CHAPTER 2

GENERAL METHODS

2.1 INTRODUCTION

This chapter presents details of the general procedures used in the experiments reported in Chapters 3-7. A variety of lateral functions have been examined in this thesis (i.e. hand, foot, eye and side-of-mouth asymmetries), and most of the details of methods for measuring the different types of lateral preference are given in the separate chapters. All of the experimental procedures and housing conditions of the marmosets described in this study were in accordance with guidelines of the Australian Animal Research Act (1989) and were approved by the University of New England Animal Care and Ethics Committee.

2.2 SUBJECTS

Twenty-one common marmosets (13 female, 8 male) were tested. These subjects make up the colony at the University of New England, Armidale, Australia. All of the marmosets were born in captivity. The six founding members (3 female, 3 male) of the colony were obtained from Foundation 41 (Sydney, Australia) when they were 6 months old. These marmosets had not been used previously for any form of testing. Tests of lateral preference with the founding marmosets were not started until they were 10-12 months of age, allowing the subjects to become familiar with their new environment and with the experimenter before testing began.

The six founding members of the colony were not closely related to one another: all common relatives were removed by at least two generations. These six marmosets formed three heterosexual pairs, which gave birth to the remaining 15

marmosets housed at the University of New England. Table 2.1 outlines the compositions of the three family groups used in this study. The ages (birth dates) and sexes of the subjects are also listed (Table 2.1). Either twin or triplet births occurred in this colony and the infant pair/trio(s) are identified in Table 2.1. Twin and triplet births are more common in marmosets than are singleton births (Kingston, 1967; Box and Hubrecht, 1987).

The six founding marmosets received less parental care than those born at the University of New England and were housed in more stressful conditions. Before coming to the colony at the University of New England, the subjects were part of a colony in a biomedical research facility examining the effects of toxicity on primates.

Table 2.1 The age, sex and family group membership of marmosets at the University of New England

Family group 1			Family group 2			Family group 3		
Subject	Sex	Birth date	Subject	Sex	Birth date	Subject	Sex	Birth date
Light Blue	F	10 Sept. 1991	**Red**	F	25 Sept. 1991	**Black**	F	25 Sept. 1991
Gold	M	2 Sept. 1991	**Blue**	M	14 Sept. 1991	**Silver**	M	24 Aug. 1991
Sage [A]	F	6 Feb. 1993	Crassus [A]	F	2 April 1994	Zhen [A]	F	7 Feb. 1995
Coco [A]	M	6 Feb. 1993	Pompey [A]	F	2 April 1994	Xing [A]	M	7 Feb. 1995
Maylin [B]	F	9 July 1993	Caesar [A*]	M	2 April 1994	Delta [B]	M	22 July 1995
Sunga [B]	F	9 July 1993	Ash [B]	F	31 Aug. 1994	Omega [B]	M	22 July 1995
Snap [C*]	F	9 Dec. 1993	Wattle [B]	F	31 Aug. 1994			
Crackle [C]	M	9 Dec. 1993						
Pop [C]	F	9 Dec. 1993						

The subjects with bolded names are the parental pairs in each group, that is the founding members of the colony. Superscripts A, B and C are used to identify the twin and triplet siblings in each family group. * indicates that the subject is deceased. Snap died at 28 months of age and Caesar died before 1 month of age.

Although these 6 marmosets were not used in the pharmacological experiments, it is probable that the level of stress in this environment was quite high. Moreover, the relocation of these subjects at 6 months of age represented an additional stressful experience. The marmosets born and raised at the University of New England were housed with their mothers (and siblings) until at least 5 months of age. They were then housed with their mothers, fathers or siblings (see Figure 2.3) in large environmentally enriched cages. No form of invasive testing has been conducted with this colony. It should be noted that early experience has been found to influence functional lateralization in rats (Denenberg et al. 1978; Sherman et al. 1980; Denenberg, 1981; Diamond, 1985; Cowell et al. 1997) and, therefore, it was considered in this study as a variable that may affect the expression of functional asymmetries in the marmosets. The founding and offspring marmosets groups will be referred to as Experience Group 1 and Experience Group 2, respectively, to avoid confusing them with the family groups discussed previously.

2.3 GENERAL HUSBANDRY AND HOUSING

General husbandry

The daily care of the marmosets was primarily the responsibility of the experimenter. This allowed the experimenter to continually assess the health, behaviour and emotional state of the subjects and to ensure that a calm environment was maintained. The marmosets were housed in temperature controlled rooms maintained between 21^{o} and 25^{o} C. Light and dark cycles were also regulated (lights on at 7.00 hours and off at 19.00 hours). The marmosets were exposed to ultraviolet light (350-390 nm) for 30 minutes per day to supplement their vitamin D intake. A skylight was placed in the rooms, when the founding marmosets were 49 months of age (midway through this project), to provide a natural source of sunlight. This meant that they were exposed to varying daylength, thereafter, although the main source of light was still the artificial light source.

Although the marmosets were not handled regularly, all were habituated to handling by the experimenter in order to reduce stress when this procedure was required. As it was observed that the marmosets were stressed when handled according to conventional handling procedures, placing one hand around the chest and another around the lower abdomen, a handling method that did not involve this degree of restriction was adopted. The marmosets are very familiar with the experimenter and it was possible to simply guide them onto the experimenter's arm and then firmly grip their tails to prevent escape. The marmosets were familiarized and were comfortable with this handling method. They were also trained to enter a perspex tube to reduce the need for handling when they had to be weighed or transported between cages.

The weight and physical condition of all subjects were monitored regularly. The marmosets were weighed fortnightly for at least the first year of life, after which they were weighed once or twice a month. The weights of individual marmosets over the experimental period are presented in Appendix 2. The mean weights (±SEM) of each heterosexual pair of founding marmosets and the mean weights (±SEM) of their offspring are also shown in Figures 2.1a, b and c. As can be seen in Figure 2.1, all adult subjects (22 months) weighed between 350-550 grams, which is in agreement with the average adult weights reported in the literature (Abbott, 1978; Poole and Evans, 1982; Clarke, 1994). Figure 2.1 also shows the rapid physical development of marmosets. Marmosets become sexually and physically mature at 10-14 months (Hershkovitz, 1977; Box, 1978) and are socially mature by 18-24 months of age (Clarke, 1994). The rapid physical development of this species allows longitudinal studies to be conducted, from infancy to adulthood, in a considerably shorter period of time than would be required with other captive nonhuman primate species in which the developmental periods are significantly extended (Fragaszy et al. 1991; Adams-Curtis

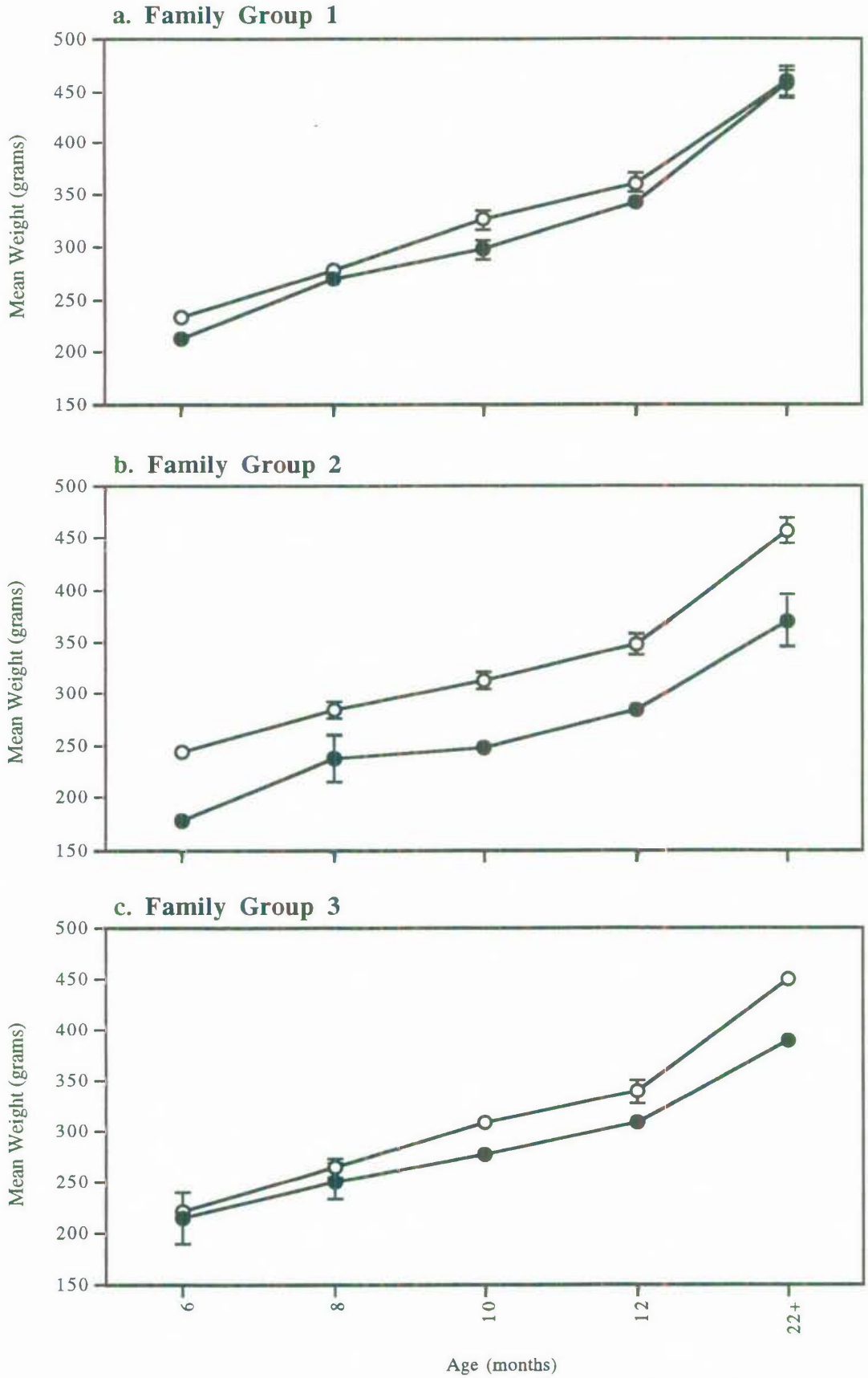

Figure 2.1 Weight Gain of the Parent and Offspring Marmosets with Age. The mean weights (±SEM) of the parents (●) and offspring (○) in Family Groups 1 (a), 2 (b) and 3 (c) are presented from the juvenile stage of development (6 months) until adulthood (22+ months). Where there are no standard error bars for the mean weights it indicates that the SEM was very small.

and Fragaszy, 1994; Garber and Leigh, 1997). The developmental stages as defined in this thesis are outlined in Table 2.2.

Table 2.2 Developmental stages of marmosets

Developmental Stage	Age	Reference
Infant	1-20 weeks	Ingram, 1977; Yamamoto, 1993
Juvenile	5-10 months	Ingram, 1977; Yamamoto, 1993
Subadult (sexually mature)	10-18 months	Hershkovitz, 1977; Box, 1978
Adult (socially mature)	18-22 months	Clarke, 1994.

There were no differences between male and female subjects in the increase of body weight with age when comparison was made between the weights of the subjects at 6, 8, 10, 12 and 22 months (Repeated measures ANOVA, gender, $F= 0.17$, $p= 0.69$, family group, $F= 1.80$, $p= 0.19$). Furthermore, no differences in the increase of body weight with age was found between members of different family groups. By contrast, a significant effect of rearing experience (see above) on body weight was found when comparison was made between members of Experience Groups 1 and 2 (Repeated measures ANOVA, $F= 16.08$, $p= 0.0007$). Also, there was a tendency for an interaction between age and experience ($F= 2.12$, $p= 0.087$). This result demonstrates differences in the physical development of subjects in the two Experience Groups. Subjects born and raised at the University of New England consistently weighed more than the founding marmosets. It should also be noted that Light Blue was pregnant at 22 months and, therefore, her weight for the 22 month period in this analysis was taken at 35 months when she was not pregnant.

Diet

The marmosets were fed a variety of foods twice daily at approximately 10.30 and 15.30 hours. Scheduled feeding times were delayed for up to 2 hours to accommodate some testing procedures (eye preference experiments), but there was always food remaining in the subjects' food bowls or on the cage floors during these delay periods. Thus, the marmosets were never food deprived. Water was available *ad libitum*. The daily dietary intake of the marmosets is detailed in Table 2.3. The recipes for the meatloaf and banana cakes are also presented. The diet was designed for nutritional value and as a form of daily enrichment. Banana was used as a food reward

Table 2.3 Diet of marmosets in the University of New England colony

	Variety of Foods Provided
	Staple: Banana cake*, meatloaf*, dog pellets, apple
	Nonstaple Foods provided
Monday	Bread with vitamin supplement (PENTAVITE), orange
Tuesday	Cheese, soft fruit (nectarine, peach, plums, paw paw)
Wednesday	Beans, boiled egg, nutrigrain cereal, blueberries/grapes
Thursday	Boiled egg, nutrigrain cereal, sultanas, mealworms
Friday	Unshelled roasted peanuts, raw potato
Saturday	Corn on the cob, soft fruit (pear, nectarine, grapes)
Sunday	Unshelled roasted peanuts, flavoured yoghurt, mealworms

**Banana Cake*
25g Uncooked rice
105g polenta
25g desiccated coconut
15g sunflower seeds
1 egg
25 g brown sugar
1 mashed banana
25g skim milk powder
25g sultanas
1 rice cake
15g dicalcium phosphate

***Meatloaf*
600g mincemeat
6 slices brown bread
20g dicalcium phosphate
2 eggs
45g bran cereal
7g vitamin C
45g dog pellets
1 cup of water

in experiments and, therefore, was not provided as part of the daily diet. There have been no cases of nutritional deficiencies in this colony and no signs of 'marmoset wasting syndrome', a disorder caused by nutritional deficiencies and stress (Diniz and da Costa, 1995).

Housing and environmental enrichment

Most of the marmosets were housed in groups (see *Cage composition*) in cages at least 1.8m x 1.5m x 1.6m high. One male was housed singly in a cage 0.9 x 0.8 x 2.2m high. The cages were placed on stands to raise them 20cm off the floor of the room (Figure 2.2). The cage structures, and positions within rooms, were designed to increase vertical space and to prevent any of the subjects having to be housed above or below other subjects. Both of these variables have been shown to increase stress and significantly influence the behaviour of captive callitrichidae species (Rylands, 1985; Box and Röhrhuber, 1987; Scott, 1991; Box and Röhrhuber, 1993). There was also a nest box (18cm x 15cm x 29cm) in each cage to provide an area for the marmosets to sleep or hide (Figure 2.2). Cages were cleaned 3 times per week.

The cages contained leafy branches, swings, tyres, polyethylene pipes and other hanging playthings (Figure 2.2). Gum arabic feeders were also placed into each cage. The gum feeders consisted of a small tree branch with 10 to 20 drilled cavities (approximately 0.5cm diameter and 2cm deep) which were used as gum reservoirs. Twice weekly the reservoirs were filled with a commercial gum arabic solution (purchased from Country Kitchens, Fort Wayne, Indiana). Gum exudate feeding is a natural behaviour of *Callithrix jacchus* in the wild, providing marmosets with an important nutritional source of complex polysaccharides, calcium and trace minerals (iron, aluminium, silicon, potassium and magnesium; Nash, 1986). The activity of captive animals is increased by providing them with the opportunity to engage in this natural behaviour (Kelly, 1993). Environmental enrichment and increased cage size have also been shown to significantly increase activity, reduce stress and increase the occurrence of many behaviours, including tree-gouging, grooming and locomotion, in

a.

b.

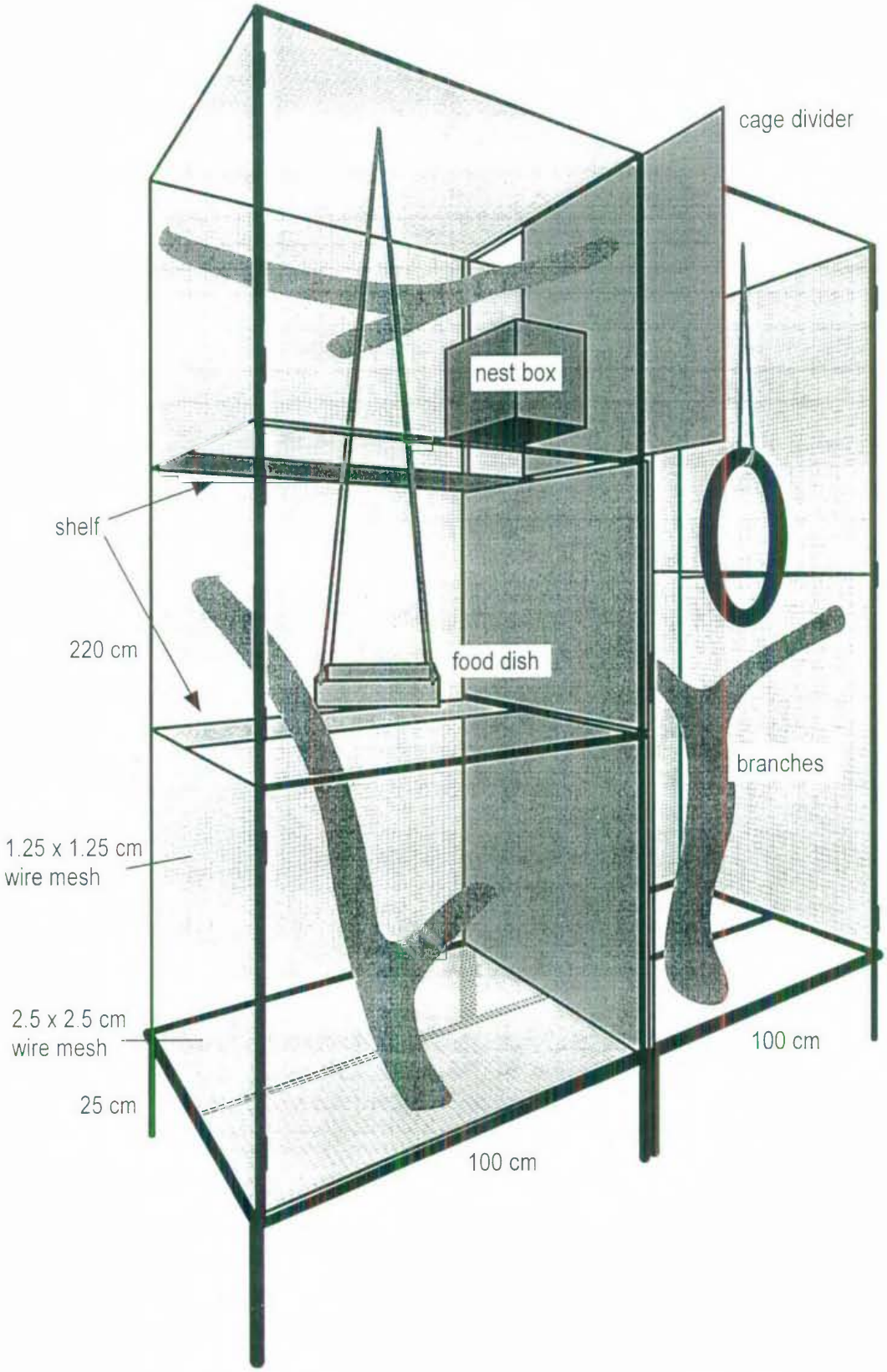

Figure 2.2 Cage design at the University of New England. Figures 2.2a and 2.2b are photographs showing the two types of cage design used at the University of New England. Figure 2.2c is a diagrammatic representation of the cage design. This figure shows the size of the cage and also the divisions in the cage which allowed the experimenter to separate individuals for testing. The nest box, in which the marmosets sleep, and some of the objects used for environmental enrichment are also depicted.

captive marmoset groups (Kitchen and Martin, 1996). Moreover, there is evidence that maintaining a novel captive environment is a necessary component for the well-being of many primate species (Snowdon et al. 1985; Johnson et al. 1991; Kitchen and Martin, 1996). Thus, the cages were environmentally enriched (changing toys and branches) at least once per month.

Group housing

Figure 2.3 is a flow diagram outlining changes in the marmosets held together in the cages throughout this study. From 6 to 11 months of age the founding marmosets were housed in a single cage (1.8m x 1.5m x 1.6m high). At 11 months, they were separated temporarily into single-sex groups and housed in two cages of the same size as described above. At 14 months of age they were housed as heterosexual pairs for breeding (Figure 2.3) in cages of the same size (1.8m x 1.5m x 1.6m high).

As the colony became larger it was necessary to move some subjects into other rooms. To maintain visual contact between the members in each family group the colony was divided and each family group was housed in one of three rooms. The ages of the subjects when they were moved from one room/ cage to another are given in Figure 2.3. The three family groups were able to communicate vocally between rooms. It was noted that the marmosets oriented themselves toward air vents that connected the rooms during inter-family group communication.

To prevent further breeding, one month prior to the birth of the final set of offspring in each family group (Table 2.1) male and female subjects were separated. As it has been shown that both parents and older siblings participate in caring for infants it was possible for the mother to raise her last set of offspring with the help of her juvenile offspring (Box, 1977b; Tardif et al. 1993; Yamamoto et al. 1996). Male offspring that were born in the final set of twins/triplets remained with their mothers until at least 5 months of age and were moved into male groups by 8 months of age

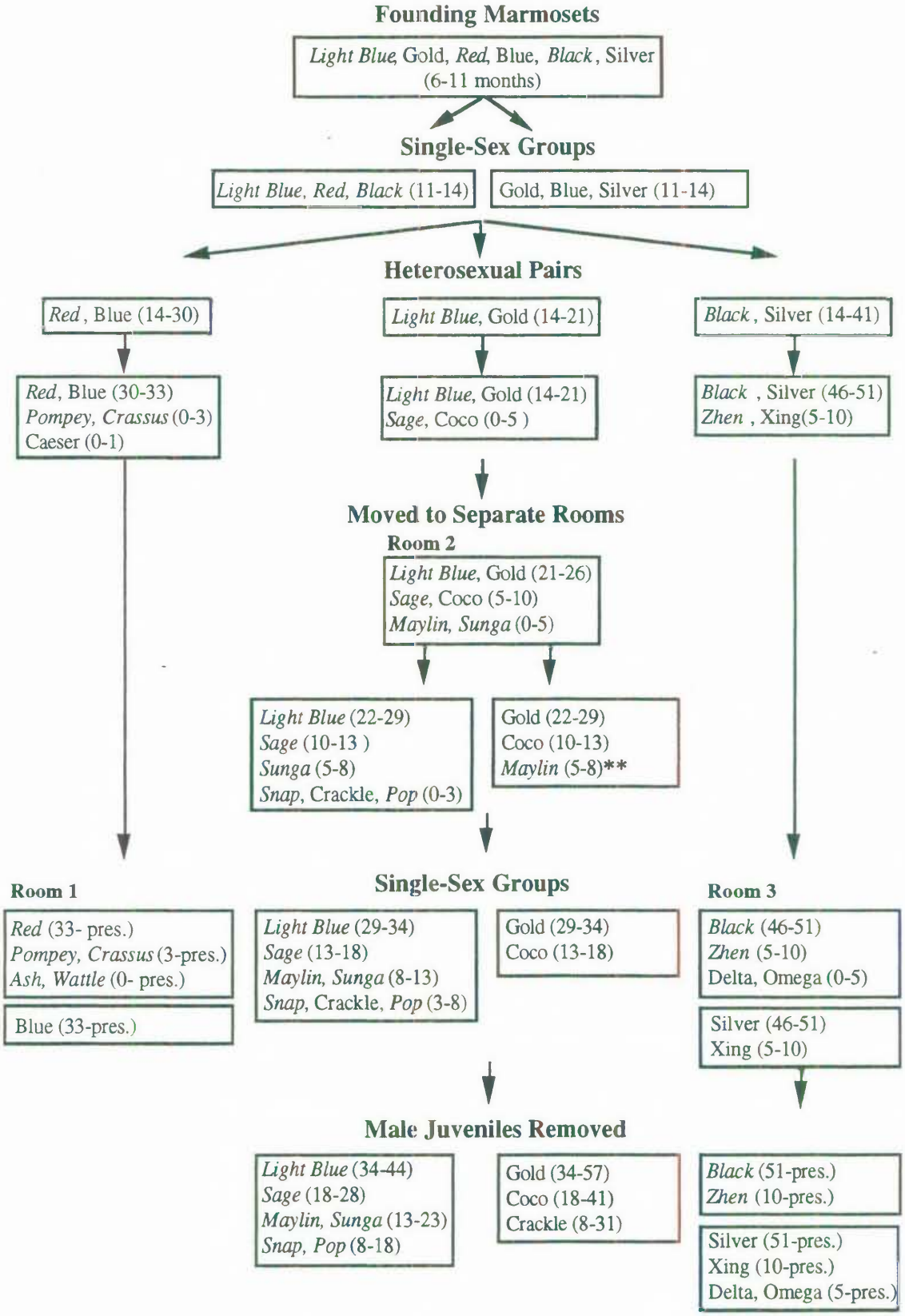

Figure 2.3 Outline of Cage Compositions. The flow chart outlines the changes in cage compositions over the duration of the experiments reported in this thesis. Female subjects are denoted in *italics* and males in plain text. **Maylin was placed with the males temporarily but removed before sexual maturity. Maylin was mistakenly thought to be male until she was 8 months old. The abbreviation pres. indicates that they were housed in these groups until now. The numbers in brackets refer to the ages of the subjects in months.

(Figure 2.3). The cages in the second and third rooms were at least as large as those described above.

Only one male in the colony was housed individually. Blue was 33 months of age when he was separated from his family group and has remained individually housed. Individual housing was necessary as Blue did not have any surviving male offspring (Caesar died at 14 days of age) and it was not possible to house him with other male groups as he fought with the other male subjects when this was attempted. It must be emphasized that although Blue is physically isolated, he still has visual, vocal and olfactory contact with the rest of his family group.

Further separations within the male and female subgroups of Family Group 1 were necessary due to aggressive behaviour asserted by some siblings toward others. In the female subgroup, Pop exhibited threatening behaviour toward Sunga. The female subgroup was separated to form two groups when Pop was 18 months of age and Sunga was 23 months of age: the two groups consisted of 1. Light Blue, Sage, Pop, and 2. Maylin, Sunga, Snap. The male subgroup (Gold, Coco and Crackle) also had to be separated when Coco began to display intimidating behaviour toward Crackle. This separation occurred when Crackle was approximately 36 months and Coco was 46 months of age. The perspex tube joining the two male cages was closed and Coco and Crackle were housed in the separated cages (0.9 x 0.8 x 2.2m high). To avoid housing one of the males individually a time sharing system was set up. Gold was swapped between cages every day, so that every second day Coco and Crackle had a companion in their cage.

2.4 TESTS OF LATERALIZATION

As one of the aims of this study was to make a detailed analysis of how individual marmosets are lateralized, a variety of lateral functions was examined (Table 2.4). Use of the left or right limb, side-of-mouth and eye were recorded on tests measuring: hand preferences for simple food holding and when reaching for static or moving objects; side-of-mouth preferences in chewing; hand and foot preferences in

the initiation of locomotory activities; eye preferences for viewing different stimuli; and asymmetries of the mouth and other features of the face in the production of facial expressions and vocalizations.

All experiments were conducted between 9.00 and 16.00 hours. The subjects were not tested if they were aroused (unless the test was designed to measure the effect of this variable on lateralization; Chapters 8 and 9). Behaviours indicative of high arousal included 'tsik', 'egg' and 'ock' vocalizations (Epple, 1968; Stevenson and Poole, 1976; Lipp, 1978; Abbott, 1978; Stevenson and Rylands, 1988), an extended period of piloerection, pacing of the cage and ballistic grabbing movements (not directed toward an object). On two occasions, when the subjects were presented with a fear-inducing object, 'tonic immobility' was observed. The two subjects were observed to lie motionless on their sides for more than 30 seconds. Similar behaviour has also been described by Lipp (1978), who refers to it as a flight (ducking) behaviour. On these occasions tests were discontinued immediately, as the situation was obviously producing very high levels of stress in the subjects. Other situations that were found to increase arousal included dominance battles between subjects; changes in cage compositions or relocation between rooms; power failures; and electrical storms. Experimental isolation also caused increased arousal in some subjects.

Most tests were conducted in the home cages (Table 2.4). The home cages were designed so that cagemates could be partitioned into one half of the cage while a subject was being tested. When tests were conducted in the home cages, most subjects were not aroused by experimental isolation (Table 2.4). However, 4 subjects (Zhen, Xing, Delta and Omega) did display aroused behaviour and would not participate in some of the tests of visuospatial reaching that involved reaching for small pieces of food on a plate, grasping string and taking food from a rotating disc (Table 2.4). Testing of these subjects was not pursued as it was considered that stress may bias experimental results.

Table 2.4 Caging and group design in testing

Experiment	Tested in home cage or experimental cage	Tested individually, as pairs or in groups
Hand preferred for simple food holding	Home	Group
Visually guided reaching through holes in the lid of a bowl (no postural demands)	Home	Pair
Visuospatial reaching for static object while maintaining a suspended posture	Home	Individual
Visuospatial reaching for moving object while maintaining a suspended posture	Home	Individual
Visuospatial reaching for moving object on a rotating disc (no postural demands)	Experimental	Individual Exceptions tested in pairs: Red, Blue; Pompey, Crassus; Ash, Wattle
Side-of-mouth preferences in chewing (hand use not required)	Home	Group
Side-of-mouth preferences in chewing (hand use required)	Experimental/ Home	Individual
Leading foot when leaping, landing and walking	Home	Individual
Eye preferences	Home	Individual
Lateralized production of facial expressions and vocalizations	Home	Group

As can be seen in Table 2.4 only two tests required the subjects to be transferred to a separate experimental cage (27cm x 29cm x 45 cm high) so that behaviours could be observed clearly and accurately. It was also desirable to test subjects individually when they were transferred to the smaller experimental cage. However, some subjects were very aroused in this isolated situation and had to be paired with companions of the same age for testing on the visuospatial reaching task. These subjects are identified in Table 2.4. Other subjects would not participate in tests in the experimental cage even when trialed in pairs (Zhen, Xing, Delta and Omega). With the purchase of a video camera with higher resolution and ease of handling (Sony Hi 8) the side-of-mouth preferred to chew could be clearly recorded in the home cage and, therefore, isolation in the experimental cage was no longer necessary for subjects that had not yet been

tested on this task. On tests with the offspring of Family Groups 2 and 3, it was possible to obtain video recordings of side-of-mouth preferences leaving them in their home cages.

Each of the tests of lateral preference was conducted when subjects reached the designated ages outlined in Table 2.5. The scoring criteria for the different types of lateral function are described briefly in Table 2.5. The number of subjects (N) that participated in each of the experiments and the chapters in which the experiments are reported are tabulated also (Table 2.5). The hand preferences of the 6 founding members of the colony had been determined for simple food holding at 10-12, 14, 15-18 and 22 months by the author prior to the commencement of this study (Hook-Costigan and Rogers, 1995). These subjects had also participated in the tests of visuospatial reaching and side-of-mouth preference in chewing prior to this study.

Recording methods

The details of each task used to measure lateral preferences are given in the appropriate chapters. However, the general recording techniques used in this thesis are discussed in this section.

Hand and eye preferences were recorded by direct observation. The experimenter was always positioned directly in front of the subjects to prevent biasing lateralized responses. It appeared that the experimenter's presence when scoring did not influence to any great extent the marmosets' behaviour as they did not interrupt play, feeding, grooming or sleeping behaviours to watch the experimenter. The marmosets were very familiar with the experimenter because of the time she spent with them involved in experiments, feeding, cleaning and general husbandry. Stevenson and Poole (1976) have demonstrated using experimental comparisons that the presence of a familiar observer has no influence on the behaviour of marmosets.

Table 2.5 Timetable and scoring criteria of experiments reported in this thesis

Experiment	Age	N	Scoring Criterion	Chapter
Hand preferred for simple food holding	0-2 5-8 10-12 14 15-18 22 25-30 31-40 41-50 51-60	15 15 21 21 21 21 17 14 10 6	From 0-2 months, subjects observed for 1-2h per day and all incidences of unimanual hand use and bimanual hand use for holding food were recorded. For all other tests, 5-15 scores collected per day for a minimum of 8 days. 100-130 unimanual hand use scores collected for each individual at each of the ages tested. Bimanual hand use for holding food also recorded.	3
Visually guided reaching through holes in the lid of a bowl (no postural demands)	14	21	5-15 scores collected per day for a minimum of 8 days. 100-130 incidences of hand use when reaching recorded for each individual.	4
Visuospatial reaching for static object while maintaining a suspended posture	16	19		
Visuospatial reaching for a moving object while maintaining a suspended posture	17	17		
Visuospatial reaching for a moving object on a rotating disc (no postural demands)	18-19	17		
Side-of-mouth preferences in chewing (hand use not required)	18	21	5-15 scores collected per day (minimum of 8 days). 100-130 scores of side-of-mouth use recorded per individual.	4
Side-of-mouth preferences in chewing (hand use required)	19	21	5-15 scores collected per day for a minimum of 4 days. 50-60 scores of side-of-mouth use recorded for each individual.	4
Leading foot when leaping, landing and walking	24+	17	Subjects' foot movements video recorded for 30 minutes/day over a minimum of 8 days. 100 incidences of each locomotory pattern were recorded per individual.	5
Eye preferences for viewing familiar food	5-8 10-12 15-18 22	15 15 15 21	5-15 scores collected per day for a minimum of 8 days. 100-130 incidences of monocular eye use recorded for each individual.	6
Eye preferences for viewing a variety of stimuli	22+	14	As above.	6
Lateralized production of facial expressions and vocalizations	22+	9-11	10 samples of 3 expressions collected per individual.	7

The ages of the subjects at testing are presented in months, and N= Number of subjects tested.

Details of behaviours such as the number of vocalizations made during testing, the leading hand and foot in leaping and landing, the side-of-mouth used to chew and asymmetries in the areas of the left and right sides of the open mouth made in the production of facial expressions and vocalizations were determined by analyzing videotapes of the behaviour. Testing sessions were recorded using either a Panasonic (NV-PS1) video camera or a Sony High 8 camcorder (CCD-TR2000E), as mentioned above. Frame-by-frame analyses were performed using a Sony Hi8 video cassette recorder (EV-S9000E). Video images were scanned into a Macintosh LC630 using a videocard program (Apple video player) for the analysis of mouth use asymmetries during the production of facial expressions.

In all of the experiments reported in this thesis, the method of scoring used to determine lateral biases prevented the recording of bouts of use of one hand, side-of-mouth, foot or eye during testing. Bouts are defined as behavioural acts repeated several times in succession without interruption by a different behavioural act (McGrew and Marchant, 1992; Martin and Bateson, 1993). Analyzing bouts of behavioural acts as if they were separate scores would violate the assumption of the statistical independence of observations (McGrew and Marchant, 1992; Martin and Bateson, 1993). Thus, scores of the focal behaviour were recorded only when they were separated by a considerable amount of time (5 to 60 seconds), by locomotion or by a different intervening behavioural act. The length of time considered necessary between behavioural scores depended on the act being measured. For example, only 5 seconds were required between monocular viewing scores as each incidence of viewing was less than 1 second in duration, whereas at least 30 seconds were required between visuospatial reaching scores.

2.5 STATISTICAL ANALYSIS

Each subject's percentage left hand, mouth, foot or eye use was calculated on each test of lateral function. The strength of preference, or the absolute preference score of each individual regardless of the direction of the lateral bias, was also

determined. These two indices of lateralization were used in most analyses. A Shapiro-Wilks test applied to each set of data (Table 2.5) indicated that the percentage left hand, foot and eye preference scores were not normally distributed ($p \leq 0.05$ in all these cases). Therefore, nonparametric statistical methods were used. All of the nonparametric procedures used are described by Siegel and Castellan (1988).

For each test of lateralized function, the significance of the preference score for each individual was determined using a z-score test (Edwards, 1963). The relative bias for each individual on each test was determined by comparing the z-score calculated to the expected binomial distribution. Each binomial z-score was calculated with respect to chance, thus testing the null hypothesis and assuming that the incidences of left and right scores should be equal. The z-score equation is, $z=(X-M)/\sqrt{Npq}$, in which X= the number of left or right responses, N= the total number of responses, M=N/2 and p and q= 0.5. An individual was considered to have a significant preference if the z score was ≤ 1.96 ($p \leq 0.05$).

Chi-squared (if number of subjects ≥ 15) and binomial significance tests (if number of subjects 10 to 15) were used to determine the relative bias of distribution of right and left preferences at the group level. Fishers exact tests (2 x 2 contingency table) were applied to determine whether the distribution of lateral preferences scores differed for male and female subgroups.

The Friedman two-way analysis of variance by ranks (F_r), with age as the repeated measure, was used to compare the direction and strength of intra-individual preferences displayed on tests of hand, foot and eye preference. Significant results of Friedman's tests were further analyzed using two-tailed *post hoc* Wilcoxon signed rank tests (T^+). Comparisons between preferences displayed by individuals in different groups (i.e. the 3 family groups) were analyzed with Kruskal-Wallis one-way analysis of variance by ranks (KW) tests. Data showing significant group effects were analyzed *post hoc* with Mann-Whitney U-tests (U).

The Spearman rank correlation coefficient (r_s) was calculated to determine the

strength of relationship between lateral preferences displayed on different tasks, using scores for both direction (percentage left) and strength of preferences. When clustering of data occurred separate correlations were calculated for each clustered group. As one of the aims of this thesis was to examine the hypothesized relationships between preferences displayed on different tests of lateral function, a considerable number of Spearman rank correlations were performed. Therefore, to limit the probability of committing a Type I error (Siegel and Castellan, 1988) and falsely rejecting the null hypothesis, a more conservative significance level ($p \leq 0.01$) was used for the correlation analyses.