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Effect of Housing on Stress-Related Parameters in NHPs

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Abstract

In preclinical studies, it is important to reduce animal stress, both for animal welfare reasons and to minimize the impact on toxicologic parameters. Effects of stress on in-life parameters (body weight, food consumption), clinical pathology (white blood cell and differential counts), organ weight (endocrine, immune, reproductive) and histopathology (lymphoid, GI tract) are well documented. Myostatin (MSTN), a member of the TGF-β superfamily, is a negative regulator of muscle deposition and has been shown to increase in response to psychological stress in mice; increased MSTN correlated directly with decreased muscle mass. Plasma levels of MSTN were measured in 15 young adult male cynomolgus monkeys during a two-week period of housing in standard four packs (pairs and trios) and after transfer into gang housing enclosures (three animals per cage) that meet or exceed EU standards. Average MSTN levels in standard housing ranged from approximately 3,900 to 7,100 pg/mL, and in gang housing from 1,700 to 5,000 pg/mL. On an individual animal basis, average MSTN levels decreased by 10-32%, suggesting potential stress reduction in the gang housing setting. The significance of this change in MSTN levels in monkeys is unknown. Potential correlation with other stress-related parameters, including body weight, clinical pathology, and behavioral evaluations, was assessed. The specific benefits of gang vs. standard housing were examined to better understand the manifestations of stress in a laboratory environment that are relevant to toxicology study outcomes.

Introduction

EU Directive 2010/63/EU replaced the original directive of 1986 pertaining to the protection of animals used for scientific purposes. Annex III of the Directive addresses housing of laboratory animals and specifies minimum standards in terms of space required per animal. Several CROs in the U.S. have included EU-compliant housing as options for EU-based pharma companies and others who want an optimal environment for study animals. Based on anecdotal evidence, it is generally understood that animals benefit from the increased vertical space and other attributes of the larger enclosures; however, reports of actual data quantifying those benefits by comparing data from standard group housing appear to be nonexistent.

The role of MSTN in muscle growth regulation is well known; upregulation results in protein breakdown and muscle atrophy, inhibition promotes protein synthesis and muscle growth. Interestingly, MSTN levels have been reported to increase as a result of psychological stress in mice (Allen et al., *Am J Physiol Regul Integr Comp Physiol* 299; 2010). In both restraint stress and cage-switching stress models, MSTN mRNA levels significantly increased as early as one day after either stress condition. Muscle atrophy could be measured one day after restraint stress and seven days after cage-switching stress and was shown to be MSTN-dependent as it did not occur in MSTN knock-out mice. MSTN expression has also been shown to increase in salmonids exposed to crowding stress (Galt et al., *Aquaculture* 483; 2017).

The work described here examined the MSTN levels of 15 young adult male cynomolgus monkeys during acclimation prior to a study. Animals were socially housed in standard four-pack caging for the first two weeks and in EU housing for an additional two weeks. Blood samples were taken weekly. Results were averaged per animal for each housing situation. Additionally, behavioral data from a pool of 140 different cynomolgus monkeys that had been in standard housing prior to EU housing were examined, and the incidence of alopecia and fecal smearing compared.

Figure 1: Standard four-pack NHP housing

Figure 2: European-style housing

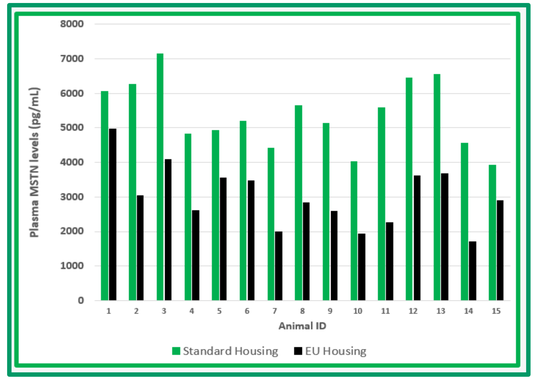

Figure 3: Effect of EU housing on individual animal levels of MSTN.
Average (one and two weeks) plasma levels of MSTN (pg/mL) in individual animals in standard group housing (green bars) compared to EU housing (black bars). MSTN levels decreased in each animal after relocation to EU housing. MSTN measured using GDF-8/Myostatin Quantikine ELISA Immunoassay kit from R&D Systems (DGDF80) according to manufacturer's instructions.

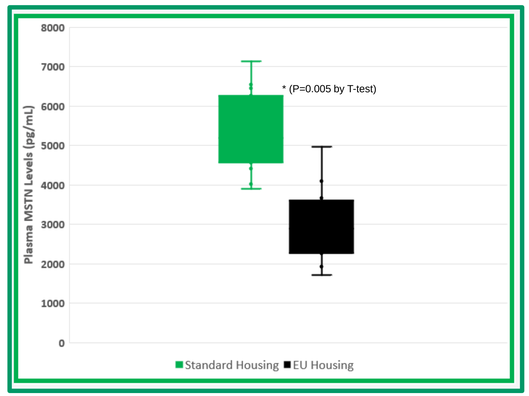

Figure 4: Effect of EU housing on group MSTN levels. Box and whisker plot of average MSTN levels of all animals. EU housing resulted in significantly lower levels of MSTN (3016 pg/mL) compared to standard housing (5378 pg/mL) at p=0.005 by T-test.

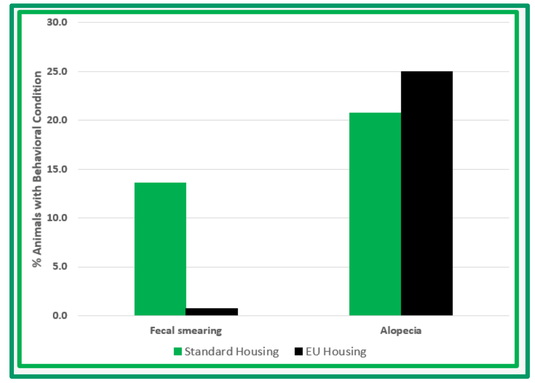

Figure 5: Effect of EU housing on incidence of fecal smearing ("painting" of cage walls with feces) and alopecia (absence of hair). Observations of 140 cynomolgus monkeys showed 14% fecal smearing and 21% alopecia while in standard caging. Following relocation to EU housing, fecal smearing decreased to <1% and alopecia increased slightly to 25%.

Results

Figure 3 shows that MSTN decreased consistently in all animals after relocation to EU housing. On an individual animal basis, average MSTN levels decreased by 10-32%, suggesting potential stress reduction in the gang housing setting. Considering that these animals changed social partners (cage mates) when they moved, it is even more surprising that MSTN levels decreased so quickly, given the expected stress associated with establishing new social hierarchies. Average MSTN levels in standard housing ranged from approximately 3,900 to 7,100 pg/mL, and in gang housing from 1,700 to 5,000 pg/mL. The group mean data in Figure 4 show that the decrease is statistically significant (P=0.005 by T-test).

These data show that the MTSN decrease following relocation is significant within one week and is sustained for at least an additional week. The down regulation may be related to a decrease in glucocorticoids, as these are known to regulate MSTN (Wang et al, *PLoS One* 11, e0156225, 2016). The significance of this change in MSTN levels in monkeys is unknown. Ongoing research of MSTN is primarily focused on its role in regulating muscle mass, but a complete picture of MSTN functions is not yet clear and may be species-dependent. Latres et al (Nature Comm DOI:10.1030/ncomms15153) report that activin A has a more dominant role in regulating muscle mass in primates (including man) than MSTN, which has the primary role in mice.

Figure 5 summarizes the effect of EU housing of two behavioral attributes that are commonly associated with stress. In a population of 140 cynomolgus monkeys that spent time in both types of housing, fecal smearing was observed in 14% of animals while in the standard housing and almost disappeared completely when relocated to EU housing. The results with alopecia were not expected. Even though alopecia resolved in many animals after transfer to EU housing, several animals *acquired* it after the transfer. The correlation of alopecia and stress is a controversial topic among primate behaviorists (Lutz et al, J Med Primatol DOI 10:1111/jmp122220, 2016). Alopecia can result from a variety of physiological conditions and is complicated to isolate in a social structure like group housing.

Conclusions

This is the first report of the effect of housing on MSTN levels in cynomolgus monkeys and presents many interesting directions to pursue. Future investigations may include analysis at the mRNA level, as well as a time course to determine how quickly the MSTN changes occur. Correlating MSTN with cortisol levels would also be useful to determine if there is an association.

In conclusion, these data suggest that relocation of cynomolgus monkeys from standard social housing to European style housing affects parameters associated with stress, including MSTN and fecal smearing.