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The effects of individual housing on 'anxious' behaviour in male and female gerbils

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Abstract

Gerbils are social animals and live in family groups in the wild, suggesting that individual housing may be a psychosocial stressor in this species. In the present study, gerbils were housed in same sex groups for 4 weeks, and then were either individually housed or remained with their cage mates for 1 week. Gerbils were tested in the black/white box (BWB), elevated plus maze (EPM) and social interaction test. Results indicated no significant differences in behaviour in the BWB. In contrast, on the EPM individually housed males showed increased anxiety compared to other groups, whilst there were no specific effects in females. In the social interaction test, however, individual housing in males increased social investigation, whilst females showed a decrease in exploration, accompanied by increased immobility. Passive immobility (freezing) was also increased in both sexes following individual housing. Thus, data from the EPM suggest that individual housing leads to increased anxiety mainly in males, whilst data from the SI suggests broadly the opposite. Therefore, individual housing results in different behavioural changes in male and female gerbils, which are dependent upon the test situation. This study highlights two key points. Firstly, it is important to use our knowledge of the species natural ecology in interpreting and designing anxiety tests. Secondly, it is important to assess behaviour in a range of situations when attempting to measure 'anxiety', particularly where tests developed for use in one species/sex are being used in another.

Keywords: Individual housing; Social isolation; Anxiety; Gerbil; Sex differences; Elevated plus maze; Black white box; Social interaction; Animal model; Behaviour

1. Introduction

Many animal models use social isolation in rodents before assessing their behaviour in standardised tests of anxiety such as the open field (OF), black white box (BWB), elevated plus maze (EPM) and social interaction test (SI). Social isolation been recognised as a means of increasing social interaction in rats for well over 40 years and was used by Grant (1963) in his classic study which described the social behaviour of the laboratory rodent [1]. Since then, this manipulation is used frequently to improve the likelihood of detecting anxiogenic or anxiolytic compounds. For example, rats are routinely isolated prior to being tested in the SI as this increases time spent interacting, thus making alterations to the level of this behaviour easier to observe [2].

Studies examining the effects of isolation in rodents can be divided into three groups on the basis of the age or developmental stage of the animal. That is: neonatal, postweaning and adult [3]. The effect of social isolation at each age has been shown to have distinct effects in terms of behaviour and neurochemistry. However, the response to social deprivation is not entirely consistent across all rodent species. Explanations for this have focused on the specific social environment and structure of each species. For example, permanent effects of postweaning isolation (such as hyperkinesis, hyperphagia and greater behavioural rigidity in complex motor tasks) are only observed in species that demonstrate high levels of social play during this stage of development (e.g. rats) but not in those who play much less (e.g. mice, gerbils and guinea pigs) [4]. Given the current study examines the effects of social isolation on adults the following literature review is focused accordingly.

In adult male rats, the effects of social isolation are wide ranging. Five to 14 days of social isolation is reported to alter a

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variety of behaviours. These include increases in: anxiety [5–10], activity [5,11], responsiveness to novelty & noise, [11,12], social investigation of an unfamiliar rat [13], aggression [3], drug sensitivity [5,14] and voluntary ethanol consumption [15,16]. The effects of social isolation on exploratory behaviour in the open field are contradictory with some reporting no alterations [17] and others reporting either increased or decreased exploration [11,18]. Social isolation is also reported to alter the metabolism and function of dopamine, serotonin, and noradrenalin although these changes are dependent upon the brain region and length of isolation [19–21].

In mice the effects of social isolation are similar to those observed in rats. That is they show increased anxiety, aggression [22] and social investigation [23]. These effects however appear to be dependent upon strain and length of isolation as DBA/2 mice isolated for 1–3 weeks failed to show alterations in anxiety like behaviour on the EPM [24]. However, studies have shown that although the behavioural effects of 7 days social isolation were not evident, the effects of clozapine differed in grouped and isolated mice [25].

The consequences of social isolation for female rodents are less well characterised. In some cases social isolation is reported to alter the behaviour of males only. For example, isolated adult male rats showed novel object conditioned place preference, but not group housed males or group or individually housed females [26]. Similarly, adult female rats showed less response to isolation as measured by EEG and CRF receptors after 6 weeks isolation than the notable alterations observed in males [27]. In mice, 3 weeks of isolation led to less weight gain and increased locomotor activity compared to group housed controls in males only [28]. In contrast, isolation may have opposite effects in males and females. For example, in rats, males found crowded housing more stressful than individual housing (as measured by corticosterone levels) whilst the opposite was apparent in females [29,30]. In keeping with this, in the free exploratory paradigm, individually housed female mice were more anxious and showed less exploration compared to group housed females whereas males broadly showed the opposite effects [31]. Similarly in the EPM, social isolation increased anxiety in females, however, in males, it had the opposite effect [28]. Finally, some researchers report that males and females respond to individual housing in a similar way, for example, isolation increased amphetamine self administration in male and female rats compared to those provided with environmental or social enrichment [32]. Thus, it seems that social isolation has the propensity to alter behaviour in both sexes but the specific effects depend upon species, strain, length of isolation and the test situation.

In contrast to the large number of studies on social isolation in rats and mice, few studies of this type have been conducted in gerbils. These small burrowing desert mammals originating from Asia, Africa and Southern Russia differ from the rat and mouse in that they form pairs which are usually monogamous in nature and that both parents play an active role in nest-building and caring for young, unlike most polygynous rodents [33,34]. As the gerbil is a social animal which lives in family groups in the wild, isolation would be expected to alter behaviour. However, results are somewhat contradictory. Isolation from day 22–50 (postweaning) resulted in no behavioural change in the open field activity when compared to group housed or partially isolated animals (1 h contact per day) [4]. In contrast, in adult gerbils, studies examining social behaviour following pair disruption have shown that separation from an opposite sex cage mate leads to alterations in social behaviour [35]. In vasectomised males, these effects differed depending on whether the gerbil was isolated from a same sex group/pair or an opposite sex pair. When isolated from a same sex group, vasectomised males showed increased exploratory & social behaviour, whilst separation from an opposite sex partner resulted in a decrease in social & aggressive behaviour. In females however, the effects of 7 days isolation were the same, irrespective of prior housing conditions, resulting in an increase in immobile in contact (freezing) when interacting with a stimulus male [36]. The studies examining pair disruption were designed to specifically examine social behaviour, rather than to use it as a means of assessing anxiety and thus, the experimental conditions employed were somewhat different from those used in the SI test originally described by File & Hyde in 1978 [37]. For example, the studies described above employed vasectomised males both as experimental and stimulus animals. Thus, the effects of individual housing on the behaviour of intact males in the SI test is unknown. Also, assessment of social behaviour occurred over a 10 min session, rather than the routinely used 5 min assessment period. Habituation to novel test environments occurs quite rapidly and therefore, behaviour during the first 5 min may differ significantly from that observed during a 10 min test.

In addition to these studies examining pair-bonds in gerbils, the popularity of this species is increasing within the anxiety field, with several studies using gerbils in the EPM & SI being published during the last few years [38–41]. Furthermore, a recent study described sex differences in tests of anxiety in gerbils, suggesting that it is important to use a range of measures [42]. Given the increase in use of this species, particularly within anxiety research, a greater knowledge of the behavioural effects of alterations in housing conditions is important. Thus, the aims of this study were to examine the effects of individual housing on the behaviour of male and female gerbils in 3 tests of anxiety, the EPM, the BWB and the SI test.

2. Methods

These studies were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and were approved by the University of Central Lancashire, Faculty of Science Ethics Committee.

2.1. Animals and housing

Gerbils (*Meriones unguiculatus*) were obtained from the Faculty of Science, University of Central Lancashire breeding colony. All animals used in these studies were housed in the same room and standard sized cages throughout the study (38 × 25 × 20 cm) under a 12 h light cycle (lights on: 07:30), in