference in performance.

2. I predicted that there would be an effect of treatment on score, with T-treatment improving performance, as in Experiment 1A. I expected that the effect of T would be similar to the effect of DHT or E2.

3. I predicted that treatment would affect response latencies to the second sample image, with T- and DHT- treatments increasing response latencies compared to the E2-treatment and vehicle.

4.6.1 Materials and methods.

Animals. The subjects were the same 8 male and 16 female great tits used in Experiment 2A. Birds were housed and fed as before and maintained on the same 9:15 h light:dark cycle and under a temperature range of 14-16°C. Birds were deprived of food at 8am each morning and testing began at 10am. Birds were tested 7 days a week.

Apparatus. See Chapter 3.

Protocol. All birds had completed Experiment 2A. Due to differences in number of trials taken to reach criterion in Experiment 2A, birds finished testing at different times. Each bird was retrained to a performance level comparable to that achieved in Experiment 2A. Birds were tested on consecutive days, and completed 20 trials per day. Again, the experiment was a within subjects design, with each bird serving as its own control. All birds underwent all hormone treatments (10μl of 8mg/ml T/E2/DHT in peanut oil) but the order in which they received the treatments was counterbalanced. There were six possible orders of the three hormone treatments and four birds were assigned to each one. Birds were first treated with the vehicle for two days (vehicle 1; a larva injected with peanut oil). Each of the hormone treatments were separated by 2 days of “control” treatment (vehicle 2 & 3) and all birds were treated with the vehicle for the final two days of testing (vehicle 4).
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4.6.2 Results

The analysis was performed using the cumulative score (i.e., score out of 20) as the performance measure of each day's testing (20 trials). To determine if the hormone treatments had an effect on performance, the following model (proc GLM) was fitted to the data, with day entered as a linear covariate:

\[
\text{Score} = \text{bird (sex)} + \text{treatment} + \text{sex + day} + \text{sex*treatment}
\]

There was no significant interaction between sex and treatment \(F(3,289) = 1.31, p = 0.27\) such that the manipulations affected each sex in the same way. Once this non-significant interaction was removed from the model, there was no significant effect of treatment on score \(F(3,292) = 0.31, p = 0.82\). Scores achieved also did not differ between sexes \(F(1,22) = 2.93, p = 0.10\); see figure 10). There was a significant effect of day \(F(1,292) = 10.75, p = 0.001\). The relationship between score and day was positive (slope = 0.44±0.13 / day): performance improved across the course of the experiment.

![Figure 10: Mean (± s.e.) performance scores for males and females. There was no significant difference between the sexes.](image)

Again, the analysis of cumulative scores was potentially too coarse to detect variation in treatment effects within a session (20 trials). Therefore, subsequent analyses were
Chapter 4. The effect of testosterone on avian spatial learning and memory abilities performed on the “raw” data (i.e., correct or incorrect score on each trial). The model (proc LOGISTIC) was fitted to the data, with the time variable created as before:

\[ \text{Score} = \text{bird} + \text{day} + \text{trial} + \text{time} + \text{treat} + \text{time*treat} \]

The interaction between time and treatment was non-significant \( (\chi^2 = 1.06, p = 0.79) \) suggesting that there was no variation in treatment effects across the day. Further analyses were carried out to determine whether hormone treatment affected other aspects of cognition, such as time to respond to (peck) the images on the touch screen, as in Experiment 2A. The following model (proc GLM) was fitted to the log transformed data:

\[ \text{Latencies} = \text{bird (sex)} + \text{sex} + \text{treatment} + \text{day} + \text{time} + \text{sex * treatment} + \text{time*treatment} \]

When the non-significant interaction terms \( (p > 0.05) \) were removed from the model, there was no effect of treatment on Choice latencies \( (F(3,6364) = 0.86, p = 0.46) \) but the effect of treatment on response latency to the 2nd sample image was marginally non-significant \( (F(3,6364) = 2.53, p = 0.06) \). Treatment tended to result in longer latencies to the second sample image, as in Experiment 2A (see figure 12).

There was no effect of sex on response latencies to either the Sample 2 or Choice touch screen images (sample 2: \( F(1,22) = 1.03, p = 0.32 \); choice: \( F(1,22) = 1.82, p = 0.19 \)). There was a significant effect of bird (sample 2: \( F(22,6364) = 19.46, p < 0.0001 \), choice: \( F(22,6364) = 12.13, p < 0.0001 \)), with variation in response latencies between birds. There was no significant effect of day in Choice image latencies \( (F(1,6364) = 0.08, p = 0.78) \) but response latencies to the Sample 2 image varied across days \( (F(1,6364) = 27.14, p < 0.0001) \). Time affected both Choice and Sample 2 latencies (choice: \( F(1,6364) = 7.87, p = 0.005 \), sample 2: \( F(1,6364) = 66.47, p < 0.0001 \)), with response latencies increasing across the course of a given day’s testing.
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Figure 12: The effect of treatment on average (±s.e.) response latencies. Treatment-effects on the Sample 2 latencies (median = 2 seconds) were marginally non-significant whereas treatment did not affect Choice latencies (median = 1.4 seconds).

Figure 13: Average response latencies (±s.e.) to the touch screen images. Latencies did not correlate with sex. Males and females took comparable amounts of time to respond to the sample 2 and choice images.
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4.6.3 Discussion

To some extent, the results of Experiment 2B corroborate those of Experiment 2A. In neither case did treatment have an effect on score. However, the effect of treatment on response latencies in Experiment 2B was in the predicted direction and corroborates the results of Experiment 2A. T-treatment tended to lengthen response latencies to the sample 2 image, although the trend was not significant. Birds fed T prior to testing achieved scores that did not differ from those achieved on E2-treatment and control days, in spite of having taken longer to respond to the sample 2 image (thereby increasing the time they had to remember Sample 1). Longer response latencies are usually associated with incorrect choices (McGregor & Healy, 1999) but T treatment seemed to reduce this effect. The treatment effects on response latency were marginally non-significant possibly because Experiment 2B was less powerful than 2A because birds were just tested for two days under each hormone treatment rather than five days.

4.7 General Discussion

Taken together, these experiments have provided provisional evidence for the role of steroid hormones in avian spatial learning and memory abilities. T-treatment had a beneficial effect on females’ performance in Experiment 1A. Females achieved higher scores after being fed T immediately before testing than on control days. Although none of the hormone treatments affected overall score in subsequent experiments, T-treatment significantly lengthened response latencies in the sample phase of the experiment. When treated with T, birds were able to take longer before responding to the touch screen image than when they had received the vehicle or E2-treatment and still perform at the same level (Experiment 2A).

The rapid effects that resulted from the non-invasive method of T administration were unlikely to be mediated through intracellular receptors in the hippocampus. Once a hormone-receptor complex changes transcription levels, a change in protein levels and/or cellular activity is not usually detectable for 30-60 minutes after hormone
treatment (Breuner et al., 1998). Therefore, it is likely that T acted through a nongenomic mechanism. In their study of behavioural effects of CORT (also administered orally), Breuner et al. (1998) came to the same conclusion. Perch-hopping in Gambel's White-Crowned Sparrows increased in response to CORT treatment and occurred concurrently with steroid elevation (i.e., once the level of CORT had returned to baseline, perch-hopping activity returned to normal), suggesting that CORT was acting through a nongenomic mechanism.

It is possible that the effect of T on response latency was not mediated by the hippocampus and actually involved activation of androgen receptors in another brain area. This explanation would at least explain why I found an effect of T on response latency but failed to find any treatment effects on overall task performance in three of the four experiments. It is possible that, like the HVc (the neural substrate underlying song in songbirds), the hippocampus becomes insensitive to T outside of the breeding season. At the end of the breeding season birds become refractory to the stimulatory effects of long days and the gonads regress, sex hormone levels decrease and feature molt ensues (Nicholls et al., 1988; Juss, 1993). During this photorefractory period androgen receptor (AR) and oestrogen receptor (ER) production in the HVc appears to be diminished (Fusani et al., 2000) and exogenous T-treatment does not induce an increase in HVc volume (Bernard & Ball, 1997). In support of my hypothesis, seasonal variation in AR mRNA expression in the hippocampus has been found in the Lapland Longspur (Meddle, pers. comm. 2003). In this species, AR numbers in the hippocampus decrease at the end of the breeding season when T levels return to basal and at this time T-treatment has no effect on behaviour (Meddle, pers. comm. 2003). The treatment effect in females in Experiment 1A may therefore have resulted because the birds were maintained under a breeding season (long-day) photoperiod. AR and ER receptor numbers in the hippocampus may have reflected the putative elevation in sex hormone levels. The lack of treatment effects on performance in Experiment 2, when the birds were maintained under a short-day, non-breeding season, photoperiod, could, therefore, possibly be explained by low AR and ER numbers in the hippocampus.
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Another, related, possibility for the discrepancy in results between Experiment 1A and subsequent experiments is that as endogenous levels of sex hormones were assumed to be higher in birds maintained under the long-day (Experiment 1), compared to short-day (Experiment 2), photoperiod, the elevation in T achieved by the manipulation may not have been sufficient to effect spatial learning and memory abilities in the latter group. However, this explanation fails to account for the failure to find any treatment effects in Experiment 1B. Nevertheless, the results of a study carried out by Williams et al. (1996) provide a possible explanation why a treatment effect may have been found in Experiment 1A but not in 1B. Ovariectomised rats (implanted with either high or low doses of E2) were trained on a RAM immediately after E2 replacement, and retrained after 12 months of chronic E2 replacement. E2 at both high and low doses improved performance of rats after short-term E2 treatment, but not after long-term treatment. Therefore, it is possible that carry-over effects of the hormone treatment administered to birds in Experiment 1A resulted in a lack of treatment effects in the same birds in Experiment 1B. This explanation is made all the more likely given that Experiment 1B commenced immediately after the completion of Experiment 1A. However, this hypothesis cannot be accepted without plasma radioimmunoassay which would permit an estimation of the time-course of steroid action.

The failure to find a consistent effect of steroid hormones is not unexpected, especially considering the variation in results of studies that have assessed steroid effects on cognition in mammals. Steroid hormones have been found to improve, impair, or not affect performance on various tasks of learning and memory (Dohanich, 2002). Their effects are task-dependent and possibly memory-dependent (Sherwin, 1994, 1996, 1996; Gouchie & Kimura, 1991). Two weeks of oestrogen treatment in ovariectomized rats impaired hippocampally-dependent spatial reference memory but enhanced hippocampally-independent cued memory in a radial arm maze (Galea et al., 2001). This finding led Markus and Zecevic (1997) to propose that whether or not steroid hormones exert an influence on ability depends on whether the task is hippocampal versus extrahippocampal (Markus & Zecevic, 1997). This supposition would facilitate an interpretation of my results if the visual task was in fact hippocampal-dependent.
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However, even then, the effect of T on performance on the spatial memory task was not consistent, evidenced by the dichotomy in results between Experiment 1A and subsequent experiments.

Other researchers make a distinction between working memory and reference memory tasks in trying to explain the inconsistent effects of gonadal steroids on cognition. Cast in this framework, available data indicate that endogenous or exogenous E2 enhances performance on tasks that depend primarily on working memory (as measured, for example, in RAM and MWM). Enhancements in performance have been reported during both task acquisition and retention in both male and female rats. Rats exposed to E2 commit fewer errors during acquisition and display better retention during short and long delays. Conversely, E2 usually fails to alter, or even impairs, performance of reference-memory tasks (Daniel et al., 1999; Chesler & Juraska, 2000). Although the touch screen task used in this study was primarily a working memory task (with the storage of information being useful only within a single trial), no enhancement of performance was found with E2. Therefore, the distinction between steroid effects on working and reference memory does not facilitate an interpretation of my results.

Despite the complexities discussed, my findings point to steroids as potential modulators of cognitive performance. Further behavioural studies are necessary and should address a number of issues raised by my research. First, the administration of different concentrations of steroids and measurement of circulating levels would determine whether the lack of consistency in my results was due, in part, to the use of too low a dose of hormone and ensure that hormones were at a physiological level, comparable to the breeding season maxima. Second, it would be useful to determine what is occurring in the hippocampus after hormone manipulation. Examination of hippocampal anatomy would facilitate an interpretation of results. Through the examination of AR and ER mRNA expression in the hippocampus one could predict whether exogenous hormone treatments had the potential to alter spatial learning and memory abilities. Finally, in future experiments, the effect of T on response latencies could be explored further using a titration procedure.
Chapter 5. Does Corticosterone affect Memory in the Zebra Finch 
(*Taeniopygia guttata)*?

5.1 Abstract

The pattern in which androgens influence spatial memory is mirrored by the effect of the glucocorticoid hormone, corticosterone (CORT): they both follow a concentration-dependent biphasic relationship. Furthermore, testosterone and CORT co-vary so may influence spatial learning and memory abilities not only separately but in concert with one another. Similar to research carried out to assess the influence of sex steroids on spatial learning and memory abilities, most work into the effect of CORT on cognitive abilities has been limited to the study of mammals. The primary aim of this experiment, therefore, was to determine whether CORT affects avian spatial memory in a similar way to that seen in mammals and, furthermore, to investigate the effects of putative interactions between gonadal hormones and CORT on spatial ability. The memory of zebra finches (*Taeniopygia guttata*) selectively bred for peak CORT level was assessed using two versions (spatial and visual) of a one-trial associative memory task. High CORT birds performed less well than controls on the spatial version of the task but there was no difference in performance between lines on the visual task. Results suggest that high levels of CORT have a detrimental effect on spatial cognition.

5.2 Introduction

It is not only androgens that influence spatial memory in a non-linear manner (see Chapter 4). The glucocorticoid hormone corticosterone (CORT), commonly termed the stress hormone, also influences spatial learning and memory abilities in a concentration-dependent fashion (Roof & Havens, 1992; Roof, 1993b; Lupien & McEwen, 1997; McEwen & Sapolsky, 1995). Glucocorticoid hormones are secreted from the endocrine glands of the adrenal cortex in response to stressors. CORT acts in concert with other components of the hypothalamic-pituitary-adrenal axis (e.g.,
adrenaline/epinephrine and noradrenaline/norepinephrine) in order to facilitate
cope with stress and promote return to the normal life history stage (see Wingfield
et al., 1998). It has a diverse effect on target organs, such as the brain, and behaviour
(De Kloet, 2000; Saldanha et al., 2000). In particular, the high density of receptors
(Type I / mineralocorticoid (MR) and Type II / glucocorticoid receptors (GR)) for
cORT in the mammalian hippocampus (Arizza et al., 1987; McEwen et al., 1986;
Fuxe et al., 1985; Reul & De Kloet, 1985; Van Eekelen et al., 1987; Van Eekelen et
al., 1988) suggests that corticosteroids may be important modulators of hippocampal-
dependent functions, and that these receptors may mediate corticosteroid effects on
cognition, learning and memory (Douma et al., 1998; Diamond et al., 1992; Lupien

5.2.1 How CORT influences learning and memory

The importance of glucocorticoids in learning and memory has emerged from
studies using both in vitro and in vivo models. Results from these studies are
complex and suggest that the influence of CORT on spatial learning and memory
abilities follows a concentration-dependent biphasic relationship. CORT is thought
to enhance spatial learning and memory abilities when at intermediate levels
(Schantz & Widholm, 2001; Shors et al., 1992), with fluctuations in circulating
levels of CORT, in either direction, adversely affecting learning and memory in both
humans and rodents (Vicedomini et al., 1986; Kirschbaum et al., 1996; Lupien &
McEwen, 1997).

For example, elevation of circulating CORT concentrations through exogenous
administration or glucocorticoid receptor agonists impairs spatial learning and
memory in both perinatal and adult rats (Arbel et al., 1994; Bodnoff et al., 1995;
Bohus, 1994; De Kloet et al., 1988; Kerr et al., 1991; Endo et al., 1996; Oitzl et al.,
1994; Vicedomini et al., 1986). The extent of the decrement in spatial learning and
memory abilities depends on the dosage of administered CORT; rats given high
doses are more impaired than those given low doses, which are more impaired than
controls (Vicedomini et al., 1986). It is not only exogenous administration of
glucocorticoids, however, that has an adverse effect on spatial learning and memory
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abilities. Daily restraint stress for 21 days (chronic stress) induces an elevation of CORT levels and is associated with impaired RAM performance in male rats (Luine et al., 1994). Conversely, if levels of CORT are abnormally low (achieved experimentally through removal of endogenous glucocorticoids by adrenalectomy (ADX)), spatial orientation learning is impaired in rats (Oitzl & De Kloet, 1992).

Moderate stress, on the other hand, can have a facilitative effect on spatial learning and memory abilities in male rats and mice (Luine et al., 1996; Micheau et al., 1985). For example, daily restraint stress for 13 days caused an enhancement of performance in the RAM 10-13 days post stress. Performance of the stressed rats significantly correlated with their stress-induced serum CORT levels: rats with higher levels of CORT outperformed those with low levels (controls) (Luine et al., 1996). 13 days of restraint stress elevated CORT to a level that enhanced performance on the RAM. This level of CORT was above that of rats restrained for 7 days and below that of rats restrained for 21 days.

Taken together, these results suggest that stress duration, and the induced differences in CORT level, may differentially affect spatial learning and memory, with shorter periods of stress (leading to moderate levels of CORT) having a beneficial effect on performance while longer durations (high levels of CORT) having deleterious effects on mammalian spatial learning and memory abilities (Luine et al., 1996).

The effect of CORT on avian spatial learning and memory abilities in birds has been examined by monitoring caching behaviours in the food-storing mountain chickadee (Parus gambeli) following exogenous CORT treatment. The rapid effects of CORT on food-storing behaviours were examined by feeding birds mealworms injected with CORT five minutes prior to either caching or retrieval. Such manipulations had no effect on caching but CORT-treated birds retrieved significantly more seeds compared to controls. Since CORT-treated and control birds did not differ in the number of seeds eaten or the total number of sites visited, it was unlikely that CORT affected activity, appetite or motivation. Rather, the results suggest that an elevation in CORT level had a positive effect on memory for cache sites (Saldanha et al., 2000). Pravosudov and Clayton (2001) experimentally
demonstrated that long-term limited and unpredictable food supply results in moderately elevated CORT levels (lower than standardised stress response) in mountain chickadees. Such small but chronic elevations in CORT levels triggered by unpredictable food supply were also correlated with enhanced cache retrieval efficiency and spatial memory performance (Pravosudov & Clayton, 2001). In a subsequent study, Pravosudov (2003) demonstrated that an elevation in CORT level not only affected cache retrieval, but also altered caching itself. Birds implanted with 90-day continuous time-release CORT pellets cached and consumed significantly more food and showed more efficient cache retrieval (inspected fewer sites to find previously made caches) and superior spatial memory (performed better on a one-trial associative spatial memory) compared with placebo implanted birds (Pravosudov, 2003). The positive effect of CORT was specific to the spatial domain as control birds performed as well as CORT-treated birds on a non-spatial (colour) version of the one-trial associative memory task. The lack of a performance difference on the colour task also suggested that the differences between CORT-treated birds and controls on the spatial task were not due to motivational differences.

An elevation in CORT, however, does not always have a facilitative effect on avian spatial learning and memory abilities. When CORT levels were experimentally elevated during early development, the ability of Black-legged Kittiwakes (Rissa tridactyla) to solve a spatial task was compromised (Kitaysky et al., 2003), possibly suggesting different effects of organisational and activational levels of CORT.

5.2.2 How CORT acts in the brain

Through the examination of the uptake and retention of adrenal steroids by brain tissue, adrenal steroid receptors have been identified in extrahypothalamic limbic brain regions of the rat (McEwen et al., 1969; McEwen et al., 1968). The greatest accumulation of adrenal steroids occur within the hippocampus compared to other brain areas (see McEwen et al., 1999). CORT binds to specific receptors in the
plasma membranes of neurones and other tissues (Koch et al., 1978; Towle & Sze, 1983; Orchinik et al., 1991; Trueba et al., 1991; Allera & Wildt, 1992; Suyemitsu & Terayama, 1975; Quelle et al., 1988) but, in most vertebrate species, these membrane-associated receptors are poorly characterised (Breuner & Orchinik, 2001). On the other hand, intracellular, ligand-activated transcription factors are well described in mammals (Breuner & Orchinik, 2001). The mammalian hippocampus contains both Type I receptors (mineralocorticoid (MR)) and Type II receptors (glucocorticoid (GR)). Type I and Type II receptors co-ordinately modulate the influences of CORT (Reul & De Kloet, 1985). The selective activation of these two types of intracellular receptor exerts distinctly different behavioural effects (Oitzl et al., 1994; Lupien & McEwen, 1997; McEwen & Sapolsky, 1995) that can explain how CORT affects learning and memory in a concentration dependent manner. CORT binds to Type I receptors with a ten-fold higher affinity than to Type II receptors (Oitzl et al., 1994; Douma et al., 1998). Therefore, at basal levels, CORT predominantly occupies Type I receptors. Granule cells of the adult dentate gyrus require adrenal steroids for their survival. Activation of Type I receptors actually enhances survival of neurons in the dentate gyrus (Woolley et al., 1991) and protects against adrenalectomy-induced cell death, thereby optimising performance on hippocampal-dependent tasks. When Type I receptor antagonists are administered, or endogenous glucocorticoids are removed, spatial orientation in rats is impaired (Douma et al., 1998; Oitzl & De Kloet, 1992). As CORT levels increase during the stress response, Type I receptors become saturated and Type II receptors become activated (Joels & De Kloet, 1992). The occupation of Type II receptors exacerbates the destructive effects of certain neurotransmitters (particularly excitatory amino acids) on hippocampal neuronal survival (Sapolsky, 1990; Lawrence & Sapolsky, 1994; McEwen & Sapolsky, 1995), and have an effect on spatial learning and memory that is similar to hippocampal lesions (Vidal et al., 1986). This is corroborated by electrophysiological recordings in vivo (Pfaff, 1971) and in vitro (Vidal et al., 1986) that show a decrease in excitability of hippocampal CA1 pyramidal cells. In addition, elevated concentrations of CORT leads to the atrophy of the dendritic branches in pyramidal neurons of the CA3 region of the hippocampus and a decrease in total dendritic
length in the rat (Watanabe et al., 1992; Magarinos & McEwen, 1995a; Magarinos & McEwen, 1995b; Vyas et al., 2002; Woolley et al., 1990).

The level of CORT can affect long-term potentiation (LTP) and prime burst potentiation (PBP), both of which are involved in the regulation of learning and memory formation (Diamond et al., 1992; Foy et al., 1987; Shors et al., 1992). Activation of Type I receptors heightens glucose uptake and leads to an increase in LTP and PBP in the rat (Douma et al., 1998; Lupien & McEwen, 1997; McEwen & Sapolsky, 1995; Pavlides et al., 1995). Conversely, stress and subsequent elevation of CORT levels result in activation of Type II receptors and blocking of hippocampal LTP (Filipini et al., 1991a; Filipini et al., 1991b; Dubrovsky et al., 1990; Foy et al., 1987; Diamond et al., 1990; Lupien & McEwen, 1997).

It is not clear how closely the pharmacology of avian receptors matches that of mammalian receptors. Two intracellular CORT receptors have been identified in radioligand binding studies in the duck (DiBattista et al., 1985) and the chicken (Beaudry et al., 1983), and a mammalian Type II receptor antibody recognises Type II-like proteins in the quail brain (Kovacs et al., 1989).

5.2.3 Sex differences in response to stress

The experience of stress is largely mediated by the hypothalamic-pituitary-axis (HPA) and sex differences exist in resting levels of the HPA secretions. Virgin
female rats have higher resting levels of CORT (Critchlow et al., 1963) and display greater diurnal changes in CORT compared to males (Handa et al., 1994). Glucocorticoid levels are responsive to hypothalamic-pituitary-gonadal-axis secretions and increase with the experience of stress to higher levels in females compared to males (Galea et al., 1997; Handa et al., 1994). This sexual discrepancy in glucocorticoid secretion varies with changes in ovarian hormone levels across the oestrous cycle in females. CORT levels are at their highest during proestrous when ovarian secretions are high (Burgess & Handa, 1992; Carey et al., 1995; Viau & Meaney, 1991). It is possible then, that oestradiol may moderate the sex difference in stress effects on spatial learning and memory.

A sexual discrepancy in stress effects has been noted in both behaviour and neurology. Firstly, 21 days of restraint stress impaired RAM performance in males (Luine et al., 1994) but enhanced performance in females (Bowman et al., 2001). To assess the role of ovarian hormones in this sexually dimorphic response to stress, Bowman et al. (2002) compared the performance of oestradiol-treated ovariectomised rats to controls (non-treated, ovariectomised rats). Oestradiol-treated animals, with or without stress, performed better than controls (Bowman et al., 2002), highlighting the importance of ovarian hormones in mediating the sexually dimorphic stress response.

Secondly, oestrogen may result in female resistance to stress-induced impairments by affecting hippocampal morphology. One possibility is that oestradiol exerts either a direct protective effect on the hippocampus or modifies the HPA cascade in females (e.g., oestrogen could influence CORT release or glucocorticoid receptor density) (Bowman et al., 2001). Indeed, female rats showed less atrophy of apical dendritic branches than males after chronic stress, although the resultant elevation in CORT led to basal remodelling in females (a decrease in the number of basal branch points compared to control females) (Galea et al., 1997). This remodelling in the female hippocampus following stress and the enhancing effects of oestrogen on the CA1 pyramidal cells may work together to protect against stress-induced impairments (Bowman et al., 2001).

Seasonal differences in behavioural and physiological components of the stress response have been documented widely in birds (Breuner & Wingfield, 2000). Type
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I- and Type II- like molecules, basal and stress-induced plasma CORT levels have been found to vary with season (Breuner & Orchinik, 2001). The study of seasonal variation in adrenocortical response to stress, particularly in Arctic breeding birds (Wingfield et al., 1982; Wingfield et al., 1995), has highlighted sex differences in CORT levels. In Arctic ecosystems the brief breeding season limits the capacity of most avian populations to renest. Therefore, to successfully reproduce in the Arctic, birds must modulate their neuroendocrine and behavioural systems. These adjustments include an attenuation of the stress responsiveness of the HPA axis to external stimuli and a behavioural insensitivity to high CORT levels. For example, basal and stress-induced CORT levels are significantly lower in the female Smith’s longspur (Calcarius pictus) than in males. This mechanism is hypothesised to increase reproductive success by preventing interruptions to parental care during transient deleterious environmental perturbations (e.g., harsh weather) (Meddle et al., 2003). However, it is not known whether CORT levels in female birds are sensitive to fluctuations in levels of oestradiol, as they are in mammals.

5.2.4 CORT and testosterone co-vary

The effects of glucocorticoids on glucose mobilisation are considered essential for facilitating short-term behavioural responses to stressors. Glucocorticoids help break down protein and convert it to glucose, help make fats available for energy, increase blood flow, and stimulate behavioural responsiveness. They decrease the sensitivity of the gonads to luteinizing hormone (LH), which suppresses the secretion of sex steroids. It is not surprising, then, that circulating levels of CORT and testosterone are correlated. The relationship between CORT and T may be “two-way” as elevated testosterone levels may sensitise the hypothalamic-pituitary-adrenal axis and cause elevated CORT (Schoech et al., 1999). A positive correlation between T and plasma CORT concentrations has been found in male dark-eyed juncos (Junco hyemalis) (Breuner & Orchinik, 2002; Ketterson et al., 1992; Klukowski et al., 1997; Schoech et al., 1999) and male pied flycatchers (Ficedula hypoleuca) (Silverin, 1998) (see figure 2).
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5.2.5 Corticosterone binding globulin

Once a hormone is secreted into the bloodstream, it rapidly becomes deactivated. Through binding to "protective" carrier proteins (globulins) in the blood, however, a hormone extends its half-life, thereby prolonging its activity. Carrier proteins facilitate transportation of hormones in the blood (steroid hormones would otherwise be insoluble) and regulate the bio-availability of steroid hormones to target tissues (Breuner & Orchinik, 2002; Hammond, 1995; Siiteri et al., 1982; Deviche et al., 2001). According to the Free Hormone Hypothesis (Mendel, 1989; Breuner & Orchinik, 2002; Silverin, 1986), before a hormone can bind to its target cell receptors, it must be uncoupled from its carrier protein. The binding of hormones to binding globulins, therefore, serves as a buffer against potential deleterious effects of excessive hormonal stimulation.

Mammalian plasma contains a corticosterone binding globulin (CBG) that exclusively binds glucocorticoids, and a sex hormone binding globulin (SHBG) that binds gonadal hormones, such as T (Hammond, 1995). In contrast, the plasma of several avian species contains CBG (Klukowski et al., 1997; Kovacs & Peczely, 1983; Silverin, 1986; Wingfield et al., 1984) but apparently lacks SHBG (Wingfield et al., 1984). Avian CBG not only binds glucocorticoids with high affinity, but also binds progesterone with high affinity, and T and E₂ with low affinity (Wingfield et al., 1984).
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al., 1984). The affinity of CORT to CBG is five greater than that of androgens. Therefore an increase in plasma CORT (such as occurs during the stress response), acutely increases free T by as much as five fold, as CORT out-competes T for CBG binding sites (Breuner & Orchinik, 2002; Deviche et al., 2001). As CORT displaces T from CBG, the resultant surge in circulating T may have a number of effects. For example, experimental elevation of T in the dark-eyed junco significantly increases CBG levels and thus the capacity of the plasma to bind CORT (Breuner & Orchinik, 2002; Klukowski et al., 1997). The competition between CORT and T for plasma binding sites in birds may have significant implications for the physiological, developmental, and behavioural actions of both steroids (Deviche et al., 2001).

The primary aim of this study is, then, to determine whether CORT affects avian spatial learning and memory in a concentration-dependent manner, as it does in mammals. To my knowledge, this is the first study to investigate the effects of putative interactions between CORT and T on spatial learning and memory abilities in birds. The competition between CORT and T, both at their respective hippocampal receptors, and also for CBG in plasma, may have significant implications for the cognitive actions of both steroids.

By taking advantage of the production of zebra finches (Taeniopygia guttata) selectively bred for peak CORT level, I was able to assess the effect of differing CORT levels on learning and memory abilities, without the need for experimental manipulation of hormone levels. Memory abilities were assessed using two versions of a one-trial associative memory task (spatial and visual). Patel et al. (1997) showed that zebra finches were able to complete a task of a similar nature, consisting of two phases (sample and choice). Each bird was allowed to locate a reward in the sample phase and, after a 30 minute retention interval, its ability to relocate the reward was assessed. In the current study, the two types of memory task differed in the cues available to the bird when relocating the reward. A visual version of the task was used to ascertain whether any hormonal influence on memory was specific to the spatial domain. Individuals from three different lines of zebra finch, selectively bred for peak stress-induced CORT level, were studied (i.e., High CORT, low CORT & controls).
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I predicted that birds selected for high CORT would be selectively impaired on the spatial task but their performance on the visual version of the task would be spared. I expected the impairment to be specific to the spatial domain as manipulations of CORT level often do not affect performance on non-hippocampal-dependent tasks (Pravosudov, 2003). In addition, as female birds sometimes have lower levels of both basal and stress-induced CORT (Meddle et al., 2003), I predicted that high CORT levels would be less detrimental to the performance of females compared to males.

5.3 Materials and Methods

Animals. The subjects were male and female sexually mature, captive bred adult zebra finches (F1 and F2 generation). The birds were selectively bred at the University of Stirling for peak CORT level, with birds being chosen with the highest and lowest CORT titres in each generation. The selection process was as follows: all offspring were ranked according to CORT levels at 6 weeks post-fledging. The top 50% in the high line became the parents of the next 'high' generation and the bottom 50% of the low line went on to produce the next low generation. Controls were picked at random from the bottom 50% of the high line and top 50% of the low line (Buchanan, pers. comm. 2003). Birds tested here were either from the "High CORT" (n = 20), "Low CORT" (n = 16) or "Control" (n = 19) line. Owing to limited laboratory space and temporal variation in the availability of the selected lines, subjects were tested in three groups. The first group of birds tested were 10 from the "High CORT" line and 10 from the "Control" line. These birds were tested between September and October 2002. Ten more birds from the "High CORT" line were tested, along with a further six "Control" birds (3 control birds died between October and December 2002), between January and April 2003. The final group of birds tested were all bred for a low CORT level and were tested between May and July 2003. Once collected from Stirling University, all birds received a unique combination of plastic coloured leg bands for identification. They were housed in single sex groups of six birds in wire-mesh cages (77cm long x 44cm wide x 44cm high) in a windowless room. Each cage had a removable sliding door (33.5cm x
27.5cm) at the front and contained three dowel perches. All the cages were located in full visual and auditory contact with each other. Birds were fed daily with ad libitum water and a seed mixture, supplemented by fresh vegetables, millet spray and dried cuttlefish bone. They were maintained on a 16:8 hr light:dark cycle and under a temperature range of 19-21°C. For both training and experiments, birds were deprived of food at 8am each morning and provided with fresh food when their session was complete. Training and testing began at 2pm.

**Apparatus.** An experimental tray (29cm X 22cm) with 48 circular wells (1cm diameter; 1cm deep) arranged in 8 by 6 array, was used to assess memory. The wells were surrounded by Velcro to which squares of felt, measuring 2.5cm X 2.5cm were attached (see figures 3a-4).

**Training.** Each bird was caught and placed in the test cage alone. This was considered the most effective way to train the birds as it avoided the risk of “local enhancement” (when a food discoverer attracts the attention of others; see Giraldeau, 1984) and avoided problems associated with frequency dependent learning (see Dickinson, 1980). If, for example, the seed was discovered by one individual who attracted the attention of another, the chances of the latter bird learning to find seed in subsequent trials would be reduced (Giraldeau, 1984). Each bird was presented with the experimental tray, with 10 wells filled with seed, for thirty minutes each day. Once an individual was eating seed from the tray, felt flaps were introduced and attached to the Velcro so that they partially covered the baited compartments. Once a bird lifted the partially covered flaps to obtain the reward, the felt pieces were attached so that they completely covered the rewarded wells. Next, when a bird removed flaps to obtain seed, the number of rewarded wells was gradually reduced to three so that it learnt that a covered well did not necessarily contain seed. The test phase of the experiment began when the bird lifted at least three flaps (with only 1 well rewarded) in a five minute period.

The duration of the training period varied greatly among birds, and among birds that reached criterion, ranged from 24-52 days.

**Testing.** The one-trial associative memory tasks. Each task consisted of two phases separated by a retention interval of five minutes. In the sample phase, the experimental tray was introduced into the cage. Only one well contained 5ml of bird
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Seed. Six other pieces of felt covered six different empty compartments, chosen at random. In the spatial task, all felt flaps were red in colour and differed only in position. Red was chosen as sexual selection studies have revealed a preference for this colour in the zebra finch (Burley, 1988). In the visual task, the reward well was covered with a piece of felt that differed in colour to the flaps covering the 6 randomly chosen wells. There were six different colours in total, with the colour of flaps on any given trial chosen randomly.

The tray was placed into the centre of the home cage. Each bird was allowed to remove as many flaps as necessary to find the food and then allowed to eat for 30 seconds. This meant that it consumed some, but not all, of the seed. The tray was then removed for a 5 minute retention interval. At the start of the choice phase, the tray was reintroduced into the cage. The bird was rewarded when it removed the “correct” flap. In the spatial task, the location of the rewarded well and the 6 distractor wells remained unchanged from the sample phase (see figure 3a). In the visual task, the colour of the pieces of felt remained the same in the choice phase but the location of all the covered wells varied so that the only reliable cue to the reward was the unique flap colour (see figure 3b). In both versions of the task, the test ended when the bird had eaten all of the remaining food or after 5 minutes, whichever occurred sooner. The number of looks the bird made to find the food, i.e., the number of flaps the bird removed in the choice phase, was recorded. Revisits to the same compartment were not counted as looks, because the food was visible once the flap had been removed. Birds completed 5 trials (5 days of testing) of both the spatial and visual tasks. Each trial was unique in terms of the spatial location of the rewarded and empty wells. In the sample phase, performance should be random, because the food was hidden, and therefore, birds should have lifted 3.5 flaps on average to find the food (because seven compartments were covered). Performance in the choice phase tests for memory in relocating the partially eaten food; if the birds had used memory to solve the task in the choice phase, then they should have lifted significantly fewer than four flaps, and a score of one would indicate perfect performance.

A pilot study had shown that when the rewarded well was positioned among the distractors in the spatial task, the bird was less likely to complete the task. After
lifting four or more flaps and not locating the reward, a bird often gave up and failed to lift any further flaps. In the test, the rewarded well was therefore consistently positioned at a distance from the distractor wells to make the task “easier” (see figures 3a & 4).
Memory tests carried out under stress. Although the high CORT birds had been bred to have higher peak levels of CORT relative to controls, it was not clear whether this variation was reflected in CORT levels at the time of testing. In order to investigate this, once the birds had completed the tasks, they were re-tested after having been stressed. Peak CORT responses were stimulated by capturing a bird and placing it into in a small drawstring bag for 20 minutes (a standard technique for engendering peak stress response). On being released from the bag, the bird was presented with the memory task. This procedure was repeated twice for each bird so that its performance on both versions of the task (spatial and visual) under putative peak CORT levels could be monitored.

Plasma analysis. On the final day of testing, after the each bird had completed both the sample and choice phase of the experiment, it was caught and sacrificed by decapitation. Blood (approximately 100μl) was collected from the neck using heparinized capillary tubes. Plasma CORT concentrations increase rapidly following exposure to adverse conditions (e.g., Schwabl et al., 1991; Astheimer et al., 1994) but not for at least 3 minutes. Therefore, all plasma was taken within 3 minutes of capture and therefore reflects circulating levels at the time of behavioural testing. Blood samples were stored in the refrigerator until they were centrifuged (14000 rpm for 10 min) in the laboratory, within 1 hour of collection. Plasma was collected and stored at —20°C until assayed for T and CORT.

5.4 Results

Only 10 of the 20 birds from the first group and 9 of the 16 birds from the second group reached test criterion, and none of the birds from the “Low CORT” line learned the task. A mean test score for performance on both the spatial and visual versions of the one-trial associative memory task was determined for each test bird. The average number of flaps each bird lifted in the choice phase, over the five days of testing, for both versions of the task, was calculated (see table 1).
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Table 1: The mean number of flaps lifted in the choice phase of both versions of the one-trial associative memory task (M = male, F = female; C = Control, HC = High CORT).

The data were analysed using SAS (SAS Institute, 1989). The initial analysis compared the average number of flaps lifted in the spatial and visual task by birds of each line in the choice phase of the experiment (data presented in table 1). The assumptions of normality of error (Kolmogorov-Smirnov normality test of the residuals) and homogeneity of residuals (plot of fitted values against residuals) were tested in Minitab. To determine whether there was an effect of group (i.e., when the birds were tested), the following model (proc GLM) was fitted to the data using SAS:

\[
\text{Score} = \text{sex} + \text{group} + \text{sex} \times \text{group} + \text{bird (sex group)} + \text{task} + \text{task} \times \text{sex} + \text{task} \times \text{group} + \text{task} \times \text{sex} \times \text{group}
\]

The interaction terms were not significant hence were excluded from the final analysis. There was no difference between the groups in the number of flaps lifted (Group effect: F \((1,15) = 0.02, p = 0.90\); see figure 5). As a result, the data from the first and second groups were pooled and all subsequent analyses were performed on the combined data set.
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Figure 5: Mean (+/- SE) number of flaps lifted in the choice phase by birds in Group one and two (n = 19). The average number of flaps lifted does not differ between groups.

The performance of the birds on the spatial and colour tasks across the 5 days of experiment was assessed by examining responses in the choice phase (as in Patel et al., 1997). The number of flaps lifted can be taken as a measure of performance; if a bird lifted an average of 3.5 or more flaps, it was performing at random (as there were 7 flaps in total). The fewer the number of flaps lifted, the better the performance. The number of flaps an individual lifted each day (i.e., not average across days) were analysed using the following model:

\[
\text{Score} = \text{sex} + \text{line} + \text{sex} \times \text{line} + \text{bird (sex line)} + \text{task} + \text{day (task)} + \text{sex} \times \text{task} + \text{task} \times \text{line} + \text{sex} \times \text{task} \times \text{line}
\]

When non-significant (p > 0.05) interaction terms were removed, there was no significant effect of sex on score (F(1,16) = 0.02, p = 0.90). Males and females did not differ in the number of flaps they lifted in the choice phase. There was no significant effect of line (High CORT vs. Control) (F(1,16) = 2.54, p = 0.13). High CORT and control birds lifted a comparable number of flaps. The effect of task was marginally non-significant (visual vs. spatial)(F(1,165) = 3.06, p = 0.08). Birds tended to perform better on the visual task than they did on the spatial task. There was no significant effect of day (F(4,165) = 0.63, p = 0.64), suggesting that there was no significant variation in performance across the course of the experiment. However, the
interaction between task and line was significant \( F(1,165) = 12.56, p = 0.0005 \). The trend was that high CORT birds performed worse than controls on the spatial task with the reverse being true on the visual task. To analyse the interaction further, the tasks were analysed separately using the following model (proc GLM):

\[
\text{Score} = \text{sex} \cdot \text{bird} \cdot (\text{sex} \cdot \text{line}) \cdot \text{line} \cdot \text{day}
\]

On the visual task, there was no significant effect of sex \( F(1,16) = 0.10, p = 0.75 \) or of bird \( F(16,72) = 0.68, p = 0.80 \), i.e., performance did not differ between individuals and sexes. The effect of line was marginally non-significant \( F(1,16) = 3.90, p = 0.07 \). High CORT birds tended to lift fewer flaps than controls in the choice phase of the experiment. The effect of day was significant \( F(4,72) = 4.39, p = 0.003 \). The number of flaps the birds lifted varied from day to day but not in a systematic way as when “day” was entered as a linear covariate it no longer had a significant effect \( F(1,75) = 2.88, p = 0.09 \).

On the spatial task, there was no significant effect of sex \( F(1,16) = 0.10, p = 0.76 \) or of bird \( F(16,72) = 1.34, p = 0.20 \). The effect of day was non-significant \( F(4,72) = 0.58, p = 0.68 \), with there being no notable variation in performance across the course of the experiment. However, the effect of line was significant \( F(1,16) = 7.80, p = 0.01 \). High CORT birds performed significantly worse than Controls on the spatial task (see figure 6). The High CORT birds’ performance on the spatial task was not significantly different from random \( \text{mean} = 3.2, \text{Confidence interval (95\%)} = 0.55 \), whereas the number of flaps lifted by the control birds was significantly different from chance \( \text{mean} = 2.0, \text{Confidence interval (95\%)} = 0.41 \).
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Figure 6: Mean (+/− SE) number of flaps lifted in the choice phase of the visual and spatial tasks (n = 19). All birds tended to perform better on the visual task than the spatial task. High CORT birds performed significantly worse than controls on the spatial task whereas there was no difference in performance between the lines on the visual task.

5.4.1 Hormone assays

The stress-induced hormone data (Matthew Evans, *pers. comm. 2002*) were analysed to ensure that High CORT birds had a significantly higher peak (stress-induced) CORT level than controls.

The following model (proc GLM) was fitted to the plasma data of all birds for which plasma hormone measurements were available (n = 39):

\[
\text{Peak CORT level} = \text{sex} + \text{line} + \text{sex} \times \text{line}
\]

High CORT birds had significantly higher peak levels of CORT than did the controls (F(1,35) = 31.05, p<0.0001, see figure 7). There was no significant effect of sex (F(1,35) = 0.66, p = 0.42), although the interaction term was marginally non-significant (F(1,35) = 3.90, p = 0.06; see figure 7). High CORT males tended to have a
higher peak CORT level than females whereas the reverse was true with the control birds where females tended to have higher peak CORT levels than did the males.

Figure 7: Average difference between the sexes of the selected lines in peak corticosterone titre ((ng/ml) mean +/- S. E.) (n = 39). High CORT birds had a significantly higher peak CORT level than controls. High CORT males tended to have a higher peak CORT level than females whereas control females tended to have a higher peak CORT level than control males.

To see if this pattern held true for the birds that reached test criterion, an analysis using the plasma data from the test birds alone (n = 19) was performed using the same model. When the non-significant interaction term (sex*line: p > 0.05) was removed from the model, High CORT birds had significantly higher peak levels of CORT than controls ($F_{(1, 16)} = 15.82, p = 0.001$, see figure 8). Again, there was no significant effect of sex ($F_{(1, 16)} < 0.001, p = 0.97$).
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5.4.2 Memory test carried out under stress

When the birds were tested after having been stressed (by placing them in a draw string bag for 20 minutes in order to elevate CORT levels), they failed to perform either the spatial or the colour test (i.e., did not lift any flaps). This suggested that the birds' plasma CORT levels were not at peak level when they were tested in the previously described tests. I therefore carried out an analysis of the plasma collected at the time of testing (i.e., plasma samples representative of levels at the time of testing and therefore referred to as “test” CORT).

The following model (proc GLM) was applied to the plasma data of all birds for which peak CORT and “test” CORT plasma data was available (n = 26). The manipulation term refers to whether the plasma data was “peak” or “test”:

\[
\text{CORT} = \text{sex} + \text{bird (sex line)} + \text{line} + \text{manipulation} + \text{line} \times \text{manipulation} + \text{sex} \times \text{line} + \text{sex} \times \text{manipulation}
\]

When the two latter non-significant (p > 0.05) interaction terms were removed from the analysis, there was a significant effect of line (High CORT vs. Control)
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$(F_{(1,23)} = 26.61, p < 0.0001)$, with high CORT birds having significantly higher levels of CORT than controls. There was a significant effect of manipulation (peak vs. test CORT levels) $(F_{(1,24)} = 58.41, p < 0.0001)$. Peak CORT levels were significantly greater than test CORT levels. There was no significant effect of sex $(F_{(1,23)} = 1.25, p = 0.27)$. The interaction between line and manipulation was highly significant $(F_{(1,24)} = 23.86, p < 0.0001)$, i.e., the extent of the difference between “test” and “peak” CORT levels depended on line. The difference in the two plasma measurements was much greater in the High CORT line compared to controls (see figure 9), indicating a higher peak CORT response in the High CORT birds.

![Figure 9: Average (±s.e.) peak and test CORT levels. The difference between the two CORT measurements (test and peak) was much greater in the High CORT line.](image)

To determine whether there was a difference in circulating levels of CORT at the time of testing between lines which might explain the performance discrepancy, the following model (proc GLM) was fitted to the “test” CORT plasma data of all birds for which is was available $(n = 26)$:

$$\text{Test CORT} = \text{line}$$

There was no effect of line $(F_{(1,24)} = 1.88, p = 0.18)$ on the level of “test” CORT. The level of circulating CORT at the time of behavioural testing did not differ
between the selected lines. When the analysis was carried out on the birds that were actually tested for which there was plasma data (n = 14 (the amount of plasma collected from 5 birds was insufficient for radioimmunoassay)), there was still no effect of line on level of test CORT ($F_{(1,12)} = 3.32, p = 0.10$).

5.4.3 **Sex hormones**

To determine whether the discrepancy in performance on the one-trial associative memory task could be accounted for by a difference in T levels (thought to be comparable with those at the time of testing) between the lines, the following model (proc GLM) was fitted to the plasma data of all birds that had T assayed (n = 25):

\[
\text{Testosterone} = \text{sex} + \text{line} + \text{sex} \times \text{line}
\]

When the non-significant ($p > 0.05$) interaction term was removed, there was no significant effect of line ($F_{(1,22)} = 1.76, p = 0.20$; see figure 10) or sex ($F_{(1,22)} = 2.88, p = 0.10$) on T level, but high CORT birds tended to have higher levels of T than controls and males tended to have higher levels of T than females (see figure 10). The analysis was also performed on the plasma data of the birds that reached test criterion, for which T measurements were available (n = 17). When the non-significant interaction term ($p > 0.05$) was removed from the model, there was no significant effect of sex ($F_{(1,14)} = 1.00, p = 0.33$) or line ($F_{(1,14)} = 0.20, p = 0.66$) on T level. T-levels did not differ between High CORT and control birds, nor males and females.
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Figure 10: Mean (±s.e.) level of T measured in High CORT and Control birds. T measurements did not differ between lines or sexes.

5.5 Discussion

5.5.1 Can CORT explain the difference in task performance?

None of the Low CORT birds reached test criterion. I do not think that their inability to perform the task resulted from their low CORT levels. I consider a more likely explanation to be that the difficulty to keep the temperature within the desired range (resulting from a rise in environment ambient temperature which coincided with the time when the last group of birds were tested) meant that the birds were less motivated. Of the birds that did reach test criterion, birds selectively bred for a high peak CORT level performed worse than controls on the spatial task whereas there was no difference in performance between the lines on the visual task. An analysis of plasma taken from each selected line revealed that although the High CORT birds had a significantly higher stress-induced peak level of CORT than controls, at 6 weeks, there was no difference in test CORT levels in adult birds. It appeared, then, that the deleterious effects of high levels of CORT were specific to the spatial domain. I predicted that performance on the spatial task would be impaired as the greatest density of adrenal steroid receptors are found in the mammalian hippocampus. High levels of CORT in the High CORT line were expected to
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activate the CORT receptors in the avian brain that presumably correspond to Type II receptors and impair performance on the spatial task, through a decrease in LTP (Lupien & McEwen, 1997; McEwen & Sapolsky, 1995). However, when birds were stressed prior to testing, all birds failed to complete the task. Thus, it seemed likely that plasma CORT was not at peak level at the time of testing. This was later confirmed by plasma radioimmunoassay which showed that CORT levels at the time of testing were significantly lower than peak CORT levels. Activation of Type II receptors in the hippocampus was therefore unlikely to be the mechanistic explanation for the impairment on the spatial version of the task.

As there was no relationship between circulating levels of CORT and task performance, but there was a difference in performance, it is possible that peak CORT levels had an organisational effect in the brain during early development. The ability of Black-legged Kittiwakes (Rissa tridactyla) to solve both a visual food-finding task and a test of spatial ability was compromised by experimental elevation of CORT during early development. Kittiwakes were tested months after having their hormone levels experimentally manipulated and, as in the current study, a performance discrepancy was found between treated birds and controls even though their CORT levels did not differ at the time of testing (Kitaysky et al., 2003). A relationship between baseline CORT and intracellular receptor numbers was not found in a seasonal study of the house sparrow (Breuner & Orchinik, 2001), so it is possible that even though the selected lines tested in this study did not differ in their levels of “test” CORT, a discrepancy in intracellular receptor numbers may have been present. Furthermore, it is possible that differences between the lines in stress-induced peak CORT levels (i.e., in response to the stress induced by being put in a bag at 6 weeks post-fledging) may have led to differences in adrenal hormone receptor densities in the brain (see figure 11).

Receptor numbers are dynamic and change in response to hormone concentrations through a feedback mechanism. When hormone levels are low, receptors are up-regulated to ensure sufficient numbers of receptors are activated. Conversely, if hormone concentrations are high, the receptors are down-regulated to prevent their over-activation (Lupien & McEwen, 1997). One may then hypothesise that there is a difference in the number of adrenal hormone receptors in the hippocampus of High
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CORT compared to control birds. This will be investigated using in situ hybridisation techniques. However, studies that have monitored seasonal variation in the avian stress response have found not only cytosolic CORT receptors (that presumably correspond to mammalian Type I and Type II receptor-like molecules) but also membrane-associated CORT receptors (e.g., Breuner & Orchinik, 2001). Breuner and Orchinik (2001) postulated that the membrane and intracellular receptors may serve different functions, with the former receptor type being mediating rapid behavioural responses to stress, whereas the intracellular receptors may control more enduring organisational, structural, synaptic and behavioural changes (Breuner & Orchinik, 2001). It is therefore possible that the selected lines may differ in the number of either or both of these receptor types.

An alternative explanation is that the lines differed at some other level of the HPA (see figure 11). The selected lines may have different levels of corticotrophin releasing hormone (CRH) in the hypothalamus which have a differential knock-on effect on CRH secretion and on corticotrophs (which may also vary in sensitivity between the selected lines) and result in differential secretion of adrenocorticotrophin hormone (ACTH). In addition, the lines may have differed in stress-induced (peak) CORT levels because of differences in CORT secretion from the adrenal gland. In future research, the relevant pathway could be identified by monitoring CORT secretion after exogenous administration of CRH or ACTH. If the same dosage of either CRH or ACTH led to a discrepancy between the lines in the amount of CORT secreted, the mechanism behind the dichotomy in peak CORT levels could be identified.

5.5.2 Can T explain the difference in task performance?

Although testosterone and CORT have been shown to co-vary with experimental elevation of T in pied flycatchers (Silverin, 1980), dark-eyed juncos (Breuner & Orchinik, 2002; Ketterson et al., 1992; Klukowski et al., 1997; Schoech et al., 1999) and with season in Gambel’s white-crowned sparrows (Zonotrichia leucophrys gambelii) (Romero & Wingfield, 1998), dark-eyed juncos (Deviche et al., 2001), Lapland longspurs (Calcarius lapponicus) and redpolls (Carduelis flammea)
(Romero et al., 1998a; Romero et al., 1998b), I did not find a positive relationship between CORT and T titres in zebra finches in this study. Contrary to prediction, zebra finches bred for a high peak CORT level did not have higher T levels than controls. However, these studies looked at a much wider range of variation than I did (i.e., experimental manipulation or seasonal variation). On the other hand, I looked at natural variation in T and CORT levels under stable conditions (i.e., breeding season photoperiod). The deficit in performance on the spatial task of High CORT birds, therefore, seems unlikely to be due to an excess of testosterone.

However, the lack of a sex difference in performance on either the spatial or colour version of the one-trial associative memory task may, in part, be explained by the lack of a difference in T levels between the sexes. Previous research has shown that T facilitates spatial learning and memory ability in rodents (e.g., Roof & Havens, 1992; Roof, 1993a; Roof, 1993b). Avian CBG binding capacity is positively related to T level. It is higher at the beginning of the breeding season than at the end (Romero et al., 1998a; Romero et al., 1998b; Deviche et al., 2001), higher in males than in females (Deviche et al., 2001; Silverin, 1986) and higher in T-treated males compared to castrates (Silverin, 1986; Klukowski et al., 1997). Differences in CBG binding capacity in the sexes or selected lines would have made comparisons of steroid effects on performance more difficult, as it is generally accepted that once bound to CBG, hormones are rendered inactive (Mendel, 1989). Therefore, differences in plasma concentrations of CBG between the selected lines or sexes would have meant that differences in levels of free CORT were likely, necessitating radioimmunoassay of not only CORT, but also CBG.

I predicted that High CORT females would outperform High CORT males on the basis that research with rats has shown that high CORT levels are less detrimental to spatial task performance in females compared to males. In addition, both basal and stress-induced CORT levels are lower in females in some species of bird (Meddle et al., 2003). In mammals, E_2 moderates the sex difference in stress effects on spatial learning and memory. Whether E_2 modulates the stress response in birds is currently unknown but it is possible that modulation of the stress response by E_2 may result in female resistance to stress-induced impairments by affecting hippocampal
morphology, as it does in mammals (Bowman et al., 2001; Galea et al., 1997), although such resistance was not observed in this study.

Figure 11: Schematic representation of the HPA showing the different levels at which the selected lines (which differ in stress induced CORT levels) may diverge.
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To summarise, the performance of birds on the one-trial associative memory tasks differed between birds with different peak CORT levels. Birds selectively bred for a high CORT level performed worse than controls on the spatial version of the task whereas there was no difference in performance between the lines on the visual task. It is possible that the spatial task performance deficit of the High CORT birds was due to the detrimental effects incurred as a result of elevated levels of CORT. As the lines did not differ in CORT level at the time of testing, it is plausible that High CORT birds incurred the cost of elevated CORT during development (organisational effect) and that their hippocampal morphology was permanently affected in some manner. Alternatively, the performance impairment could result from an activational effect of high peak CORT although this hypothesis was not tested as peak CORT levels were not measured in the sexually mature zebra finches. No firm conclusions can be drawn as to the organisational effects of adrenal hormones on brain development, specifically the hippocampus, until further studies are carried out. In situ hybridisation techniques will be applied to examine adrenal steroid receptor and sex hormone receptor mRNA expression in the hippocampus.

The selection lines provide an ideal model for assessing the effect of CORT on cognitive development in the future as the problem of altered endogenous activity of other hormones (e.g., CRH or neuropeptides vasopressin (AVP)), associated with experimental hormone manipulation, can easily be avoided. However, the value of manipulation studies should not be questioned as it is possible that selection for peak CORT level selected for some other correlated trait (e.g., anxiety) and that it is this other trait that affected performance in this experiment, not CORT. Of course, there would still be a relationship between CORT and spatial ability but it would not be causal.

My results suggest that CORT is detrimental to avian spatial learning and memory abilities. However, to confirm whether the relationship between CORT concentration and spatial ability is indeed biphasic as it is in mammals it is necessary to also investigate the spatial ability of birds selectively bred for a low peak CORT level relative to controls. One could possibly increase the motivation of the birds to complete the task in the future by depriving birds of food for longer durations and by using a more desirable reward in the test.
Chapter 6. General Discussion

6.1 Introduction

Until recently, research into sex differences in spatial learning and memory abilities focused on evolutionary and ecological explanations for the sexual dimorphism (Silverman & Eals, 1992; Gaulin & Fitzgerald, 1989; Sherry & Hampson, 1997; Lovejoy, 1981; Gray & Buffery, 1971; Geary, 1995). However, mechanistic explanations for the sex difference have since been proposed and it is becoming increasingly clear that steroid hormones play an active role in mediating spatial learning and memory abilities (Barrett-Connor et al., 1999; Beatty, 1979; Daniel et al., 1997; Daniel et al., 1999; Dawson, 1972; Dawson et al., 1975; Dohanich, 2002; Duff & Hampson, 2000; Galea et al., 1995; Hausmann et al., 2000; Isgor & Sengelaub, 1998; Kimura & Hampson, 1994; Kritzer et al., 2001; Luine & Rodriguez, 1994b; Luine et al., 1998; Markham et al., 2002; Miles et al., 1998; Sherwin, 1994; Sherwin, 1996; Tan et al., 2003; Gouchie & Kimura, 1991; Neave et al., 1999; Ostatnikova et al., 1996; Roof & Havens, 1992; Roof, 1993a; Roof, 1993b; Silverman et al., 1999; Wolf et al., 2000). This research concentrated largely on steroid effects in mammals. However, if spatial ability is indeed sensitive to the effects of steroid hormones then effects might be expected to be seen in other taxa.

Most research into avian spatial learning and memory abilities has concentrated on ecological correlates to variation between species. For example, a number of comparative studies have examined memory differences in food-storing and non-storing birds (Brodbeck, 1994; Brodbeck & Shettleworth, 1995; Clayton & Krebs, 1994b; Clayton & Krebs, 1994a; Clayton, 1995; Clayton, 1998; Hampton et al., 1995; Hampton & Shettleworth, 1996; Healy, 1995; Healy & Krebs, 1996; McGregor & Healy, 1999; Shettleworth & Westwood, 2002; Shettleworth, 1995; Shettleworth et al., 1990), and parasitising and non-parasitising species (Astié et al., 1998; Sherry et al., 1993; Reboreda et al., 1996; Clayton et al., 1997). The influence of steroid hormones on avian spatial learning and memory abilities has received little attention. The primary goal of the research described in this thesis was, therefore, to gain insight into whether such abilities in birds are affected by steroid hormones. To
this end, I found an effect of testosterone (T) and corticosterone (CORT) on the ability of two species of songbird to perform one-trial associative spatial memory tasks. In the following discussion I will summarise my findings and make suggestions as to how unresolved issues might be addressed by future research.

6.2 Summary of results

- **Cue preference in great tits.** I was unable to find a sex difference in cue use in a one-trial associative memory task. Great tits of both sexes exhibited a preference for the location cue over the colour cue in a food-finding task.

- **Sex differences in spatial abilities in great tits?** I found no sex difference in the ability of great tits to perform a visual or spatial DNMTS touch screen memory task. All birds achieved longer retention intervals (i.e., superior memory persistence) on the spatial than the visual memory task. The longest retention intervals were attained when the distractor was far from the target in the choice phase of the experiment.

- **The effect of testosterone on spatial learning and memory abilities in great tits.** I found that manipulation of T levels differentially affected the ability of males and females to perform a DNMTS touch screen memory task. Females achieved higher scores on T-treatment days than on control days, whereas scores achieved by males were not affected by treatment. T-treatment lengthened response latencies in the sample phase of the experiment in both males and females. When treated with T, birds were able to take longer before responding to the touch screen image than when they had been given the vehicle or E₂ and still perform at the same level.

- **Does corticosterone affect memory in the zebra finch?** I found that the performance of zebra finches bred for a high peak CORT level was selectively impaired on a one-trial associative spatial memory task. High CORT birds performed less well than a control line on the spatial version of the task but there was no difference in performance between the lines on the visual memory task.
Given that I did not find a sex difference in cue preference in the great tit (Chapter 2), the failure to find a sex difference in spatial learning and memory abilities (Chapter 3) was not surprising. Both males and females preferred to use location cues over colour cues in a food-finding task and their ability to use spatial cues was actually superior to their ability to use visual cues in a DNMTS task, evidenced by birds achieving longer retention intervals in the spatial task than in the visual memory task. However, these results were surprising given the results of comparative studies of memory in food-storing and non-storing birds. Non-storing birds (like the great tit) have not been seen to exhibit a cue preference and respond to colour and location cues equally. In contrast, food-storing species consistently show a preference for location cues (Brodbeck, 1994; Brodbeck & Shettleworth, 1995; Clayton & Krebs, 1994; Shettleworth & Westwood, 2002). I investigated cue preference and memory in birds maintained under a breeding season (long day) photoperiod. In contrast, the majority of studies that have examined memory differences between food-storing birds have been carried out with birds maintained under a short-day, winter photoperiod, corresponding to the time of peak food hoarding and recovery, and hippocampal enlargement, in storing species. It is possible, then, that the putative elevation in sex hormone levels in the birds in my experiment, maintained under a breeding season photoperiod, accounted for their reliance on location cues and superior performance on the spatial, compared to the visual, memory task. An autumnal reliance on both colour and location cues by non-storing species may have arisen from lower levels of endogenous sex hormones in non-storing compared to storing species. In rodents, T is thought to play a role in cue preference. Male rodents and T-treated females rely on spatial cues whereas female controls rely on both spatial and appearance cues (Williams et al., 1990; Williams & Meck, 1991). In addition, T underlies a superior spatial ability in rodents as the often reported sex difference, favouring males, in maze learning ability is abolished when neonatal female rats are treated with T propionate and when voles are tested during the non-breeding season (Dawson et al., 1975; Frye, 1994; Joseph et al., 1978; Roof, 1993a; Roof & Havens, 1992; Stewart et al., 1975). A higher level of T in food-storing compared to non-storing species during the time of peak food hoarding and recovery may therefore be adaptive. If this hypothesis were true, I
would not expect to find differences in spatial memory or cue preference between food-storing and non-storing species during the breeding season (or in birds maintained under a breeding season photoperiod). As there is seasonal regulation of both T and E₂ in songbirds (Wingfield & Farner, 1978), studies carried at different times of the year could address the role of activational sex hormones without the need for experimental manipulation.

The failure to find a sex difference in the titration experiment in Chapter 4 (where birds were maintained under a long-day, breeding season photoperiod) does not rule out the role of T in avian spatial learning and memory abilities. Indeed, activational effects of steroids are thought to have less substantial effects than organisational influences (Beatty, 1979; Williams et al., 1990; Roof, 1993b; Luine & Rodriguez, 1994a; Kanit et al., 1998). Studies of organisational effects of steroids on mammalian spatial learning and memory abilities have been facilitated by the examination of the intrauterine environment (Galea et al., 1994). Organisational effects of sex steroids on cue preference and spatial ability in birds could be examined by comparing offspring that hatched from eggs with different levels of yolk androgens. Allocation of androgens to eggs by the mother varies with mate attractiveness and laying sequence (e.g., Birkhead et al., 2000; Eising et al., 2001; Gil et al., 1999). Therefore, the cue preference of offspring fathered by males of varying attractiveness or of offspring from early laid versus late laid eggs could be compared to examine organisational effects of sex steroids on avian cue preference. If T were to underlie a superior spatial ability, I would expect birds hatched from eggs with high levels of yolk androgens to outperform those from eggs with lower levels.

In future experiments, other aspects of behaviour, such as activity levels, could be monitored in T-manipulated birds to determine possible reasons why T-treated birds take longer to respond to the touch screen images. In addition, the time-course of steroid action should be determined by sampling plasma at different intervals after larva ingestion. Although we know how long it takes levels of exogenous T to peak in the plasma of the white-crowned sparrow (Sperry 2001, pers. comm.), we do not know how fast the activation effects of the steroid are (i.e., how long it takes the steroid to bind to receptors in the hippocampus and affect cognition).
Birds could also be tested on different types of memory task to determine if the effect of steroid hormones is more prominent in reference memory or working memory tasks, for example. Changes in hippocampal morphology in response to hormone treatments could also be examined. T-treatment of female rats increases number of cells in and thickness of DG-GCL (Roof, 1993a; Roof & Havens, 1992) and it is not known whether the equivalent avian brain area, the dorsomedial region of the hippocampus (Szekely, 1999), is sensitive to the effects of steroid hormones.

The role of steroid hormones in avian spatial learning and memory should also be investigated in other species as steroid effects may not be consistent, especially considering the results of a recent cross-species analysis of neural sites implicated in memory function (Saldanha et al., 1999). The pattern of androgen metabolism in the hippocampus was found to vary between food-storing and non-storing birds. For example, the mean 5α-reductase activity across non-storing birds was significantly lower than that of food-storing species, suggesting that the hippocampus of food-storing songbirds is exposed to higher levels of 5α-DHT than the hippocampus of non-storing species. As the relationship between T and spatial learning and memory abilities is biphasic in mammals, with too much T having a detrimental effect on spatial ability (Roof & Havens, 1992; Roof, 1993b; Joseph et al., 1978), it is possible that aromatase may function to rid the hippocampus rapidly of excess androgen in the hippocampus of birds in which a good spatial memory is essential (i.e., food-storers) (Saldanha et al., 1999). Whether variation in androgen metabolising enzymes in the hippocampus exists between other species (e.g., parasitising and non-parasitising species) remains to be tested but would certainly suggest that steroid hormones are indeed involved in avian spatial learning and memory abilities.

The results of Chapter 5 suggest that high levels of CORT have detrimental effects on spatial cognition. Replication of these results and testing of birds selectively bred for a low peak CORT level would enable one to determine whether CORT affects avian spatial learning and memory abilities in a concentration-dependent fashion as it does in mammals (Schantz & Widholm, 2001; Shors et al., 1992; Vicedomini et al., 1986; Lupien & McEwen, 1997; Kirschbaum et al., 1996; Arbel et al., 1994; Bodnoff et al., 1995; Bohus, 1994; De Kloet et al., 1988; Kerr et al., 1991; Endo et al., 1996; Oitzl et al., 1994). Through examining adrenal and sex
hormone receptor mRNA expression in the hippocampus of the selected lines, in addition to experimentally elevating levels of CRH or ACTH to see if there is a discrepancy between the lines in the amount of CORT secreted, the mechanism behind the effect of selection on peak CORT levels could be identified.

6.3 Summary and conclusions

The research described in this thesis has gone some way to describing how steroid hormones affect avian spatial learning and memory abilities. I have found T to improve the ability of female great tits to perform a DNMTS task and lead to longer response latencies in a spatial task in both males and females. I have found high levels of CORT to have a detrimental effect on the ability of the zebra finch to perform a one-trial associative spatial memory task. There is a huge literature on the effect of both steroids on mammalian spatial learning and memory abilities and my experiments are amongst the first to draw parallels between these steroid effects in mammals and the effects of gonadal and adrenal steroids in birds. There are, however, many unanswered questions, providing an exciting avenue for future research.
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