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Show Me the Meaning of Being Lonely (and Its Effects on Seizure Burden and Comorbidities)

Effects of Single Cage Housing on Stress, Cognitive, and Seizure Parameters in the Rat and Mouse Pilocarpine Models of Epilepsy

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Many experimental approaches require housing rodents in individual cages, including in epilepsy research. However, rats and mice are social animals; and individual housing constitutes a stressful situation. The goal of the present study was to determine the effects of individual housing as compared to conditions maintaining social contact on stress markers and epilepsy. Control male mice socially housed during pretest and then transferred to individual cages for 6 weeks displayed anhedonia, increased anxiety, and biological markers of stress as compared to pretest values or mice kept socially housed during 6 weeks. Pilocarpine (pilo)-treated mice housed together showed increased levels of anhedonia, anxiety, and stress markers as well as decreased cognitive performance as compared to the control group. The differences were more significant in pilo-treated mice housed individually. Anxiety correlated linearly with cognitive performance and stress markers independently of the experimental conditions. In the male rat pilo model, seizures were 16 times more frequent in singly housed animals as compared to animals kept in pairs. Daily interactions with an experimenter in otherwise singly housed animals was sufficient to produce results identical to those found in animals kept in pairs. We propose that social isolation produces a severe phenotype in terms of stress and seizure frequency as compared to animals maintaining social contact (at least in these 2 models), a factor that needs to be taken into account for data interpretation, in particular for preclinical studies.

Commentary

Quantification of seizure activity and severity in experimental rodent models of epilepsy is commonly obtained through electroencephalogram (EEG).[1] While this technology yields critical information on the brain's activity, certain practicalities of the instrumentation and animal housing conditions may produce confounding variables. For example, if implantable radiotelemetry devices are not available, a wired recording system can be used in which the lead wire extends from the animal's headcap and connects to the amplifier, comprising a fragile system that warrants protection. To prevent cage mates from gnawing at the tethers, experimental animals are often housed individually. This protective measure, however, induces social isolation, which itself is known to alter activation of the hypothalamic-pituitary-adrenal (HPA) axis and consequently levels of the stress hormone, corticosterone.[2] Furthermore, epilepsy can drive hyperactivation of the HPA axis,[3] and reciprocally, stress is a major precipitating factor for increased seizure incidence and severity.[4] Considering that most EEG-monitored epileptic rodents are singly housed, it may be the case that some phenotypes of seizure parameters and associated comorbidities are influenced by isolation experienced by the animals. Therefore, the authors of the present study set out to determine the extent to which phenotypic measures of epilepsy and associated stress are enhanced by social isolation.

Both male mice and rats were tested using the pilocarpine post-status epilepticus model of epilepsy. The experiments tested epileptic (pilocarpine-injected) versus control (saline-injected) animals as well as housing conditions: social versus isolated for both mice and rats, and an additional handling condition for rats. In this case, handling involved an experimenter stroking, holding, and feeding the rat without gloves every day. Whereas mice were socially housed in cages of 3, the social cohort of rats was housed in pairs in which one rat was implanted with a wireless EEG radiotelemetry device. Stress for both species was assessed through measurements of trunk blood levels of the biological stress markers, adrenocorticotropic hormone (ACTH), and corticosterone, as well as brain-derived neurotrophic factor (BDNF), a transcription factor that has been shown to decrease with stress and has been associated with depression.[5]

Stress increases anhedonia, which can be reflected in a decreased preference for sucrose water in rodents.[6] Isolated epileptic mice showed the greatest decrease in preference for the sweetened water, followed by the socially housed epileptic mice. Isolated control mice also showed decreased preference but not as strongly as their epileptic counterparts, suggesting that while isolation alone increases anhedonia, epilepsy produces a more severe phenotype and the added condition of isolation further exacerbates the comorbidity.

High levels of stress also impair performance on memory tasks and increase anxiety.[7] Furthermore, memory impairments and increased anxiety are common comorbidities of epilepsy.[8,9] Therefore, the researchers tested the mice on a novel object recognition task. The results indicated that epilepsy impairs memory performance regardless of housing condition, and that social isolation further decreases performance. Similarly, in an Elevated Plus Maze task, epileptic mice displayed greater anxiety levels, which were further enhanced by social isolation. Isolated housing alone also increased anxiety in control mice, confirming other studies.[10] For all groups of mice, levels of blood corticosterone and ACTH increased with anxiety while levels of BDNF and memory performance decreased. Epilepsy was found to increase levels of ACTH and corticosterone in mice, but did not alter levels of BDNF. Not surprisingly, these phenotypic relationships were most severe in the isolated epileptic mice, followed by the socially housed epileptic mice. In summary, epilepsy alone induced the greatest increase in all stress markers, with the exception of BDNF levels, and social isolation further enhanced these phenotypes.

In addition to an increased stress phenotype, as reflected in elevated corticosterone and ACTH and decreased BDNF levels, isolated epileptic rats showed a significant increase in seizure frequency, duration, and severity compared with both paired and handled rats. Seizure severity parameters were similar between the paired and handled rats, suggesting the interesting prospect that daily handling of rats used in EEG experiments could attenuate the enhanced seizure burden observed in isolated rats, and thereby mitigate the confounding effects of social isolation. An important consideration, however, regarding the increased seizure phenotype in the isolated epileptic rats is the interactive and reciprocal relationship between neuronal hyperactivity and elevated levels of stress hormones. The increased seizure phenotype in the isolated condition may in turn further activate the HPA axis, leading to excess release of stress hormones, contributing to a positive stress-seizure feedback loop. This relationship might confound the observation of an increased stress phenotype, preventing definitive conclusion that it is due to social isolation alone.

This study reveals that some of the observed phenotypes in rat and mouse models of pilocarpine-induced epilepsy are perhaps due to, or at least exacerbated by, social isolation. Acknowledging the interactions between the disease and housing conditions is thus an important consideration for experimental design in animal studies. Likewise, the results from this study can perhaps lend translatable insight into management of epilepsy in patients, taking into consideration lifestyle factors of the individual such as frequency of social interactions. Additional research is needed, however, before these measures can be factored into determining the optimal type of treatment for a given individual. For example, determining whether there is a sex difference by testing female animals is critical as several lines of evidence indicate the presence of a sex difference in rodent stress response mechanisms.[11] For example, corticosterone is increased in isolated female mice yet decreased in isolated males.[12] Additionally, stages of the reproductive estrous cycle have been shown to differentially impact regulation of the hypothalamic-pituitary-gonadal (HPG) axis in female epileptic mice,[13] and there are strong interactions between the HPG and HPA axes.[14] It would therefore be of interest to determine whether the reproductive cycle and social isolation interact to affect stress, cognitive, and seizure phenotypes in female animal models of epilepsy.

In the present study, the handling condition was only tested in rats. Therefore, it remains unclear whether a similar paradigm could also help mitigate epilepsy and comorbidity severity in mice. Because at least some strains of mice are prone to handling-induced seizures,[15] it is possible that mice may not benefit as much as rats from increased encounters with human experimenters. In the case of group-housed male mice, aggression from a dominant mouse in the cage can increase measures of stress[16]; therefore, social contact may mitigate stress in male mice but only when excessive aggression between cagemates is not observed. Altogether, determining which groups benefit from the stress-mitigating effects of one housing condition over another will provide valuable insight into the contributions of species, sex, strain, and disease model to observed phenotypes in rodent models of epilepsy, with better likelihood of extension from bench to bedside.

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Supplemental Material

Supplemental material for this article is available online.

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