More than numbers matter: the effect of social factors on behaviour and welfare of laboratory rodents and non-human primates

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Abstract

With the development of laboratory animal science, increasing attention has been given to the possible influence of housing and husbandry on the behaviour and welfare of laboratory animals as well as on the scientific integrity. With the present paper, we aim to contribute to this knowledge by reviewing existing literature on how social factors influence laboratory rodents and non-human primates. We use social ecology in the wild as a starting point to understand experimental studies of these social species. Laboratory studies show that preweaning social experiences, and lack thereof, affect the development of social skills and capacity to cope with stressful situations in both primates and rodents. Studies of deprivation, of preference and of demand indicate that both rodents and primates are highly motivated to interact with conspecifics. When housed alone, rodents and primates typically show a more 'anxious' reaction in behaviour tests, and are more profoundly affected by certain stressors, although there seem to be some differences in how rodent males and females react to different social situations. However, for social housing to be beneficial for the animals, compatible and stable groups are crucial. When forming groups of monkeys in captivity, the age and sex of individuals and their relative age difference, the taxonomic membership of the animals as well as the introductory technique are factors of importance for success. Kinship is also of importance for the compatibility of both rodent and primate groups.

Social instability through changes of group composition is apparently stressful, resulting in behavioural and physiological alterations in both rodents and primates. The effects of social conditions around testing have been given much less attention, but several studies show that animals react differently in behaviour tests when tested in group than when tested individually. Altogether, the most commonly used laboratory rodents and primates belong to social species, and their behaviour and welfare are strongly affected by previous and present social environment. Factors such as group composition and stability, rank and previous social experience therefore need to be taken into account both when designing housing systems and when planning experiments and interpreting results.

Keywords: group, individual, social isolation, social housing, rat, mouse, guinea pig, macaque, primate, rodent

Introduction

With the development of laboratory animal science as a research area, increasing attention has been paid to the biology, behaviour and welfare of the common laboratory animal species. The welfare of an animal is affected by health and ability to cope with the environment (e.g. Fraser and Broom, 1990) as well as affective state (e.g. Duncan, 1993) and ability to express motivated behaviours (e.g. Dawkins, 1998). The physical and social environment in which laboratory animals are housed obviously have important consequences not only for welfare but also for experimental results and the quality of research (e.g. Balls, 1994; Claassen, 1994; Würbel, 2001; Olsson et al, 2003; Sherwin, 2004). Although this fact is increasingly being recognised, the conditions for social housing are still to a considerable extent dictated by what is convenient in the animal facility and of the research protocol in question, rather than considerations of animal biology and welfare. Nevertheless, the scientific literature contains extensive information about how the social living conditions affect laboratory animals. The studies are of two main types: those designed to increase understanding of the species in question and those where the animals were used to model general principles of behaviour or human biobehavioural phenomena.

In the present review, we aim to review the existing information of how group size and other social aspects such as individual housing, early social experiences and group composition and stability affect the behaviour, health and welfare of laboratory rodents (rats, mice and guinea-pigs¹) and non-human primates (the main focus being on macaques, the most commonly used laboratory monkeys). Besides being the animals that the authors work with, it can be argued that these are the two most important species groups: rodents because they are by far the most common animals used in research and non-human primates for their particularly complex requirements as regards social housing environment. After giving an introduction to the natural social behaviour and social groups for the species in question, we address the following aspects of the social situation in the laboratory setting: early social environment and experiences, individual housing, group composition and stability, group size and social density and social conditions around testing.

1. Species-characteristic social behaviour

There is no “prototype primate” or “prototype rodent”, and no single set of rules will satisfy the needs of all members of a given species, let alone all species. Nevertheless, studies of wild populations, as well as captive populations in semi-natural settings, have indicated which characteristics are particularly relevant for the appropriate functioning of social groups and for the well-being of animals kept in the laboratory.

1.1 Rodents

The social organization of wild house mice (*Mus musculus*) may vary between different populations. Commensal populations live in territories with stable and plentiful food supply with up to 10 mice/m², in groups composed of a single dominant male, a few subordinate males and several breeding females with offspring. Feral populations are less dense and are typically unstable with a high turnover rate (Bronson, 1979). Overall male mice are territorial, with two types of territory holders: exclusive territorial and dominance territorial (Hurst, 1987). Subordinates and subdominants may nest alone or

¹ For an ethological view on the social environment of the less common laboratory rodent species, see the recent review paper by Sørensen et al (2005).

subordinates nest communally or with females or juveniles. Young males may either disperse or challenge the territory holder, an event that is likely to end either with the challenging male overthrowing the territory holder or being killed. Females may stay in the colony, where they will remain subordinate to the breeding females and some will never come to reproduce themselves, but may help with the rearing of other young (reviewed in Latham and Mason, 2004). The Ratlife project (Berday, 2003) elegantly demonstrated the adaptive capacity and diversity of social behaviours in laboratory rats (*Rattus norvegicus*) in a semi-natural environment, showing a polygynous society with promiscuous mating and low levels of aggression. Under favourable conditions, such colonies can grow to several hundred individuals (Barnett, 1975). At puberty, both male and female rats show dispersal, generally moving into areas which are less densely populated or inhabited by low-status residents (Calhoun, 1962). Domestic guinea pigs (*Cavia aperea*) as well as their wild ancestors live in polygynous groups. In wild cavia, the daughters integrate into their maternal group, whereas male offspring will be socially incompatible with the dominant male as they reach sexual maturity. In contrast, in domestic guinea pig colonies, both male and female offspring integrate into the social group in which they were born. The clear preferences of females for particular males contributes to the social organisation (reviewed in Sachser 1998, Sachser et al 2004). In summary, overall the social organization of rodents is dynamic and partly determined by environmental aspects such as resource availability.

1.2 Non-human primates

Studies of wild primate populations may indicate characteristics important to the well-being of captive primates. Those that influence social behaviours the most (reviewed in National Research Council, 1998) are the mating system (solitary, monogamous, single-male or multi-male groups), migration patterns, group size, group composition, spacing patterns, food availability and diet, reproduction, age at sexual maturity, patterns of parental care, communication, and type of dominance structure (egalitarian or despotic; de Waal and Luttrell, 1989). There are considerable differences between species, and it is beyond the scope of this review to go into all socio-ecological details for different species. For example, bonnet macaques (*Macaca radiata*) show substantial group cohesion, including alloparenting behaviour in unrelated females and little intragroup

aggression (Reite et al., 1989). In contrast, the closely related pigtail macaques (*Macaca nemestrina*), have highly protective mothers, far less social cohesion and exhibit more aggression in intragroup encounters (Kaufman and Rosenblum, 1967). Generally, monkeys are xenophobic, and react to strangers with hostility and aggression (Lindburg, 1991). They maintain bonds with alliance partners, mates and kin through social grooming. Social grooming reduces heart-rate, as shown in a study on pigtail macaques (Boccia et al, 1989).

Some aspects of primate socio-ecology and behaviour can have profound implications for captive primate management. For example, in wild single-male groups, aggressive take-overs by new males are common and are sometimes associated with infant deaths. This male strategy is thought to increase male reproductive success by reducing the interbirth interval in females (Blaffer Hrdy, 1979). Thus, in captivity, while small infants are present in the group, it is probably unwise to replace a resident gorilla, langur or guereza.

Other aspects of captive primate husbandry involve olfaction. Some primates are highly sensitive to chemical stimuli, and communicate through scent-marking. There is great complexity in these behaviours, and the secretions can yield information about the scent owner's species, gender, and hormonal status. This information is important in contexts such as reproduction, dominance and territoriality. In callithrichids, scent marks from a breeding female contribute to the suppression of ovulation in non-breeding adult females in the group. Consequently, in scent-marking species such as the prosimians, too thorough or frequent cage cleaning might seriously disrupt patterns of social information and thus compromise animal well-being. (National Research Council, 1998).

Migration usually occurs in either gender, or both. The process of migration may involve both the risk of aggression in the natal group as well as in the new group, in conjunction with a higher predation risk during migration. Up to 80% of migrating macaque males may die from starvation and injury, and longtailed macaque males (*Macaca fascicularis*) showed significant increases in urinary cortisol levels during immigration (Visalberghi and Anderson, 1993).

Overall, natural group structure and migration patterns help explain patterns of dominance, aggression and friendships manifested in captive primate groups. Therefore, knowledge of the socio-ecology, communication patterns and natural history of the species in question is fundamental to achieve successful captive primate management.

2. The pre-weaning social environment – effects of disturbed socialization

2.1 Rodents

When the mother leaves the nest, rat pups increase their locomotory activity and emit ultrasonic vocalisations (Hall, 1998). Long-term effects of maternal separation in rats have been extensively studied using two different paradigms: early handling and maternal deprivation (reviewed in Hall, 1998). Early handling implies brief daily separations during the first 2-3 weeks postnatally, and results in a decreased behavioural and endocrine response to stress persisting into adulthood (Hall, 1998; Würbel, 2001). There are strong indications that this effect is mediated through the increased maternal behaviour directed towards the pups when the female is reintroduced (reviewed in Mason, 2000; Würbel, 2001). In maternal deprivation protocols, mother and pups are separated for several hours and the effects on offspring vary. It has been argued that maternal deprivation and early handling produce opposite effects in offspring reactivity (e.g. Hall, 1998), however recent work by Würbel and coworkers suggest a more complex picture. Under natural conditions, the mother would have to leave the pups to forage, so the brief separations in the early handling protocol may well reflect the natural adaptation, whereas the prolonged absence in the maternal deprivation set-up that disrupt the suckling pattern is an abnormal situation likely to be maladaptive (see Würbel, 2001; Macri et al, 2004).

Mendl (1988) reviewed a large number of studies of mice and rats for the effect of litter size on different physiological and behavioural variables. He found that offspring birth

weight is lower and growth rate and physiological development slower in large litters, effects that may persist into adulthood, when females from larger litter show delayed sexual maturity and reduced fecundity. Mothers of larger litters spend less time with and are more likely to kill offspring. There are also some indications that offspring from larger litters show more social behaviour. Because of the effects of litter size on growth and development, standardizing litters the first days after birth is standard practice in studies of reproductive toxicity (Agnish and Keller, 1997), although this has been challenged as unnecessary and unbiological (Palmer and Ulbrich, 1997).

Although taking place at the age when the pups stop suckling naturally (21-23 days; e.g. König and Markl, 1987), the standard weaning procedure of abrupt separation must be considered a premature separation, as in nature young rodents only migrate from their home colony at sexual maturity several weeks after weaning (Calhoun, 1962; Latham and Mason, 2004).

2.2 Non-human primates

Primates have long developmental periods and are capable of extensive behavioural modification as a function of experience and learning from older individuals (Bernstein, 1991). Deprived of this opportunity, serious, and in many cases irreversible, behavioural problems occur.

Nonhuman primates who have experienced socially deprived conditions during infancy often develop idiosyncratic behaviour interpreted as a replacement for maternal activities, such as self-clasping, self-mouthing and rocking. These developmentally induced stereotypies do not respond to treatment in the same manner as other atypical behaviour patterns with a different etiology, and typically persist in later and socially adequate housing situations (Mason, 1991). Depending on the degree of early social deprivation, the severity of the developmental abnormalities varies. Hinde (1971) found that 3 months' isolation could be recovered from, but 6 months' isolation produced permanent effects and 12 months' isolation destroyed all social abilities in rhesus macaques (*Macaca mulatta*). Animals that had experienced early social deprivation became asocial, neurotic, hyperaggressive and/or socially indifferent and deficient in

sexual and parental behaviour. They also showed an inability to cope with stress as evidenced by self-biting, eye-poking, coprophagy and other stereotypic behaviours. In general, there were no cognitive deficits. (Hinde, 1971; Mason, 1991). However, Anderson and Mason (1978) found that the development of higher orders of social cognition (responding to status relations between other individuals) is dependent on early social experience. Physiological effects of early social deprivation has also been demonstrated, such as an increase in basal cortisol levels in macaque infants (Champoux et al., 1989), and compromised immunocompetence in macaques that have undergone forced separations as infants (Visalberghi and Anderson, 1993).

In nature, primates in general spend the first few months clinging to their mothers (except the prosimians). As they gradually move away from their mothers and explore their surroundings, other individuals such as peers play an important role in social development. Rhesus monkeys raised with their mothers but denied access to peers showed remarkable social deficiencies, including hyperaggressiveness and impaired affiliative behaviour. Again, the severity of this effect depended on the period of deprivation, 8 months' peer deprivation producing a more pronounced effect than 4 months' (Hinde, 1971). Rhesus macaques raised with peers but not their mothers quickly started developing physical attachments to each other, and developed more normally. Apparently, peer-peer interactions compensate reasonably well for the lack of mothering (Hinde, 1971). However, longtailed macaques raised with peers but not mothers developed phobic behaviours in the presence of large novel objects, in contrast to animals raised with their mothers (Timmermans et al., 1986).

Some studies examined the effects of temporal removal of the mother on infants' immediate behaviour and future development. Although there seem to be species differences as well as considerable individual variation, Hinde (1971) reports that short but repeated removals of the mother (for as little as 2 hours every fortnight during the first 8 months) lead to significant differences in dominance at age 3 years. Animals subjected to this procedure were low in social dominance as compared to normal animals (Hinde, 1971). In conclusion, monkeys are highly sensitive to even minimal disturbances in their early social environment, both access to peers and mother are important.

Even though single housing of laboratory primates is a system being abandoned in many countries today (CoE GT 123, 2004), premature maternal separation (at around 6-8 months) still takes place in many macaque breeding facilities around the world, despite the documented negative effects on wellbeing. Although perhaps not as critical as the total isolation experiments described previously, the effects of premature maternal separation are severe enough to make this procedure a model used in studies of depression, immune deficiency, and stress (reviewed in Reinhardt, 2002). One of the reasons behind the practice of premature maternal separation is the idea that females with force-weaned infants begin cycling sooner, thus maximizing reproduction output in the colony (unpublished observation, KW). However, there does not seem to be any scientific support of this notion: in a study on captive baboons, naturally-weaned mothers exhibit their first post-partum oestrus approximately one cycle earlier than mothers of force-weaned infants (Cary et al., 2000).

3. When there is no group – individual versus group housing

Individual housing of social species is not an uncommon phenomenon in laboratory animal facilities. It may be part of an experimental paradigm, to study the effect of deprivation of social stimuli or to induce stress, or it may be called for by other scientific or practical reasons, such as need for individual monitoring of food distribution and intake or after surgical procedures involving cannulation or sutures which can be damaged by other animals. However, it seems that individual housing is not always necessary in this type of situation. Control over individual food intake in primates in a group setting might be accomplished by careful training procedures (Schapiro et al., 2003), and rats in a learning set-up where individual housing is usually applied learned equally well irrespective of housing (Molina-Hernández and Téllez-Alcántara, 2004). Some surgical procedures (e.g. head-cap implants in primates (Reinhardt, 1997; Roberts and Platt, 2004); telemetry device implantation in rodents (Meijer et al., 2002)) may allow for continued social housing with no apparent ill effects.

Another common reason for housing animals individually is aggression. In many of the laboratory animal species, including mice, rats and primates, group-housing of males may result in aggression problems. Sometimes the term “isolation” is used; however as discussed in van Loo et al. (2000), single-housed animals can usually smell, hear and even see animals in the same room, and the ‘isolation’ is therefore limited. Nevertheless, individual housing deprives animals of social stimuli, which in turn can cause profound alterations of behaviour. The nature of the alterations is fairly generalizable over the social mammalian species studied, but depends on factors such as the age at onset and duration and type of deprivation (Hinde 1971; Mason 1991; Bernstein, 1991; Hall, 1998). The effects of maternal deprivation have already been discussed in section 2, and the present section focuses on isolation in adolescent and adult animals.

3.1 Rodents

There are few studies of the acute effect of social isolation in rats at any age (Hall, 1998), but two effects appear clear: the stress reaction and the effect on social behaviour. Acute social isolation acts as a stressor, resulting in increases in corticosterone, alterations on a number of neurotransmitter systems, increased anxiety-behaviour in the elevated plus maze as well as increased voluntary ethanol-intake (reviewed in Hall, 1998). Increased anxiety-behaviour was also found in male mice isolated 24h prior to an elevated plus-maze test (Ferrari et al., 1998). In guinea-pigs of both sexes, plasma levels of cortisol were elevated and oxytocin reduced in individually housed animals compared to male-female pairs (Machatske et al., 2004). Acute isolation also affects social behaviour, in that isolation-housed mice and rats show more social interactions than group-housed animals in test encounters with peers (Terranova et al. 1993; Hall. 1998; Douglas et al., 2004). Hurst et al. (1999) found that when initially group-housed rats of both sexes were regrouped into individual or group-housing, individually housed animals showed much more escape-related behaviours than group-housed rats, suggesting that individual housing is aversive and that the animals seek social contact. This was confirmed in an operant study where female rats showed much greater motivation for access to social contact than for any other resource (Patterson-Kane et

al., 2002). Also male mice showed preference for social contact, preferring to sleep in close proximity to a familiar male (van Loo et al., 2004).

Increased locomotor activity in response to a novel environment is frequently reported for isolation-reared rats, and it has been postulated that these animals are 'hyperreactive'. This is consistent with the observation of increased anxiety as revealed in a series of standard tests and a potentiated stress response (Hall, 1998). Hyperreactivity was also found in several behaviour tests on mice after long-term isolation (Völkar et al., 2005). On the other hand, some studies found no difference between rats reared and housed either socially or individually in behavioural response to a novel open field (Holson et al.; 1991), and several studies report a lower rather than higher level of anxiety-like reactions in some tests of isolated animals (Guo et al. (2004): elevated plus-maze, light-dark box; Völkar et al. (2005): elevated plus-maze). The different reaction patterns in tests of anxiety between animals housed in group and individually are also reflected in different reactions to anxiolytic drugs (e. g. Manzanique et al. 2002). As the studies differ in strain, timing and duration of individual housing, housing group size for controls and type of tests used, it is difficult to point to any one reason for the discrepancy in results. Part of the explanation may be the apparent existence of different behavioural strategies in rodents, differing widely in their response to psychological challenge (e g Korte et al, 2005).

In isolation-housed (but not isolation-reared) rats, the increased anxiety seen in a novel environment is reversed if the animals are rehoused in groups (reviewed by Hall, 1998). Indeed, present housing condition may influence the way animals react to a stressor: the typical persistent behavioural and physiological reaction to social defeat is greatly reduced in rats housed with familiar mates compared to individually housed animals (Ruis et al., 1999; Von Frijtag et al., 2000).

When studied in their home cage, the behaviour of isolated male rats differed from group-housed in that they showed more tail attention and chasing, more bar chewing, more drinking and more self-grooming. The isolated rats were furthermore less mobile during both dark and light periods (Hurst et al., 1999). Females showed a similar reaction pattern, although less pronounced (Hurst et al., 1998). When compared to

males pair-housed with ovariectomized females, Späni et al. (2003) found that individually housed male mice had slightly higher heart rate and a different activity pattern with more frequent changes and more frequent but shorter phases of sleep or resting. After isolation housing, male rodents react more aggressively when confronted with a stranger; in fact, isolation-induced aggression is used as an experimental paradigm in mice (e.g. Crawley, 2000). However, Hurst et al. (1999) found that if male rats had had prior contact with other rats through a barrier, this aggression was significantly decreased. Sharp et al. (2002) found that housing male rats in groups of four rather than individually reduced both basal cardiovascular stress measures and the magnitude and duration of the physiological and behavioural reaction to husbandry and experimental procedures. A similar, although less pronounced, pattern was observed in female rats (Sharp et al., 2003)

There are probably sensitive periods that determine the long-term effect of social isolation, and several findings suggest that the period prior to or during puberty is critical in rodents. Rats that had been single-housed during weeks 4 and 5, followed by pair-housing, were less prone to engage in social behaviour as young adults than rats that had never experienced isolation (van den Berg et al., 1999a). The same researchers (1999b) reported that a 30-min daily play session prevented development of post-weaning isolation effects on male rats' later reaction to tests of social and sexual behaviour. Avitsur et al. (2003) reported an altered submissive response to an aggressive winner in isolation-reared mice, showing active escape rather than species-typical submission. Sachser et al. (1998) found that for male guinea pigs, social experience at puberty was crucial for adaptive interaction with unfamiliar conspecifics: when males lacking this experience were introduced into a new colony; increased agonistic interactions were accompanied by persistent increases in adrenocortical activity, especially in subordinates.

3.2 Non-human primates

3.2.1 Adolescent single housing, post-weaning isolation, isolation rearing

In a review on the effects of social manipulations, Schapiro (2002) found that species-typical behaviour in rhesus macaques could be increased and abnormal behaviour decreased by enrichment and social housing rather than single housing. The socially housed animals also showed changes in a number of immune parameters in comparison with single housed animals (separated from their natal group at age 1 year), with pair-housed animals requiring the fewest veterinary interventions and days of treatment for diarrhea (Schapiro, 2002).

In contrast to rodents, monkeys reared in social deprivation are usually less explorative than monkeys with an adequate social background (Harlow and Zimmerman, 1959). This observation is supported by primate attachment theory: the mother being a secure 'home base' from which the infant makes excursions to explore. Deprived of the attachment figure, infants become less exploratory (Timmermans et al 1986).

There is conflicting evidence as to the effects of social deprivation in adolescence on the effect of future breeding and parenting success in primates. Schapiro et al. (1994) found age and prior social experiences to be important determinants of parental success and social competence. Rhesus macaques with restricted social experience in an early study with a small sample size exhibited impaired parental competence. In a later publication, however, Schapiro's larger sample sizes disproved this finding (2002, personal communication), seeing no differences in reproductive output between the experimental group and animals with a normal social background. However, in matrifocal societies like the macaques', the opportunity for development of parental behaviour seems to coincide with the birth of a younger sibling. Depriving immature animals of the opportunity to interact with infants by removing them before the birth of siblings has been shown to negatively affect future parental competence (Pryce, 1993). However, exposure to viable mother-infant dyads, even for a short period, may help females acquire appropriate maternal skills (Goin and Gust, 1998).

3.2.2 Adult social isolation: isolation housing in primates

Self-directed biting or aggression towards the physical environment is more common among singly housed primates than in a social setting, particularly among adult male macaques. Independent of social history, 10% of singly housed rhesus macaques

develop self-biting behaviours leading to tissue damage (Jorgensen et al, 1998). To a certain extent, this can be remediated by a complex and stimulating environment, and completely reversed in an adequate social setting (Reinhardt and Rossell, 2001). Dorey et al. (2004) found that self-injurious behaviour (SIB) in a singly housed olive baboon at a private zoo was maintained since the behaviour was reinforced by human attention. A training program involved teaching alternative attention-getting behaviour, resulting in a decrease in SIB.

The opportunity for tactile contact with conspecifics has been shown to contribute substantially to the behavioural health of primates. If, for some reason, the research protocol does not allow group- or pair housing, so called grooming bars might cater for this need (Crockett et al., 1997). In some countries, single housing often involve small cages, and the reduced mobility, lack of control or predictability of the environment, the inability to get out of sight of a nearby animal, and the restricted visual field might also affect animal well-being adversely. If single housing cannot be avoided, cages should be arranged so that animals within visual range are compatible. If the primates continuously threaten one another, they should be moved out of direct visual contact (National Research Council, 1998).

4. The social group and its composition

4.1. Group formation

4.1.1 Rodents

Hurst et al (1996) studied single-sex groups of rats in pens, and found that aggression declined rapidly in males, where subordinates tended to retaliate aggression, but not in females, where subordinates responded with escape attempts. This may suggest that female subordinates were less able to use an adaptive behavioural strategy in the captive situation, where they were unable to move away from the dominant. Familiarity or genetic relationship between group mates is an important factor affecting whether a

stable group can be established. When housed in groups of siblings, dominance status did not affect male mice' reaction in tests of anxiety / exploration, nor signs of HPA-activity or immunoresponsiveness (Bartolomucci et al., 2001; 2002). On the other hand, when two previously unfamiliar mice were housed in a set-up where they could see and smell each other and had short daily physical contact, all mice - irrespective of dominance status - showed increased corticosterone levels and altered reaction in the open field test compared to sibling-grouped control mice (Bartolomucci et al., 2001).

In male mice, the age at group formation may strongly affect social stability. Bartolomucci et al. (2004) studied the agonistic and exploratory behaviour as well as physiological profile of outbred CD-1 mice that had been regrouped either at weaning, as adults or not at all. While treatment had no effects on females, the males regrouped at weaning (26-28 days) reacted differently than the other two groups. These males showed higher aggression and the subdominants did not differ from the dominants on the measure of testosterone activity, indicating that the hierarchy was not stable in this group. When compared to the sibling group, animals regrouped at weaning or as adults showed lower basal corticosterone levels. However, when an inbred strain (Balb/c) was studied, with weanling non-sibling groups formed at 21 days of age, van Loo et al. (2000) found no difference in aggression between sibling and non-sibling groups. Inbred animals are genetically similar in a way that is likely to interfere with their individual recognition (Nevison et al., 2000), which in combination with the earlier weaning may explain the latter finding of low aggression.

4.1.2 Non-human primates

Historically, an overwhelming proportion of research monkeys have been singly caged. Apart from the practical reasons, such as ease of cleaning and better monitoring of individual animal food intake, etc, there have also been serious concerns about possible negative side effects of social housing. The primatological literature contains many examples of failed social manipulation attempts, some with disastrous consequences. The xenophobic reaction to strangers shown by many primates (Southwick et al, 1974) partly accounts for this. Joint attacks and aggression focused on one or a few targeted individuals is fairly common in monkey groups, which in the wild leads to peripheralization and migration (Visalberghi and Anderson, 1993).

Reinhardt (1990a) addresses the concerns and risks associated with pair formation in captivity. The author concludes that, unless precautions are taken such as early identification of incompatible pairs, there is a risk of physical trauma resulting in injury and death during primate pair formations. Around 6% of rhesus as well as stump-tail macaque pairs were found to be incompatible. The author found no documentation of an increased risk of disease transmission in paired compared to singly housed monkeys. On the contrary, 25% of single-housed rhesus monkeys received veterinary treatment in one year, whereas only 10% of pair-housed animals did. If compatible, both animals experienced a weight gain from pairing in rhesus macaques; thus undernourishment of the subdominant individual was not a documented problem.

Previous familiarity could be argued important when forming pairs in captivity, since this would imply the existence of an established ranking order. However, Schino et al. (1990) compared familiar versus unfamiliar long-tailed macaques in the 2-h period after pairing and found that unfamiliarity per se was not a good predictor of post-pairing social tension. Rather, the nature of dominance interactions, usually evident within moments, was decisive. If the unfamiliar animals showed a clear-cut dominance relationship, they did not differ from the familiar pairs in terms of displacement activities and grooming exchanged. However, unfamiliar animals with no clear-cut dominance relationship showed less affiliative behaviour and more displacement activities (Schino et al., 1990).

In general, pair-housing macaques in captivity is not considered problematic provided proper precautions are taken (Reinhardt, 1990a+). Formation of larger groups, however, is another matter. In nature, new rhesus groups are characteristically formed by fission: the gradual splitting off of a subgroup from a larger unit (Bernstein and Mason, 1963). In captivity, groups are often created by introducing strangers (fusion). The successful formation of such groups may depend on the age and sex of individuals and their relative age difference, the taxonomic membership of the animals as well as the introductory technique. In general, immature animals can be readily introduced and not be aggressed. Adult males are usually the most difficult to introduce into a group already containing adult males. Aged adults are usually more compatible than young or

prime adults. The amount of hostility at introduction can be reduced by previous familiarity, even after a period of separation of several years (Bernstein, 1991). In contrast to pair formation, group formation of captive primates is not a straightforward process. Several techniques have been tried and the evidence is conflicting as to the best method; the simultaneous introduction of all future group members (Bernstein, 1991); incremental release in hierarchical subgroups over a period of weeks (Westergaard et al., 1999) or systematically pairing each possible dyad of a future group before grouping (Line et al., 1990; Reinhardt, 1990b).

Some strategies used during the critical process of captive pair or group formation involve preparing the environment, such as giving the newly formed group a context shift: a new territory, a complex environment with foraging opportunities, visual barriers and multiple escape routes to allow subordinate individuals some escape possibilities, and initial prolonged human presence to divert and interrupt escalated aggression (Bernstein, 1991; Fritz and Howell, 2001; Westergaard et al., 1999). The use of noncontact familiarization is often advocated as a means to reduce aggression during pair formation (e.g. Reinhardt, 1994). In contrast, Bernstein (1991) questions the use of noncontact familiarization, warning that it might exacerbate the initial xenophobic response because of the animals' opportunity to aggress with impunity through the barrier, and that the animals build a history of exchanged aggression. He argues the main use of this method to be the possibility to predict whether or not the animals will fight.

4.2 Social (in)stability and stress

4.2.1 Rodents

The social stress paradigms in rodents have been developed primarily as tools for research on human diseases related to stress. Given that social factors are the main sources of stress in humans, it has been argued that animal models of social stress would be more appropriate for such studies than models involving physical stressors only (Martinez et al., 1998). Social defeat and subordination are naturally occurring situations in social species, and are presumably particularly stressful for male rodents if

the subordinate cannot escape the dominant as he would do under natural conditions. The resident/intruder paradigm and the colony model are the two main models of social defeat and subordination in rodents. In resident/intruder studies, resident animals are prepared for victory over intruders by being of a more aggressive strain, heavier, with previous experience of victory and coming from a social situation rendering them more aggressive (reviewed in Martinez et al., 1998). Introducing the intruder into the resident's home cage, the former is exposed to defeat which can be of varying duration and repetition. In the colony model, a stable mixed-sex group is maintained, in which one male is dominant and the remaining are subordinates (e. g. Blanchard et al., 1995; 2001). Overall effects of social stress on social behaviour of losers/subordinates include a decrease in social interactions, decrease in sexual behaviours and increase in submissive and defensive behaviours. Non-social effects include motor inhibition and decrease in locomotion and exploration, increased anxiety, pain inhibition (analgesia) and an increased tendency to self-administrate ethanol. Defeated animals also show an increased HPA-activity but a reduced activity of the hypothalamic-pituitary-gonadal axis. There is an increase in blood pressure and heart rate, reduced body weight and alterations in body temperature as well as immunosuppression (Martinez et al., 1998). However, caution is needed in interpreting studies of experimental social stress situations as there is a great variation in which type of control group is used for comparison. Control groups may even include individually housed animals, a treatment that is sometimes used as a stressor in itself. The question is further discussed by Martinez et al. (1998), who recommend that the control group should consist in animals that "have experienced a social interaction but without the experience of defeat".

Haller and coworkers (Haller et al., 1998; Baranyi et al., 2005) have studied the effect of social instability on the behaviour and physiological reaction of female rats, with the aim of developing a social stress protocol for female rodents. Female rats subject to daily alterations between individual housing and housing in a group of varying composition showed a decrease in weight gain and an increase in adrenal weight and plasma corticosterone compared to animals kept in stable male-female pairs (Haller et al., 1998). Interestingly, the same protocol did not affect corticosterone levels or adrenal weight in males. Social instability further affected reaction in a social interaction test, resulting in less social non-agonistic behaviour and more aggression compared to

females that were kept in stable groups (Baranyi et al., 2005). Even though increased levels of aggression immediately after regrouping was found in one case (Baranyi et al., 2005), the physiological stress pattern developed also in situations of low aggression. However, it is not clear how control animals were handled in these studies, making it difficult to exclude a possible confounding between amount of handling and social instability.

In male mice that had been regrouped, Bartolomucci et al. (2001) found that mice that had experienced loss of status showed immune hyporesponsiveness. A correlation between (presumed)² loss of status after introduction in a new group and altered immunoresponse was also found by Avitsur et al. (2003).

Male mice scent mark their territories, and disturbing these marks that play a role in dominance and aggressive interactions may disrupt the social stability of a group of males. Routine cage cleaning profoundly changes the scent environment, and after cage cleaning, there are typically bursts of aggression even in a group of familiar males. How much of the cage contents are replaced affect the amount of aggression. Results are conflicting as regards whether to exchange all or only parts of the substrate (e. g. McGregor and Ayling, 1990; Gray and Hurst, 1995), but van Loo et al. (2000) found that transferring nesting material from the soiled to the clean cage clearly reduced aggression.

4.2.2 Non-human primates

Natural groups of primates usually contain individuals of both genders, and of mixed ages. Captive groups that deviate too much from the natural group composition may experience trouble in the regulation of e.g. aggression. In a study by Dazey et al., 1977, female pig-tailed macaques showed significantly more aggression in female-only groups than in the presence of an adult male. Furthermore, loss of the male's control over his group through an enclosure where individuals could get out of the dominant male's sight resulted in a dramatic increase in aggression among the females (Erwin, 1979).

² The authors did not measure social status but assumed that a single mouse introduced into a cage of two residents would become subordinate

Natural primate groups undergo changes in composition when animals are born, die and migrate. Sometimes turnover might be quite high, especially for males, the gender that usually migrates. Events such as male take-overs may be quite turbulent and involve outbreaks of heavy aggression and infanticide (Blaffer Hrdy, 1979). In captivity, repeated changes in social group composition can exacerbate aggression, as shown in a study on rhesus macaques (Kaplan et al., 1980). Once a compatible group has been established, changes should therefore not be made unless necessary. During group formation, a number of physiological parameters may be affected temporarily. At the time of pair formation, the blood pressure and cortisol levels increase, reproductive hormones are suppressed and immunological functioning is decreased in rhesus macaques, even in successful pairings where no wounding is seen (Visalberghi and Anderson, 1993). Artery atherosclerosis can be experimentally induced in dominant male macaques by repeated social reorganizations. Animals that have been singly housed may require up to 15 months for stress indices to return to baseline levels following group formations (Visalberghi and Anderson, 1993).

As reported in rodents above, primate immunocompetence may also be affected in response to changes in the social environment. Capitanio (1998) reported that the number of separations both before and after inoculation of rhesus macaques with simian immunodeficiency virus (SIV) were inversely related to survival. In vervet monkeys, the subordinate males' behaviour is strongly inhibited by the alpha male, as shown in a removal study by Hector and Raleigh (1992). When the alpha male disappeared, subordinates rapidly started behaving in a manner most likely to enhance their ranking opportunities. Female aggression was also highly influential in determining male ascendancy to dominant rank. Thus, changes in group composition may have both physiological and behavioural consequences, potentially adversely affecting the collection of scientific data.

5. Effect of social status

In nature, dominance hierarchies provide predictability and stability to social relationships (Bercovitch, 1991). High rank thus gives priority to resources and a higher feeding success (Saito, 1996). High- to middle-ranking primates have a slight lifetime reproductive advantage over low-ranking animals (Ellis, 1995).

5.1 Rodents

In pen-housed rats, Hurst et al (1996) found that rank affected non-aggressive behaviours, in that dominant animals slept more and subordinates spent more time exploring along the pen walls. The effect was more pronounced in females, among which aggression was relatively high throughout the study, while the rapidly declining aggression in male groups suggests they were more successful in adopting an adaptive group-living strategy. Compatible groups may also explain why Bartolomucci et al. (2002) found no difference between dominants and subordinates in exploratory behaviour, body or organ weights, basal corticosterone levels or immune responsiveness when housing male mice in sibling groups of three. Both stability and previous social experience are important factors, as demonstrated in a series of experiments on guinea pigs reviewed in Sachser et al. (1998). In colonies of different sizes and with different social systems (see 6.1), alpha and non-alpha males did not differ in measures of adrenocortical and adrenomedullary activities. Ferrari et al. (1998) found that dominants and subordinates in stable groups of 10 male mice differed in anxiety behaviours as measured in the elevated plus-maze, with subordinates showing lower levels of anxiety than dominants. Indeed, social foraging theory (e. g. Ekman, 1987) would predict subordinates to be more risk-prone. Through effects on hormonal status, social status may also affect immunocompetence. In groups of randomly bred male CFLP mice, individuals that received many aggressive attacks had lower concentrations of serum IgG and impaired resistance to a parasite infection (Barnard et al., 1996). Social status may also affect drug sensitivity, as discussed by Lathe (2004).

5.2 Non-human primates

As reported in rodents above, captive primate studies have also found a differential susceptibility to drugs according to dominance status (Morgan et al., 2002). Physiological effects correlating with subordination in captive primates include thymic involution and adrenal hypertrophy (Tamashiro et al., 2005), as well as a dominance-related variation in relative cortisol levels (see below).

Whether subordination in a natural social group with an established dominance hierarchy is a potentially stressful situation is currently under dispute. Creel (2001) argues that increased glucocorticoid secretion may indeed be a cost of *dominance* in cooperatively breeding birds and mammals, including primates. In other papers examining effects of dominance status on different aspects of behaviour and physiology in captive primates, the situation for subdominant animals is sometimes referred to as stressful (Shively, 1998, Tamashiro et al., 2005). In a meta-analysis across seven different primate species, Abbott et al. (2003~~2~~) identified two factors correlating with higher relative cortisol levels in subordinate captive monkeys: 1) being subject to higher rates of stressors, and 2) being denied opportunities for social support. However, one major concern about these studies is that they were conducted on captive groups and neither of them provide adequate information about weaning age, socialization history, group composition, group formation techniques, compatibility or cage structuring and enrichment. As previously discussed, all of the above are factors which potentially influence the stress-response, and it is thus possible that *sub-par* housing has exacerbated stress-responses. Coping strategies when dealing with stressors differ within a population, yielding differences in both physiology and behaviour (Korte et al., 2005), and the animals' differential reaction to stress is thus potentially mirrored in their ability to attain and maintain dominance (as seen in the study by Hinde, 1971). Indeed, the Abbott et al. analyses (2003~~2~~) showed variability between species – and gender - in subordinate relative cortisol levels ranging from 45% to 154% of levels measured in dominant animals.

6. Effects of varying group sizes and densities

Overall, housing recommendations for laboratory animals are based on weight and species of the animals and to the best of our knowledge generally with very limited

underlying empirical indications of appropriate group size for the species and sex in question. This observation is particularly relevant as regards the concept of ‘crowding’, as discussed below.

6.1 Rodents

Andrade and Guimarães (2003) studied the effect of pair- or group-housing on elevated plus maze behaviour after restraint stress. Animals that were housed together with one member of their previous group after restraint showed a decrease in the percentage of time spent in open arms as compared to restrained and non-restrained animals that were housed with their intact group. The authors discussed this in terms of an attenuation of the anxiogenic effect of restraint when animals were group-housed; however since the animals were tested within 24h of change of housing condition, it is possible that they tested the effect of disruption of group rather than the effect of pair versus group.

In pen-housed guinea-pigs, the social organisation was found to change when population size and density increased. In the initial mixed-sex groups of six animals, the social organization was mainly characterized by a linear male dominance hierarchy. In larger groups, mixed-sex subunits formed, where the alpha-male showed social bondings with all females of his subunit. There was no difference between animals in the different social systems in neither amount of aggressive behaviour nor endocrine status (Sachser et al., 1998).

There is not one single definition of crowding, and exceeding present housing recommendations is the only common denominator of crowding in experimental studies. However, what is defined as crowding by the human experimenter is not necessarily the same as crowding from the animal’s point of view. Given that free-living male mice will establish non-overlapping territories of several m² (Latham and Mason, 2004), even the presence of other males in separate cages within the same room may possibly be experienced as crowding. Many older studies of crowding have used enclosures more in the range of natural territory size but far larger than normal rodent cages (see discussion in van Loo et al., 2001), and there are fewer studies within the normal laboratory setting. Van Loo et al. (2001) systematically varied the housing

density for male mice in three different group sizes (3, 5 or 8 mice; 80 or 125 cm² / mouse). They found consistent effects of group size on agonistic behaviour. In larger cages, agonistic encounters were of longer duration and in the medium-size group they were also more frequent. In addition, mice in larger cages showed more wounds, and mice in the two larger groups had more wounds than mice in the smallest group. These results are consistent with the findings of Fullwood et al. (1998) of higher mortality due to biting wounds in larger cages. An increase in aggression with increasing cage size can possibly be explained by larger space allowance increasing territorial tendencies in male mice. Fullwood et al. (1998) studied the effect of space allowance by housing groups of three male mice in cages of varying size (32.2, 64.5, 96.8 or 129 cm² per mouse) and found no space allowance effect on body weight. However, mice in the smaller cages had higher plasma glucocorticoid levels and heavier adrenal weights, as well as greater lymphocyte proliferation and NK cytotoxicity. Housing mice in groups of 2, 4 or 8 mice in cages of the same size (195, 97.5, or 48.75 cm² per mouse), Peng et al. (1989) found a group size / housing density effect on glucocorticoid and lymphocyte concentration at days 1 and 7, but not day 14, after group formation in male mice, suggesting that the observed effects were primarily a result of instability at group formation.

6.2 Non-human primates

Studies have shown that primates show a big interest in and knowledge of the relationships of others (Van Lawick-Goodall, 1968; Seyfarth and Cheney, 2002). One measure of social complexity might be the number of possible different dyadic relationships within a group, calculated as $n(n-1)/2$. Not only is there in the larger group a greater number of potential friends or enemies, but there is also with increasing group size an exponential increase in the number of potential aggressive alliances, mates and friendships between other animals in the group, that each individual needs to monitor. Apparently, the need to be vigilant about group members' whereabouts influences the accessibility of subdominant individuals for human-animal interaction, such as training or accepting treats from the hand of the trainer. The levels of this interaction are usually lower, or more complicated, in the larger groups than in smaller groups (unpublished observation; KW).

One concern might be that the larger the group, the more injuries might be sustained. Elton (1979) found that crowding produced a sharp increase in aggression and individual pathology in baboons. On the other hand, Baker et al. (2000) found that management and risk of serious wounding in chimpanzee groups (sizes ranging from pairs to groups of 12 animals) was more influenced by the sex composition and rearing history of the individual animals, rather than group size per se. The provision of shelter or visual barriers reduced aggression among members of stable groups of pigtailed macaques (Erwin, 1977; Maninger et al., 1998) and rhesus macaques (Reinhardt and Reinhardt, 1991). Thus it seems that effects of crowding are a function of many factors such as group size, individual life histories and socialization, how dominant individuals exert their privileges, cage size and structural enhancement, and escape possibilities for subdominant individuals.

In callithrichids, if there is a crowded captive colony situation, where groups have visual access to one another, it is not infrequent with high levels of abortions and infant loss. This phenomenon is seemingly caused by the chronic arousal associated with the close proximity of neighbouring conspecific groups. Wild callithrichids are highly territorial. However, groups of different species of callithrichids often mix and travel together in the wild and can safely be housed with visual access to one another in captivity. (National Research Council, 1998).

7. Social support and effects of social conditions around testing

Sachser et al. (1998) describe two types of social systems: one system based on dominance hierarchies established and maintained by agonistic behaviours and another based on social bondings established and maintained through sociopositive behaviours. Social bondings are found between mothers and offspring in most mammalian species, and between adult individuals in some species. The two systems are not necessarily mutually exclusive, since a hierarchy (defined in terms of differences in access to resources) may also exist between bonding animals. In species with social bondings, the bonding partner can act as a stress-reducing social support in stressful situations.

7.1 Rodents

Guinea pigs form male-female bondings (Sachser et al. 1998), and the presence of the bonding partner may reduce stress reaction. For example, the immediate cortisol increase showed by male guinea pigs when removed from their colony and placed in an unfamiliar enclosure was significantly reduced if the male was accompanied by a female with which he had bonded (Sachser et al., 1998). In pre-weaning males, this support effect could be produced by the presence of any conspecific, including sibling infants and unfamiliar females, but in adult males only the presence of the bonding partner reduced endocrine stress reaction. The nature of the male-female relation is apparently crucial: a social support effect was clear when the female was the one within the colony with whom the male had most amicable interactions (Sachser et al., 1998), but very limited when the only male-female link was cohabitation during the previous 24 h (Machatske et al., 2004).

Social condition at testing may affect the outcome of behavioural tests. Fear behaviour in rats in a situation which had previously induced freezing was reduced in rats tested in pairs, in particular with a nonfearful pair member, compared to when tested alone (Davitz and Mason, 1955). Genaro and co-workers (1999, 2004) compared rats tested individually or in the home group in an apparatus for exploration, and found a tendency for group-housed animals to explore more than animals tested individually. Sherwin (2003) found that when tested in groups, mice showed less motivation for accessing a running wheel than when tested individually, whereas the social context did not influence mice' work for access to additional space. Michel and Tirelli (2002) found that whether mice were housed individually or in groups affected results in a 2-week protocol to study cocaine-induced contextual sensitisation and conditioned locomotion. The effect of testing condition may confound that of housing condition in a way that is not always considered. Hall (1998) reported that the difference in corticosterone response to an open-field test between isolation-housed and group-housed animals was due to the acute effect of testing group-housing animals alone (a novel social situation for them), as the difference disappeared when group-housed animals were tested in pairs. Observed effects that are in fact artefacts, resulting from a greater discrepancy between test and control situation for group-housed animals tested alone than for single-

housed animals tested alone, are probably not infrequent, and differences in contrast between different situations for the two groups may account for at least some of the results reported above. Nevertheless, when submitting rats that were housed either individually or in groups and tested either individually or in groups to noise, Taylor (1981) found social condition at testing to have a more potent effect on freezing than social housing condition.

7.2 *Non-human primates*

Not only are infant and juvenile primates sensitive to separations, but separating adult individuals from important social partners, such as a mating partner or closely bonded female groups, leads to psychopathological stress reactions that might interfere with research protocol. The presence of a compatible social partner may buffer stress reactions in fearful situations such as exposure to a snake, as demonstrated in a study on squirrel monkeys (Coe et al, 1982). Not only frightening stimuli but novelty per se might provoke this reaction. When placing monkeys in a novel environment, the stress reaction can be reduced by providing the animal with a preferred partner, as seen in common marmosets (Gerber et al., 2002) and rhesus monkeys (Gust et al., 1994). The same observation has been done in black tufted-ear marmosets (*Callithrix kuhli*) (Smith et al, 1998); however, the authors found that the animals reacted to separations from social partners only in the novel environment, not in the home cage. Rukstalis and French (2005) found that vocal buffering in the absence of physical, visual or olfactory contact was sufficient to moderate urinary cortisol excretion in isolated black tufted-ear marmosets.

By providing an individual in a test situation with a familiar social partner rather than testing under conditions of social isolation, arterial blood pressure is reduced in baboons (Visalberghi and Anderson, 1993). Indeed, the animal's performance in the test situation might actually be improved by the presence of a compatible partner (Washburn and Rumbaugh, 1991).

8. Different housing for males and females

8.1 Rodents

The different socio-ecological strategies between males and females are helpful to understand gender difference in reaction to social housing conditions in the laboratory. Brown and Grunberg (1995) housed male and female rats in same-sex groups of varying size and space allowance. They found that male rats had higher plasma corticosterone levels when housed in groups than when individually-housed and that corticosterone levels increased with increasing density. Females on the other hand had highest corticosterone when housed alone, and increasing social density did not increase corticosterone levels³. This is consistent with Westenbroek et al. (2003), finding isolation acting as a stressor in female rats, while social housing increased the negative effects of footshock stress in males. Dronjak et al (2004) however concluded that long-term isolation is a stronger stressor than long-term crowding in male rats. Gender differences have also been found in mice: Group-housed females showed an ‘anxiety’ reaction pattern in the free-exploration arena, whereas the impact of individual versus group housing was less pronounced in males but showed an opposite trend compared to that of females (Palanza, 2001). The results reported by Hurst et al. (1996) (see 5.1) suggest that rank affects the gender-specific reaction to social conditions, an aspect that was not taken into account in any of the studies reported above.

8.2 Non-human primates

Apart from the general xenophobic reaction and establishment of dominance, which both explain aggression in newly formed captive primate groups, one should also consider how the particular species socializes in nature as well as patterns of migration. There is great variability between species. For example in long-tailed macaques,

³ Thus the title of the paper by Brown and Grunberg is misleading: there is no evidence that ‘crowding’ calms females

females remain in the group where they were born, and form close-knit kin sub-groups. Females thus have the natural propensity to associate and create strong bonds with other females. Males, on the other hand, migrate around sexual maturation, and may for a time join a loose association of bachelors before joining another, unrelated group. In the new group, males usually form strong alliances with females, and avoid other males. Usually, the predominant social interaction with other males is aggression. (Lindburg, 1991; Poirier and Smith, 1974; Wheatley, 1999). Thus, not surprisingly, it has been very difficult to successfully directly introduce unfamiliar adult longtailed macaque males in captivity (Crockett et al., 1994). However, careful monitoring of compatibility, and clear-cut dominance interactions, in a period of non-contact familiarization before pairing has lead to success in pair-housing adult longtailed macaque males (Lynch, 1998) in the absence of females.

Discussion

Animals to be used in laboratory research should experience an acceptable welfare (for ethical reasons) and show normal behavioural and physiological reaction patterns (to guarantee the quality of research). For this purpose, it is crucial that the animals undergo appropriate social experiences during their early development, and that they are kept in appropriate groups throughout life. Even though there may be differences between species, sexes and ages as for what is the appropriate group, one thing is clear: it is a group. Non-human primates, rats, mice and guinea-pigs are all social animals. For these animals, individual housing, and in particular individual rearing of developing animals, has profound consequences for behaviour and stress physiology. As Harlow and coworkers demonstrated in the late 1950s, maternal and early social deprivation in primates causes profound alterations of behaviour, particularly deficiencies in social behaviour and difficulties in coping with stressful situations. As reviewed in this paper as well as by Hall (1998), a similar pattern, with altered social behaviour and stress coping capacity, emerges after early social deprivation in rodents.

Monkeys are particularly sensitive to inadequate social aspects of early rearing, which may result in severe and irreversible behavioural as well as physiological abnormalities (Mason, 1991). In addition, monkeys with a more normal social background still react

adversely to separations, including immunological effects that may persist for weeks (Capitanio, 1998). It could be argued that there is a need for proper management or control of these influences in order to minimize the risk of potential confounds in experimental designs. Given these potential scientific confounds, as well as the ethical perspective, forthcoming European legislations are taking the stand against single housing of primates. In the final version of the new CoE Appendix A species-specific provisions for non-human primates (CoE GT 123, 2004), single housing of primates will no longer be allowed unless for veterinary reasons or in order to ensure good science. Furthermore, it argues that monkey infants should be left in their natal group until they have become independent, ranging from 8-12 months depending on the species.

However, group-housing will only be beneficial for the animals if compatible groups of an appropriate composition can be formed, and these groups maintained stable. In addition, compatibility between individual group members is important for a functioning group. In wild populations of macaques and baboons, it would appear that the matrifocal kin-groups (consisting of related females) are more compatible (in terms of more prosocial behaviours such as grooming, and lower levels of physical aggression) than unrelated females within the group (Lindburg, 1991). Kin recognition mechanisms in primates are thought to rely on early familiarity; “be friends with whoever associates with mom” (de Waal, 1996). Thus, early familiarity during infancy through kinship seems to be highly important for future compatibility in primates. Also, in captive settings, a decided dominance hierarchy is crucial in order to avoid aggression related to dominance disputes, thus ensuring compatibility. The main reasons why primate group formation procedures in captivity are notoriously difficult and risky are the animals’ xenophobic reactions as well as the establishment of dominance hierarchies, which often involves escalation of aggressive signals culminating in physical aggression, i.e. the sequential assessment game (Maynard Smith, 1974). Most successful pairing strategies have taken these two factors (familiarity and a decided dominance hierarchy) into account (Reinhardt, 1990a+). Group formations are less straightforward and many different approaches have been advocated and tested with variable outcomes (Bernstein, 1991; Line et al., 1990; Westergaard et al., 1999). In order to avoid the potential risks of group formation,

another approach might be for young animals to be kept in their natal group, which may later be fissioned into smaller subgroups or pairs, consisting of familiar, related and compatible animals.

The question about stable and compatible groups is equally important in rodents. As several of the social stress studies show, changing group composition provokes a stress reaction, often accompanied by increased aggression. Even though little is known about what affects such compatibility, kinship seems to be one important factor at least in male mice. It is however often practically difficult to maintain sibling groups together. Commercial breeders will generally not supply information about kinship or social background of animals. Even when breeding takes place in-house, since litters vary in size, common practice is to standardize groups of rodents at weaning into group sizes which are convenient for the cage sizes used. Unless repeated, such regrouping is usually considered unproblematic in the case of nonaggressive animals, and it is also often believed that regrouping before sexual maturity is problem-free in more aggressive animals such as male mice. However, there are no studies of how regrouping affect the behaviour of weanlings and Bartolomucci's and coworkers' (2004) finding of indications of an unstable hierarchy in male mouse groups created at weaning suggests that regrouping may be more problematic than previously believed, at least in some strains. This observation is particularly pertinent given the increasing use of genetically modified mice, which may be more aggressive (Nelson and Young, 1998) and in addition have smaller litter sizes, thus increasing the 'need' for regrouping for economical reasons.

Among the commonly used laboratory rodents, male mice stand out as the most complicated category for which to form compatible groups. As male mice may naturally be despotic territory-holders (Latham and Mason, 2004), it has been argued that individual housing would not be stressful for male mice (Brain, 1975). However, as a territory holder, the male would naturally be accompanied by females and young offspring, and even subordinate males without a territory will live together (Latham and Mason, 2004), so living alone is not a normal situation for mice. Nevertheless, problems with intermale aggression are frequently reported and as they may have detrimental consequences are to be taken seriously. Based on literature as well as own research, van

Loo et al. (2003) give the following indications for housing male mice: avoid individual housing, optimize group size to three animals, use nesting material and transfer this scent-marked nesting material to the clean cage at changing.

With the possible exception for male mice, group size and density seem to be of secondary importance for the behaviour and welfare of rodents. Conflicting results and/or lack of a clear effect of housing density as long as this is kept within normal housing recommendations are by some researchers taken to conclude that laboratory rodents can be housed at high densities without any negative consequences, an argument that is obviously attractive from the viewpoint of financial and space economy. From the ethological point of view, the same finding begs the question of whether the standard size cages always imply crowding, as in these cages subordinates are possibly unable to keep a sufficient distance to dominant individuals. In primates, aggression can be reduced by placing visual barriers within a cage, thus allowing subdominant individuals some measure of privacy (Maninger, 1998, Reinhardt and Reinhardt, 1991). However, similar measures may increase aggression in territorial rodents such as male mice (see review in Olsson and Dahlborn, 2002). In real life, since laboratory cages come in standard sizes, experimenters (or animal care personnel) tend to choose between a smaller group in a smaller cage or a larger group in a larger cage. Other important considerations in this choice situation are how the total space available affects the possibility to engage in species-specific social and non-social behaviour, and how inter- and intra-cage variation may affect experimental outcome. When several animals from one cage are tested subsequently they will vary in arousal, as the first animal may be picked up half asleep whereas the following one or two will come from an already active and disturbed group, with consequent effects on test outcome (Izidio et al., 2004).

A general problem when attempting to review the literature on laboratory rat and mouse behaviour from an ethological viewpoint is the vast amount of literature reporting studies that were carried out with a different objective and which use a wide variety of methods. Conflicting results are commonly reported, and it is difficult to understand the background of the differences without carrying out a systematic review of each of the different aspects (such as individual housing, crowding or social stress), something

which goes beyond what is possible in a general paper like the present. Whenever possible, we have made use of the systematic reviews available, such as regarding individual housing of rats (Hall, 1998) or social stress (Martinez et al., 1998); however it became increasingly clear during the writing process that further reviews or even meta-analyses would be useful. An additional problem is that conclusions on stress are frequently based on glucocorticoid measures only: Changes in glucocorticoid levels are related both to positive and negative events (see Rushen and de Passillé, 1992 and Dawkins, 1998 for a discussion), and in the absence of correlated behaviour measures corticosteroid levels alone are difficult to interpret.

Conclusions

Being social animals, non-human primates, rats, mice and guinea-pigs are all profoundly affected by social conditions under which they are housed. Animals that are individually housed, especially during early development, show alterations in social behaviour and capacity to cope with stress. Inappropriate and/or unstable groups will also affect animals negatively. Social factors influencing the animals have consequences also for the research based on the animals, as behaviour, endocrinology and immune system may be affected. The effects of social conditions around testing have been given less attention, but several studies show that animals react differently in behaviour tests when tested in groups than when tested individually. In conclusion, factors such as group composition and stability, rank and previous social experience need to be taken into account both when designing housing systems for laboratory animals and when planning experiments and interpreting results.

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