Consequences of housing conditions and interindividual diversity in rodent models of acquired epilepsy

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1 | INTRODUCTION

In an elegant study, Manouze et al1 report that single housing of pilocarpine-treated male outbred rats and mice resulted in social deprivation and a more severe epilepsy phenotype, with increased seizure frequency and increased severity of comorbid behavioral alterations compared to group-housed animals or animals that had daily social contacts with an experimenter. The authors conclude that social isolation is an important factor that needs to be considered in data interpretation in preclinical epilepsy research. In a commentary2 by the senior author, Christophe Bernard, in this issue of Epilepsia, Dr Bernard extends the discussion of the data presented by Manouze et al 1 by suggesting that single housing may complicate data interpretation of preclinical studies on epilepsy models. Furthermore, he points to an additional factor, interindividual diversity, which is often disregarded in preclinical studies using mice or rats. I absolutely agree with those suggestions, but would like to extend the discussion, which is quite important for translational scientists, with several thoughts.

2 | SOCIAL DEPRIVATION IS A WELL-KNOWN RISK FACTOR OF EPILEPSY AND ASSOCIATED COMORBIDITIES IN HUMANS

Several large epidemiological studies have shown a strong positive correlation between the prevalence of epilepsy and social or economic deprivation.3–5 Furthermore, mental health comorbidities of epilepsy are associated with socioeconomic deprivation.6 Thus, the interesting and important findings of Manouze et al1 in the pilocarpine model of temporal lobe epilepsy (TLE) are clinically relevant. Furthermore, in my own experience, these findings have a practical preclinical consequence because, particularly for complex and laborious pharmacological studies, the use of single-housed rodents with high frequency of spontaneous recurrent seizures (SRSs) and prominent behavioral and cognitive alterations reduces the group size that is needed to obtain significant treatment effects, which, of course, is an animal welfare issue, which is discussed in the following.

3 | GROUP HOUSING, PARTICULARLY IN MALE RODENTS, MAY INCREASE DATA VARIABILITY AND LEAD TO ANIMAL WELFARE ISSUES

One aspect that is missing in the study of Manouze et al1 and the commentary of Bernard2 is the impact of sex of the animals. As in most experimental epilepsy studies, Manouze et al1 used male rats and mice. Group-housed males, but not group-housed females, will establish a dominance hierarchy.7 Circulating testosterone levels in dominant males are, on average, five times as high as in subordinates, and so data variability in group-housed males may increase due to their dominance status and corresponding hormone levels.7 Furthermore, because of animal welfare, the issue of group or single housing for rodents used in epilepsy studies is more complex than discussed by Manouze et al.1 Although group housing should be considered best practice, the removal
of individual animals for surgery or behavioral testing can result in the disruption of an established social hierarchy. Moreover, the appearance of disturbed behavior (aggressiveness or passivity) in experimental animals (e.g., animals displaying overt seizures, or that are instrumented) may provoke hostile responses from cage-mates. The tendency for epileptic rats to be submissive may affect the social hierarchy in group housing, which could create confounds for some kinds of experiments. In any case, group housing needs careful monitoring to detect adverse effects that may be detrimental to the welfare of the epileptic animals or to the reliability of the experimental results. When individual housing is inevitable due to excessive aggressive behavior, the presence of enrichment objects such as nesting material can mitigate some of the negative consequences of social isolation.

The problems associated with dominance hierarchy in group-housed male rats or mice can be avoided by using female animals. It has long been argued that the estrous cycle in female rodents leads to a source of variability, so most neuroscientists choose to avoid the issue altogether and exclude female animals from their research. However, a meta-analysis of nearly 300 published neuroscience articles that used mice as research subjects in an array of physiological, cellular, hormonal, and behavioral measures revealed that data collected from female mice—regardless of the estrous cycle—did not vary more than that from males, and in some instances data from males varied more than female data. More recently, the US National Institutes of Health has developed policies that require applicants to report their plans for the balance of male and female animals in preclinical studies, unless sex-specific inclusion is unwarranted, based on rigorously defined exceptions.

### 4 ALTERNATIVES TO GROUP HOUSING FOR REDUCING SOCIAL DEPRIVATION AND THE IMPACT OF ENVIRONMENTAL FACTORS DURING REARING OF RODENTS

As outlined by Manouze et al. and Bernard, group housing is not the only way to prevent social deprivation and the associated stress. Habituation to the experimenter before the onset of experiments, daily interactions (e.g., friendly handling) with the experimenters during the experiments, visual and olfactory contact with other animals in the laboratory and stable, and an enriched environment in the cage can allow maintaining single-housed animals with minimized social deprivation.

However, an important factor not mentioned in the study of Manouze et al. is the animal maintenance by the vendor from which the rodents are obtained. In a large study on strain and substrain differences in a TLE model produced by electrical induction of status epilepticus (SE), we found that Sprague-Dawley (SD) rats from Charles River markedly differed from SD rats from three other vendors in pre-SE (premorbid) behavior, SE induction, and SE-induced epilepsy, behavioral alterations, and neurodegeneration. In a subsequent study, these differences in rats from different vendors were substantiated for the pilocarpine model of TLE. Interestingly, the high premorbid anxiety-like behavior of SD rats from Charles River was also described for Wistar rats from this breeder compared to this strain from other vendors. We suggested that, in addition to genetic differences in SD and Wistar rats from different vendors, environmental conditions during rearing of the rats at Charles River may affect the subsequent behavior of rats from this vendor; we therefore compared the housing and handling conditions at the vendors used in our studies. At the time of this comparison, all vendors used group housing of the animals after weaning, but Charles River did not use environmental cage enrichment and socialization to humans (handling to habituate rats to humans), which may have caused the high anxiety-related behavior of the rats after arrival in our laboratory. Even after thorough handling habituation in our laboratory, the SD and Wistar rats from Charles River still exhibited higher premorbid anxiety in standard tests than such rats from other vendors. Furthermore, male Wistar rats from Charles River exhibited enhanced stress vulnerability in a model of chronic mild stress compared to Wistar rats from other vendors, and this enhanced stress vulnerability was resistant to chronic treatment with antidepressant drugs. On the other hand, SD rats from Janvier, which was the only vendor that used group housing, environmental cage enrichment, and intense socialization to humans, exhibited a low premorbid anxiety level and low stress response but also low response to SE induction. These data indicate that handling habituation and environmental enrichment in rats during rearing at the vendor, and possibly genetic and epigenetic factors, may outreach the influence of group housing on models of acquired epilepsy.

### 5 INTERINDIVIDUAL DIVERSITY IS NOT A NEW PHENOMENON IN EPILEPSY MODELS

When Manouze et al. tested the animals for anxiety-like behavior before experimental manipulation, there was notable interindividual diversity. Furthermore, animals could react very differently to social isolation. The authors concluded that this heterogeneity can be used to generate diverse groups of animals after an insult, for example, animals susceptible or resilient to depression, thus mimicking human conditions. Furthermore, Bernard concluded that averaging results from
animals exhibiting interindividual diversity may result in missing important information, for example, the identification of responders and nonresponders.

Heterogeneity in anxiety or cognitive performance at baseline in rodents and its potential consequence for subsequent induction of epilepsy, associated comorbidities, and drug response are not a new observation but have been known for decades. For instance, Adamec et al. reported interindividual differences in the premorbid affective state of male Wistar rats that contributed to the behavioral effects of amygdala kindling. Their experiments suggested that premorbid anxiety state interacts with amygdala kindling to determine behavioral outcome, and that the outcome may be either anxiogenic, anxiolytic, or no effect at all depending on the level of anxiety at the time of kindling.

Over the past some 30 years, we found that interindividual diversity affects the drug response in different rodent models of TLE, including amygdala kindling and epilepsy developing after electrical or chemical SE induction. By observing responders and nonresponders to phenytoin's antiseizure effect in large groups of amygdala-kindled rats in the early 1990s, we discovered the first animal model of drug-resistant epilepsy, which is now widely used to identify mechanisms of drug resistance in TLE. In subsequent studies in SE-induced TLE models with SRSs, we found large interindividual variability in the consequences of SE that affected the pharmacological response of the SRSs; nonresponse to antiseizure drugs was observed in rats with high SRS frequency, hippocampal damage, behavioral and cognitive alterations, subunit differences in the γ-aminobutyric acid type A receptor, and high expression of the drug efflux transporter P-glycoprotein at the blood-brain barrier. We also demonstrated that many factors can account for such interindividual diversity in response to SE induction and antiseizure drugs, including genetic and epigenetic factors. Similar findings were reported in patients with drug-resistant TLE; therefore, the interindividual diversity in epileptic rodents resulted in clinically relevant findings. Such interindividual diversity also explains why not all animals treated with a potentially antiepileptogenic compound during the latent period after SE respond to this treatment. In the absence of predictive biomarkers, this directly affects the statistical power of studies on antiepileptogenesis, thus necessitating large group size for identifying positive treatment effects.

In conclusion, Christophe Bernard and colleagues have highlighted important features of preclinical experiments that are often underrated or even ignored in experimental epilepsy research. This may lead to false conclusions and add to the “reproducibility crisis” in the biomedical sciences. Both social isolation and interindividual diversity and their effects on seizure susceptibility and drug response are common in rodent epilepsy models (and patients) and, if recognized and dealt with, can enhance the translational power of preclinical studies.

CONFLICT OF INTEREST

The author has no conflict of interest to disclose. I confirm that I have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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