Beyond the Illusion of Controlled Environments: How to embrace Ecological Pertinence in Research?

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The controlled environment is a chimera; it is time to rethink our models. Here, I have chosen the prism of preclinical research on substance use disorders (SUD) to present, in a non-exhaustive manner, advances enabled by the use of rodent models, the crises faced by animal experimentation, the reflections and responses provided by laboratories, to finally propose rethinking our models around questions of ecological relevance, in order to improve both ethics and scientific quality. Although my discussion is illustrated by the situation in preclinical research on SUD, the observation drawn from it and the proposals made can extend to many other domains and species.

Unlocking the neurobiology of substance use disorders: insights from rodent models.

In 2018, approximately 269 million individuals worldwide received diagnoses of Substance Use Disorders (SUD) (1). Defined by the Diagnostic and Statistical Manual of Mental Disorders V (DSM-5) as persistent drug use despite significant harm and adverse consequences (2), SUD extends its impact beyond the individual, affecting families, friends, and society at large. Consequently, there is an urgent imperative for a deeper understanding of the pathological mechanisms underpinning SUD (3), to facilitate the development of novel treatments.

The DSM criteria for SUD have facilitated the modeling of behavioral traits in laboratory rodents through operant tasks. These tasks simulate various aspects of SUD progression, including increased motivation, loss of control over drug consumption, and susceptibility to relapse. Rats exposed to extended access drug self-administration procedures mimic loss of control over drug intake (4–7), while punishment-associated paradigms replicate compulsive drug seeking despite adverse consequences (8,9). Around 20% of rats persist in drug-seeking behavior despite punishment, mirroring proportions observed of humans SUD cases in drug users. Rodent models also aid in studying vulnerability to relapse (10–12) and changes in affective state associated with SUD progression, through recordings of ultrasonic vocalizations

(13–18). These models have uncovered crucial psychological and neurobiological mechanisms underlying SUD, such as alterations in mesolimbic dopamine neurotransmission and reward circuitry, which contribute to intense drug craving and heightened susceptibility to relapse (19,20,22,23). They also revealed the reduction in dopamine receptors during withdrawal, exacerbating the negative effects of drug use and elevating brain reward thresholds (24–26). These findings not only enhance our understanding of SUD but also offer insights into potential therapeutic interventions.

Recent preclinical research has delved into the modulatory impact of the environment on the development, maintenance, cessation, and relapse of SUD, alongside its associated neurobiology (27,28). Studies have demonstrated that while the lack of alternative options constitutes a significant determinant in human vulnerability to SUD development (29), most rats turn away from drug self-administration when presented with alternative rewards (30–35). Moreover, the congruence between the drug used and environmental setting is pivotal: heroin tends to be consumed at home in humans, while cocaine is favored outside the home (36,37). Corresponding findings in rats indicate psychostimulants are preferred outside the home-cage, whereas opiates are consumed within it (36,38,39). Social context also significantly modulates drug use both in humans and rodents (28,40,41). Positive social environments, such as parenting in humans or nurturing maternal care in rats, act preventatively against the risk to develop SUD (42,43). Conversely, adverse social interactions, like child abuse in humans or repeated social defeat in rats, or a lack of positive social connections, such as loneliness in humans or rearing in isolation/maternal separation in rats, heighten this risk (44–48). Furthermore, the immediate social context of drug use impacts intake (49), contingent upon the specific substance used (50–52), the relationship dynamics between the subject and the observer peer (53) and the peer's behavior, such as whether they are self-administering or not (49,50,53–55). Although the neurobiological mechanisms underlying this social influence remain poorly understood, it is plausible that the rewarding value of social interaction can potentiate or compete with that of drugs, mediated through interactions within the mesolimbic dopaminergic and oxytocinergic systems (56–59). In sum, these findings underscore the profound influence of environmental context on drug use and its neural underpinnings.

Facing the crisis: challenges in preclinical research on substance use disorders.

Despite the significant advances facilitated by the use of animal models, the translation of these findings into effective human treatments has been limited (60,61). This crisis of validation has perpetuated the notion that animal models, particularly in psychiatric disorders like SUD, exhibit poor validity, prompting numerous pharmaceutical companies to discontinue drug development programs (62). Moreover, mounting concerns pervade animal experimentation research. Outside laboratory confines, public trust in science is eroding (63), while debates over the ethical use of laboratory animals continue to unleash passions (64–66). Internally, within laboratories, it is reported that over 50% of preclinical findings lack reproducibility (67,68). Collectively, these crises pose a fundamental question: What is amiss in animal experiments?

Research laboratories have responded to the lack of reproducibility by the current global gold standard of rigorous standardization (69). Intended to minimize variability within and between experiments, these excessively standardized methods ultimately exacerbate the reproducibility crisis rather than alleviate it. (67,70,71). Furthermore, it raises questions about the translational predictive validity of such preclinical research: 'We would never perform a human drug trial in 42-year-old white males with identical educational levels, identical socioeconomic statuses, identical jobs, identical houses with identical (locked) thermostats, identical wives, identical diets, identical exercise regimes, in the same small town in Wisconsin, who all incidentally had the same grandfather' (72)? In preclinical studies, a homogeneous population (with identical genetic, age, weight, and gender characteristics) inhabiting controlled environments undergoes daily testing at consistent times, utilizing widely employed behavioral tasks. Given this setup, one must question the *real-world* relevance of our findings. Moreover, in attempts to minimize environmental variability, these gold standard methods overlook a crucial fallacy: there exists no truly controlled (i.e., neutral) environment for behavior. Consider, for instance, studies on SUD development conducted on rodents separated prematurely from their mothers—a common practice among rodent breeders. Investigations into an alternative social reward may yield skewed results when subjects are isolated in their home-cages, as is often the case. Studies examining abstinence and relapse may be compromised when drug access is restrained by experimenters. Do we not have enough confidence in our own findings regarding the influence of the environment on behavior and its neurobiology to utilize them for enhancing our own models?

Exploring the neuroethological approach: advantages and limitations for neuroscience.

In recent years, within the fields of ethology and animal welfare, one may be asked: 'How STRANGE are your study animals?' Here, STRANGE stands for 'Social background; Trappability and self-selection; Rearing history; Acclimation and habituation; Natural changes in responsiveness; Genetic make-up; and Experience' (73). This questioning echoes the self-critique of human-evolutionary biologists, a decade earlier, who interrogated whether their samples constituted 'the weirdest people in the world' (WEIRD standing for: Western, Educated, Industrialized, Rich, and Democratic) (74). Both WEIRD and STRANGE highlighted that most behavioral research is conducted using (human and non-human) subjects that are not representative of a general population and can even be outliers in broader comparisons. Nonetheless, it is crucial to note conceptual distinctions between WEIRD and STRANGE. While the former pertains to characteristics of a specific demographic group, the latter encompasses a range of factors influencing behavior within laboratory settings as opposed to natural environments (73). As awareness grows regarding the disparity between laboratory subjects and wild animal populations or human societies, ethologists advocate for greater diversity among study subjects and more naturalistic experimental settings (75,76).

In neuroscience as well, there is a growing chorus of dissent against overly artificial and simplified laboratory tasks (77–79). As an alternative to this reductive behaviorist approach, the neuroethological approach, combining ethological research with methods of neural imaging and modulation, advocates for a deeper appreciation of the environment, especially the social context. This approach takes advantage of recent technical advances - like open-source machine-learning methods for automatic tracking of individual body parts and poses (80–82) and automatic detection of specific behaviors (83–86), and wireless technics of neuromodulation (87,88) and recording of neural activity (89) controlled by radio frequency devices - to propose studying the neurobiology of spontaneous behaviors of animals living in setup that reproduce the ecological characteristics of wild animals. Overall, the wider implementation of such approach in neurosciences laboratory would provide a breakthrough in discovering neurobiological mechanisms underlying naturalistic behavior, while improving the ethics of animal experimentation.

However, it is imperative to meticulously scrutinize the limitations of this approach. A pertinent query arises: Can human SUD, among other psychiatric disorders, be sufficiently modelized based on observations of rodents in naturalistic environments? If artificial settings indeed

engender artificial behaviors, does substance abuse manifest as a natural behavior observable in rodents beyond the confines of the laboratory? Likewise, when employing rats to emulate the impacts of social reward as a substitute for drug use in humans, it's noteworthy that unlike their counterparts in controlled laboratory settings (90), rats inhabiting semi-natural environments often exhibit pronounced aggression towards their congeners (91–93). Consequently, laboratory rats may present a more fitting model for investigating human social reward and interactions. Indeed, exploring animal behavior within semi-natural habitats embodies a novel comparative approach to human neuroscience but does not allow for the proper modeling of all human behavioral features.

Let's diverge further and contemplate the notion that human behaviors, such as drug abuse, may not spontaneously occur in 'nature-like settings' either. After all, few of us rise at dawn to engage in gathering, hunting, or territorial defense. Instead, we awaken to alarms, commute to work, and partake in grocery shopping. Overall, we all live within societies that are *human social constructions*. Yet though to a lesser extent than laboratory rodents, we possess limited agency within these societies. This environment shapes our daily activities, social interactions, learning capabilities, mental and physical health, etc. This social ecology frees us from the quest for individual survival or fitness, that are the heart of ethologic research. In return, we face other problems: global warming, inequalities, pandemics, social inequalities and violence, the management of aging and dependency, neurobiological disorders, like social disorders or SUD, etc. Issues that are at the heart of preclinical neuroscientific research.

To develop better models of the human condition, we need to question what aspects of this social environment influence our behavior? For instance: can daily sudden and jarring sound of alarm clock use lead to chronic stress? How do these aspects of our environment shape our neural architecture? Moreover, to what extent could these environmental stimuli impact other species? Studies on the influence of the environment on SUD have already provided some clues. Overall, the introduction of environmental complexity, as proposed by the neuroethological approach, is necessary to improve our animal models. But, ultimately, should the ecological relevance of animal models we aspire to establish reflect the natural living conditions of these animals or those conducive to eliciting the studied human behaviors?

In conclusion, our exploration, centered on SUD research, has delved into the utilization of animals as models for understanding the human condition and the advancements achieved in behavioral neuroscience. However, we are currently confronted with the constraints of this

approach, evident in the crises challenging the field. To advance preclinical neuroscience research both scientifically and ethically, a reassessment of our models is imperative. Drawing insights from diverse fields of research and embracing the neuroethological approach can guide us in this endeavor. Nevertheless, it is equally crucial to scrutinize the unique intricacies of our approach.

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