

Perspective

Blueprints for measuring natural behavior

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SUMMARY

Until recently laboratory tasks for studying behavior were highly artificial, simplified, and designed without consideration for the environmental or social context. Although such an approach offers good control over behavior, it does not allow for researching either voluntary responses or individual differences. Importantly for neuroscience studies, the activity of the neural circuits involved in producing unnatural, artificial behavior is variable and hard to predict. In addition, different ensembles may be activated depending on the strategy the animal adopts to deal with the spurious problem. Thus, artificial and simplified tasks based on responses, which do not occur spontaneously entail problems with modeling behavioral impairments and underlying brain deficits. To develop valid models of human disorders we need to test spontaneous behaviors consistently engaging well-defined, evolutionarily conserved neuronal circuits. Such research focuses on behavioral patterns relevant for surviving and thriving under varying environmental conditions, which also enable high reproducibility across different testing settings.

CONVENTIONAL BEHAVIORAL TESTING AND ITS DRAWBACKS

You are alone. Locked in a strangely shaped, grey-walled space with no way out. You were transported here by a creature of another, definitely more powerful species that could easily do you harm. Your stress response is through the roof. Everything looks and feels strange as your surroundings are like nothing you have ever seen before. You do not know that, but now you are expected to perform; solve a task or behave in a specific way testifying to your motor, memory, or social skills. How well do you think you would do under such circumstances? More importantly, what are the chances your behavior measured in that situation would actually reflect the actions and abilities you normally present in your everyday life? This kind of scenario sounds more like a bad dream than a purposely crafted experimental setup meant to faithfully assess specific functional characteristics of a living individual. Nevertheless, this is exactly how most laboratories approach testing animal behavior and its neural underpinnings.

The most commonly discussed and, at the same time, most problematic aspect of conventional behavioral testing is its notorious irreproducibility (Figure 1) (Mandillo et al., 2008; Button et al., 2013; Gilmore et al., 2017; Kafkafi et al., 2018; Bodden et al., 2019; Richter, 2020; Saré et al., 2021; Andrews et al., 2018). Indeed, the problem has been recognized as a significant threat to both the reliability of scientific discoveries and their potency to influence policymaking and the direction of societal changes (Morrison, 2014; *Nature Special Issue on Challenges in irreproducible research*, 2018). It has been argued that in animal research the prevalence of irreproducible data can be as high as 50 to 90% (Prinz et al., 2011; Collins and Tabak, 2014; Freedman et al., 2015). The well-recognized source of the issue is the variable quality of the testing procedures, stemming from the stressfulness of the behavioral assays, and the lack of unified data interpretation practices (Morrison, 2014). As a result, significant efforts have been put into establishing guidelines for high-quality experimental design and analysis (Kilkenny et al., 2010; Nosek et al., 2016; Sert et al., 2018; Smith et al., 2018). Now more than ever, adhering to the high reproducibility standards should be our priority (Gulinello et al., 2019). Unfortunately, due to its very nature, behavior is a complex phenomenon, and measuring it in a standardized way poses significant challenges. Thus, recognizing and eliminating the factors confounding behavioral assessment is the indispensable first step toward improving reproducibility (Camp et al., 2012; Gaburro et al., 2011; Pernold et al., 2019). Let us take a closer look at randomness and biases introduced by the stressfulness of the experimental procedures. The top three factors significantly intensifying experimental stress in animal subjects and thus promoting variable, erratic responses are (1) contact with humans before and during the testing procedures, (2) artificial and unfamiliar design of the testing environment, and (3) isolation anxiety (Beery and Kaufer, 2015; Chesler et al., 2002; Crabbe

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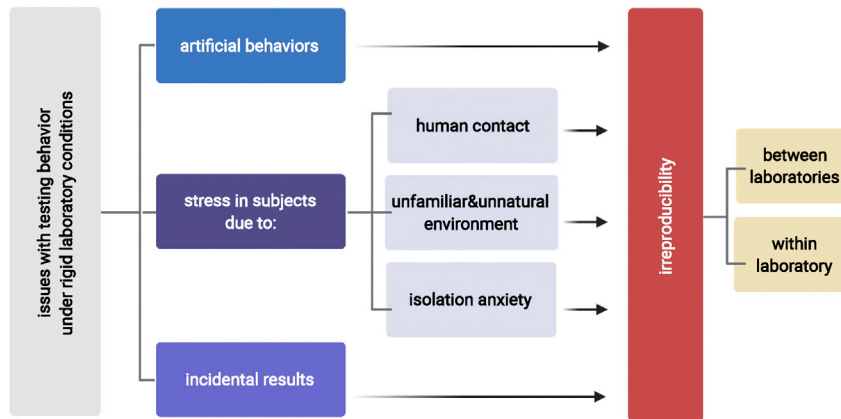


Figure 1. Issues arising from testing behavior under rigid laboratory conditions lead to notorious irreproducibility of results obtained in the field of behavioral neuroscience

et al., 1999; Heinrichs and Koob, 2006; Puścian et al., 2016; Sandi and Haller, 2015; Sorge et al., 2014). The influence of the last of these factors stems from the fact that most conventional assays test lone and sometimes also singly housed subjects. Although, as it was shown time and time again, isolating social animals such as rodents leads to deterioration of their health and cognitive functions (Zelikowsky et al., 2018; Mumtaz et al., 2018; Arzate-Mejía et al., 2020; Begni et al., 2020; Mudra Rakshasa and Tong, 2020).

On top of those considerations, there is a bigger issue of our ability to evaluate the data obtained with the use of the traditional behavioral assays. The problem with being able to interpret the data in the context of the role-specific behavior plays under naturalistic conditions is a serious limitation of classic behavioral assays. Meanwhile, the conventional wisdom in the community is that although classic behavioral tests have their problems, at least they are simple, and the interpretation of the data is very straightforward. We argue that nothing could be further from the truth. In fact, standardization of data reporting for even the very well-established assays leaves a lot to be desired. In many behavioral tests, there is little agreement as to what exact measures to use, and the between-laboratory variability in data analysis is huge (Andrews et al., 2018; Bodden et al., 2019; Chesler et al., 2002; Crabbe et al., 1999; Mandillo et al., 2008; Miyakawa, 2020; Morrison, 2014; Rudeck et al., 2019).

Even more importantly, the reasoning underlying our understanding of data is oftentimes based on the assumptions as to how animals “should” behave, rather than rooted in the ethology of a given species. The danger of such an approach was highlighted by the arguments of Paul Willner, who argued that one of the critical criteria for validation of the animal models of human disorders is their face validity. Face validity refers to the usefulness of a given test for measuring what the researchers aim to measure (Willner, 1986, 1997). For example, in one of the most commonly used assays of social behavior, the three-chambered apparatus, researchers are supposed to assess how sociable a tested animal is, based on its propensity to approach an unfamiliar conspecific, restrained under a small wire cup (Crawley, 2004; Duncan et al., 2004; Yang et al., 2011). In our view, this kind of thinking about behavior is fundamentally flawed. From the point of view of natural behavior, readily approaching an unknown individual in a situation that is already ambiguous might simply be dangerous. In fact, field studies performed in rodents show that they tend to avoid encounters with unfamiliar conspecifics and, if forced to interact openly, often become aggressive (Lopucki, 2007). This piece of knowledge about the ethology of the species shines a new light on the interpretation of data from conventional behavioral assays of sociability and is just an example of how a better understanding of natural behavioral repertoire may—or dare we say, should—inform our laboratory practices. We argue that similar problems with data interpretation can be identified across many popular behavioral assays. Pursuing investigation of not only artificially evoked but also presumptively construed behavioral responses may lead to conclusions that have little to do with naturalistic reality. Persistent usage of such a tool may thus result in us missing the point.

Another rarely discussed issue is the limited utility of the conventional behavioral tests for studying individual differences. In majority of cases, although tested individually, animals are thought of as clones,

expected to behave similarly, which can be then reflected as oscillating around the group mean. The greater the individual differences, the bigger the reason for concern on the part of the researchers, as the significant data variability hinders the conventional statistical approach. Not so long ago the argument that the more complex testing environment might increase the variability of behavioral outcomes was a dominant reason for not even considering its implementation. Yet, as shown by [Wolfer et al. \(2004\)](#), enriching the environment does not hinder the reliability of the obtained results, while having significant advantages for animal welfare ([Balcombe, 2006](#); [Bracke and Hopster, 2006](#); [Loss et al., 2021](#); [Voikar, 2020](#)).

Of course, individual differences measured with one trial assay are highly artificial and more often than not an artifact of a method itself. To tackle real behavioral specificity, animals must be provided with a significant level of freedom in when and how they respond to the testing situation. For that purpose, combining housing and experimental environments seems to be the optimal choice. Indeed, systems allowing for continuous behavioral assessment of the individuals kept in the home-cage settings are more and more popular ([Anpilov et al., 2020](#); [Codita et al., 2012](#); [de Chaumont et al., 2012, 2019](#); [Endo et al., 2021](#); [Forkosh et al., 2019](#); [Galsworthy et al., 2005](#); [Howerton et al., 2012](#); [Knapska et al., 2006, 2013](#); [Krackow et al., 2010](#); [Pérez-Escudero et al., 2014](#); [Puścian et al., 2014, 2016](#); [Schaefer and Claridge-Chang, 2012](#); [Shemesh et al., 2013, 2016](#); [Voikar et al., 2010](#); [Weissbrod et al., 2013](#)). Such an approach also provides a considerable benefit of the possibility to measure higher-order behavioral strategies. As in most automated testing systems animals live in social groups, they have a chance of performing behaviors that would otherwise be obsolete in a singly tested individual. Moreover, the considerable complexity of the homecage/experimental environment makes it feasible to observe a much richer behavioral repertoire. Standard housing cages used to keep laboratory animals provide greatly impoverished living conditions in comparison to any natural habitat. Many automated assays help solve this problem due to the implementation of enrichment, sometimes even resembling the most notable features of the rodent natural habitats ([Blanchard et al., 1995](#); [Puścian et al., 2016](#); [Foster, 2017](#); [Herman and Tamashiro, 2017](#); [Bove et al., 2018](#)). Taken together, arranging an intricate social and spatial environment gives insight into the complex actions. Such investigation is simply impossible to execute by using conventional behavioral assays.

ARTIFICIAL ENVIRONMENT EQUALS ARTIFICIAL BEHAVIOR

Although behavior itself is a complex phenomenon, investigating its neural underpinnings adds another layer of complexity. The dynamic character and intricate workings of the brain call for well-defined and standardized experimental approaches, enabling dissection of the neural circuits involved in governing specific actions. A common approach to solving this problem is the utmost simplification of the experimental tasks. This strategy has been extremely successful in studying the neuronal background of well-defined behavioral responses. A good example are elementary defensive responses, such as freezing in the face of a threat when an animal is put into a small cage without any escape route. Behavioral testing of such responses is easily replicable, shows low individual variability, and reliably mimics what is observed in the natural environment, e.g., a rodent freezes when a predator is close and there is no chance of running away ([Clugnet and LeDoux, 1990](#); [Iwata and LeDoux, 1988](#); [Maren, 1996, 1999](#); [Phillips and LeDoux, 1992](#)). Thus, measuring simple responses does not necessarily equate to performing misleading and unsound behavioral testing, as long as it is rooted in the understanding of their natural function.

However, we argue that an oftentimes contrived environment may lead to presenting rigid or atypical behavioral patterns ([Figure 2](#)). More problems arise when we aim to study more complex and variable behaviors, for example, responses aimed at obtaining food. Most commonly, they are studied in the operant tests that require a well-defined response, such as lever pressing, for the animal to get access to the reward ([Balleine, 1992](#); [Dickinson and Balleine, 1990](#); [DiFeliceantonio and Berridge, 2012](#); [Urstadt and Berridge, 2020](#)). However, motivation to get food depends on many factors, including when an animal last ate and how afraid it is of the experimental environment. The food-acquiring behavior is thus naturally variable. To reduce this variability, experimenters deprive animals of food and use shaping procedures to facilitate learning of the desired response ([Skinner, 1938](#)). Although a lot was learned using this approach, artificially increasing motivation leads to the loss of information on the natural drive to find, get, and consume food. In addition, food deprivation is known to alter brain function, thus putting in doubt the conclusions regarding neural mechanisms studied under such conditions ([Bubenik et al., 1992](#); [Claassen, 1994](#); [Karami et al., 2006](#); [Talhati et al., 2014](#)).

Another often neglected problem is the individual variability of the studied responses. One can easily imagine that various individuals of the same species apply diverse strategies to acquiring food. We can expect

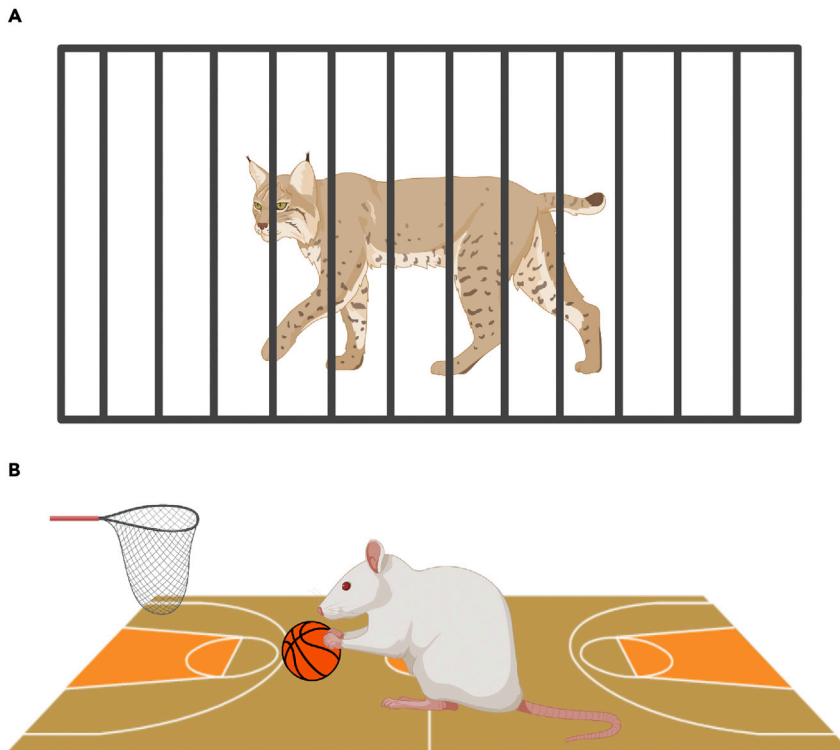


Figure 2. A contrived environment leads to rigid behaviors

(A) Stereotypic behaviors, such as pacing the fence, are often observed in captive wild animals living under conditions far more simplistic than those of their natural habitats.

(B) By the means of extensive shaping, it is feasible to teach laboratory animals to perform very elaborate behaviors, which never spontaneously occur in nature. Here a laboratory rat is taught to “play basketball” (see also: <https://www.youtube.com/watch?v=drmulHw5CM>).

even more divergence in social interactions, which by definition involve at least two animals, who affect each other’s responses in a very dynamic way. Further, animals living in a group naturally form social relationships, which have a strong impact on their behavior. Thus animals—even those very similar in terms of their genetic background, such as mice of a given inbred strain—do care who they interact with. Nevertheless, neuroscientists often try to reduce behavioral variability by simplifying the testing conditions and depriving animals of novelty and even isolating them. We argue that neuroscience is at a point where we can efficiently investigate the neural underpinnings of individual variability in animal behavior. However, to do it reliably we need well-standardized tests with a long observation time allowing for collecting a sufficient amount of data to assess specific phenotypes.

ACCESSING INDIVIDUAL PHENOTYPE

By creating stable, well-structured habitats and recording many instances of spontaneous behavior in group-housed animals we gain access to what they do voluntarily. Thus, instead of simplifying testing environments, nowadays many researchers make them more spacious and complex (Tecott and Nestler, 2004). The two main trends in measuring animal behavior more systematically are (1) tracking animals in utmost detail, usually without prior assumptions about the function of the actions taken by the subjects and then classifying behaviors using various (supervised or unsupervised) clustering algorithms and (b) designing testing environments resembling vital features of ecological habitats to elicit natural behavioral patterns. The latter approach is historically rooted in the field studies and ethology of the species most commonly used in the neuroscience research, that is rodents (Amrein et al., 2004a, 2004b; Dell’omo et al., 1998; Fiore et al., 1995; Galsworthy et al., 2005; Giorgio et al., 2012, 2012; Lipp et al., 2001; Peters et al., 2015; Puścian et al., 2016; Spruijt et al., 2014; Spruijt and DeVisser, 2006; Vyssotski et al., 2002).

Within the first framework, experiments are often conducted in spacious, highly environmentally enriched arenas, where animals can interact with numerous objects and conspecifics (Anpilov et al., 2020; Arroyo-Araujo et al., 2019; Balzani et al., 2018; Codita et al., 2012; de Chaumont et al., 2012, 2019; Endo et al., 2021; Forkosh et al., 2019; Galsworthy et al., 2005; Genewsky et al., 2017; Goulding et al., 2008; Howerton et al., 2012; Knapska et al., 2006, 2013; Krackow et al., 2010; Pérez-Escudero et al., 2014; Pernold et al., 2019; Puścian et al., 2014, 2016; Robinson et al., 2018; Schaefer and Claridge-Chang, 2012; Shemesh et al., 2013, 2016; Singh et al., 2019; Voikar et al., 2010; Weissbrod et al., 2013). Scientists track animal behavior with the use of cameras or radio-frequency-based identification (RFID) antennas. Other, more rarely used technologies include electromagnetic detection, sensor plate-, and infrared-based systems (Burman et al., 2018; Iannello, 2019; Recordati et al., 2019; Voikar and Gaburro, 2020). Camera-based technology allows for highly detailed analysis of every move animal makes and thus is the most commonly employed approach. However, two main limitations of video tracking occur when subjects enter the secluded spaces and when many animals interact with one another in closeness, proximity (Voikar and Gaburro, 2020). Sometimes the latter problem can be solved by color-marking the subjects. One of the most exciting developments in this field are scripts and software packages allowing for precise tracking of the chosen points on animals' bodies, such as paws or snouts, and even reconstructing movements based on such data (Kane et al., 2020; Mathis et al., 2018, 2020; Mathis and Mathis, 2020; Nath et al., 2019). On the other hand, in RFID-based experiments, each animal is injected with an electronic chip encoding its individual number that can be registered by the antennas placed anywhere within the testing environment. Thus, this technology allows for registering animal behavior that remains either out of sight, e.g. in shelters, tunnels, feeders, running wheels, or when one wants to register many subjects crowded in a small space. The latter is a common problem in research on social behavior in large groups of rodents. To make the most of both approaches, and at the same time avoid their perils, some researchers combine the two technologies to improve quality of animal tracking (de Chaumont et al., 2019).

On the other hand, in experiments conducted in systems mimicking essential characteristics of natural habitats researchers root their testing methods in what is already known about natural behavioral patterns and assess actions that are known to be ecologically relevant. For example, they use nose-poking as the basis for instrumental conditioning in mice, as this behavior (in contrast to e.g. lever pressing) is readily performed as a form of exploration and does not require shaping (Krackow et al., 2010; Endo et al., 2011; Knapska et al., 2013; Puścian et al., 2014; Kiryk et al., 2020; Iman et al., 2021). Some testing environments imitate borrows and tunnels as found in natural rodent habitats, to obtain activity and social behavior patterns resembling those found in wild animals (Blanchard et al., 1995; Puścian et al., 2016; Foster, 2017; Herman and Tamashiro, 2017; Bove et al., 2018). Moreover, housing animals in spaces similar to those found in nature in combination with testing naturally occurring behaviors allows researchers to design tasks best suited to engage well-conserved neural mechanisms. As most such assays utilize RFID technology for animal identification combined with other systems allowing to e.g. limit access to an attractive resource, such as food reward, located somewhere within the territory to only one animal at a time. In addition, instrumental conditioning with natural actions (operant behaviors) is also used in such research. For example, mice have to poke in a specific hole to obtain access to sweetened water. Notably, some recent experiments allow for measuring patterns of complex group behavior in a reliable, replicable way (Puścian et al., 2016; Winiarski et al., 2021).

Notably, it has been shown that implementing fully automated behavioral testing can be a successful strategy for improving reproducibility (Arroyo-Araujo et al., 2019; Krackow et al., 2010; Pernold et al., 2019; Robinson et al., 2018). Arguably, the presented approaches to studying behavior pose new challenges. The amount of data generated by such assays, especially when video-tracking is employed, requires a substantial space for data storage. In addition, novel approaches to big data analysis constitute an exciting, however still only developing, area in behavioral neuroscience. Moreover, to access the brain mechanisms underlying specific behaviors we need relevant methods of neuronal imaging and manipulation, which can be employed in the automated testing systems i.e., wireless and well aligned to behavioral responses.

TOWARD VALID MODELS OF HUMAN DISORDERS

The issues with conventional behavioral testing are significantly slowing down the progress in proposing novel therapeutic approaches to neurodevelopmental, neurodegenerative, and psychiatric disorders. Indeed, if researchers encounter significant problems at the initial phase of phenotype assessment of the animal models of the impairments, it is to be expected that reproducible *in vivo* testing of new

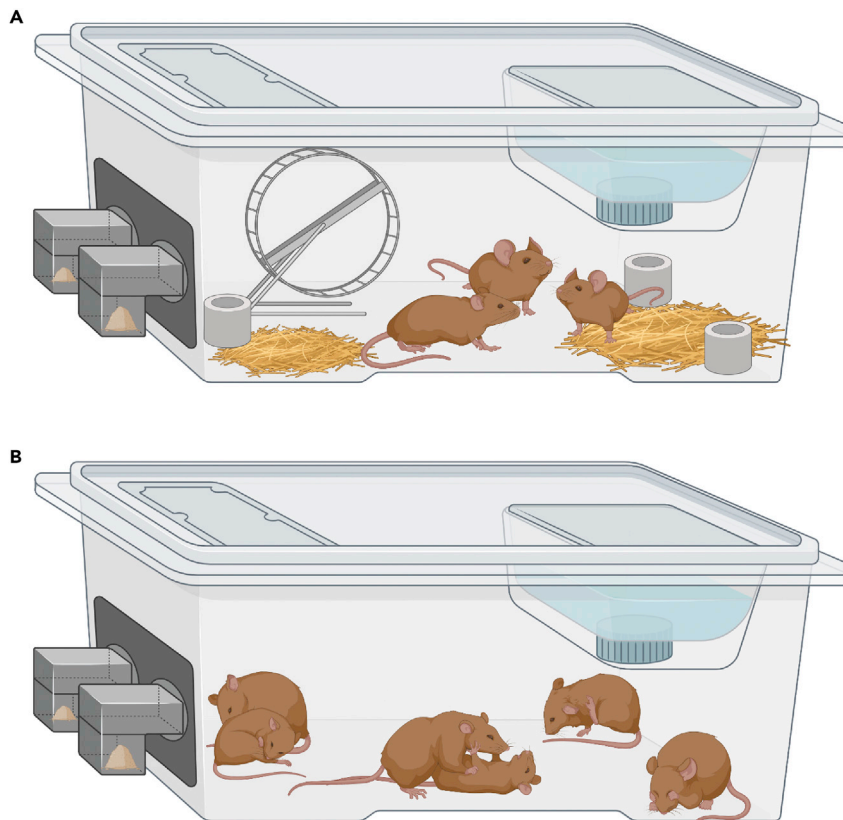


Figure 3. The behavior of the individuals depends on the environmental and social constraints

The diagrams illustrate differing social interactions under varying housing conditions.

(A) Mice amicably interact in the enriched, socially-adequate environment.

(B) Animals of the same strain present aggressive behaviors and isolate themselves as a result of being subjected to overcrowding and impoverished conditions.

therapeutic strategies poses a major challenge. In our view, the lack of compelling developments in this area over the last decades is in no small part due to the oversimplified approach to studying behavior (Figure 3).

One of the most notable consequences of this problem are inconsistencies in reporting phenotypes of mouse models of autism. Sadly, it is common that researchers from different laboratories reach opposite conclusions when assessing the behavior of animals with identical genetic mutations and background. An example of this issue is well illustrated by the studies focused on the sociability of neuroligin-3 mutant mice, which are considered one of the most relevant animal models of this disorder (Tabuchi et al., 2007; Chadman et al., 2008).

However, at this point scientists are able to design reliable and replicable assays using automated testing equipment, allowing for long observation times and taking multiple behavioral measures simultaneously. Importantly, by using such tests, we are now capable of evaluating phenotypes that would otherwise be difficult or impossible to capture. The latter issue can be illustrated by two examples. The effects of fluoxetine, the SSRI commonly used to treat depression, on the behavior of various mouse models of this disorder are often variable. Measuring the effects of fluoxetine in animals housed in either enriched or impoverished conditions for a long time revealed that the variability in the drug effects could be explained by the type of environment in which it is administered, with an enriched environment exerting beneficiary effects (Alboni et al., 2017). Such studies require long observation of animals living in a stable, friendly environment, an approach executed most efficiently with the use of automation (Puścian et al., 2021). The second example illustrates how automated tests can help to stratify the phenotypes of experimental animals. The long-term observations of alcohol drinking behavior in laboratory mice group housed in automated

systems show that the propensity to alcohol addiction differs across individuals. Based on such data, researchers are able to select mice prone to alcohol addiction, which offers a model much closer to clinical observations than that based on testing the average behavior of randomly chosen animals (Radwanska and Kaczmarek, 2012). Thus, strategies enabling testing voluntary behavior can be effectively used to study the underlying mechanisms and potential therapeutic approaches (Stefaniuk et al., 2017; Beroun et al., 2018).

We propose that especially in the case of studying animals with significant behavioral deficits, which can be exacerbated by stress, testing should be performed in the setups allowing for the spontaneous manifestation of symptoms. As previously discussed, engaging well-defined and well-evolutionarily conserved neural circuits is most probable under such conditions, which may be critical for our ability to reliably test novel therapeutic approaches.

BLUEPRINT FOR MEASURING NATURAL BEHAVIOR

Long-term assessment of animal behavior in the complex physical and/or social environment is most effectively achieved by the employment of automation. Simultaneously, recording the actions of many subjects for days or even weeks generates a vast amount of data that need to be carefully analyzed. As previously mentioned, such a strategy poses a technical challenge but is necessary to overcome variability, which unavoidably increases with test complexity. The researchers collect more data, observe animals for extended periods, record more behavioral measures, and replicate experiments to tackle the variability. The latter is critical when testing animals within social groups. As the group composition may affect social behavior and learning efficacy (Kiryk et al., 2011; Puścian et al., 2016), it is vital to test the hypothesis in several cohorts of animals and, when possible, carefully control group composition. For instance, when evaluating genetically modified animals and their wild-type counterparts, the experiments conducted in groups of mixed genotypes may yield different results than those performed in subjects of particular genotypes tested separately (Kiryk et al., 2011; Kalbassi et al., 2017; Sledziowska et al., 2020). Notably, longer observation time and simultaneous recording of multiple behavioral measures enable scientists to reveal the behavioral patterns that are stable over time (Krackow et al., 2010; Codita et al., 2012). Further, to capture the whole picture of a given phenotype, one needs to test males and females in parallel (Shansky and Murphy, 2021). To efficiently collect and analyze such a significant amount of data, we need high-throughput, cost-effective and reliable assays, and data analysis pipelines. Fortunately, the fully automated behavioral systems meeting these criteria have already been available for some time (Anpilov et al., 2020; Arroyo-Araujo et al., 2019; Balzani et al., 2018; Codita et al., 2012; de Chaumont et al., 2012, 2019; Endo et al., 2021; Forkosh et al., 2019; Galsworthy et al., 2005; Genewsky et al., 2017; Goulding et al., 2008; Howerton et al., 2012; Knapaska et al., 2006, 2013; Krackow et al., 2010; Pérez-Escudero et al., 2014; Pernold et al., 2019; Puścian et al., 2014, 2016; Robinson et al., 2018; Schaefer and Claridge-Chang, 2012; Shemesh et al., 2013, 2016; Singh et al., 2019; Voikar et al., 2010; Weissbrod et al., 2013).

As discussed, testing groups of animals in a relatively complex environment poses certain challenges. Is it really necessary to add additional layers of complexity by performing field experiments or testing outbred or even wild animals? We argue that the answer to this question is yes. To understand behavior, i.e., when and why given patterns of actions occur, we need to observe diverse animal strains, if possible in their natural habitats, and learn how animals behave in their everyday lives (Lahvis, 2017a, 2017b).

It is noteworthy that some behaviors are especially difficult to interpret when tested in the laboratory. For instance, social hierarchy in a safe laboratory environment full of food may not be as vital as it is under markedly more difficult natural conditions. The threat of predators, scarcity of food, and low temperatures—in the presence of such challenges better access to resources may be crucial for survival. What makes particular animals leaders or subordinates within the group (Figure 4)? What are the advantages and costs of being a leader? In the laboratory, scientists most commonly study social hierarchy in male mice, often enhancing it by introducing several aggressive encounters with other animals (Wang et al., 2011; Fan et al., 2019). Such conditions rarely occur in the natural conditions in which rodents rather avoid direct fights (Lopucki, 2007). The tube test commonly used to evaluate (and establish) social hierarchy offers clear-cut results, by ordering mice within a group, which some researchers consider a practical advantage when studying underlying neuronal pathways. Nevertheless, how do we know if neural mechanisms discovered under such conditions are also involved in a naturally occurring hierarchy? Which behaviors from the repertoire recorded in the laboratory are also beneficial in the real world? What circuits play a role in obtaining and maintaining the territory? Finally, are inbred laboratory animals suitable models to study the

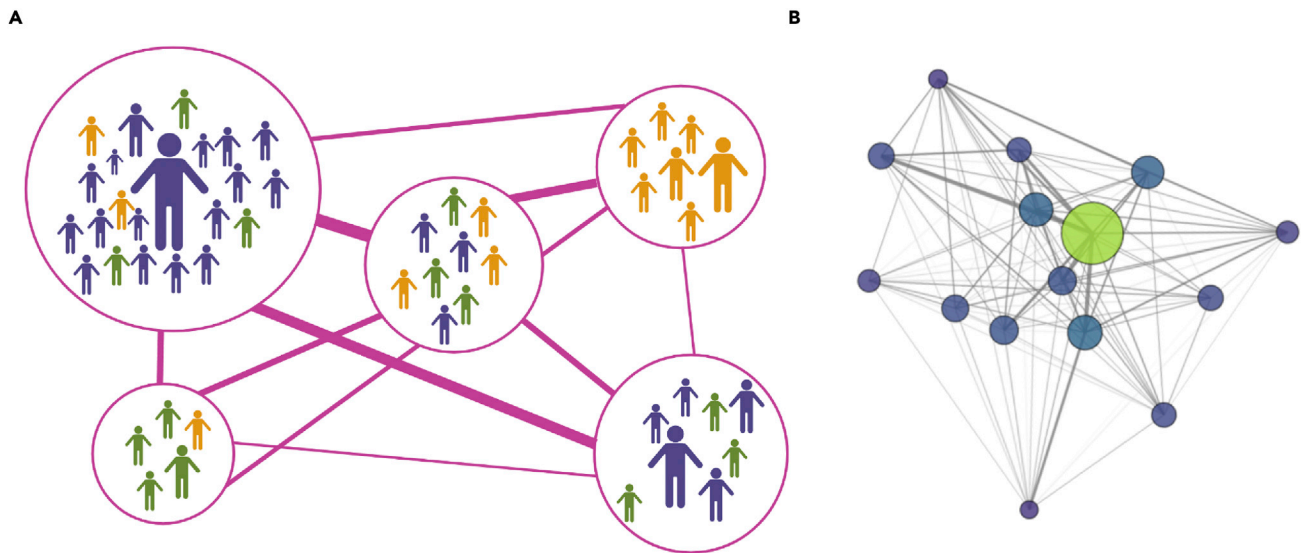


Figure 4. Graphic representation of social networks in humans (A) and mice (B) illustrating varying within-group positions and relations

Social network structures in both species are visualized as node-edge graphs. The nodes represent subgroups (A) or individuals (B). (B) The bigger the node, the higher the position within the social structure. The thickness of the edges between the nodes represents the strength of the social connections—the thicker the lines, the more frequent the interactions.

mechanisms of dominance and leadership? In our view, these questions can be addressed only by combining the knowledge gathered in the field studies with wild animals as subjects, with discoveries made by testing both inbred and outbred animals under conditions resembling the most essential characteristics of natural habitats.

SUMMARY AND OUTLOOK

Studying artificial behavior results in eliciting artificial, and to a great extent randomly, ad-hoc-recruited circuits; this in turn leads to significant within- and between-subject variability hindering our ability to draw conclusions. Indeed, because behavior studied in the artificial experimental designs is highly variable and hard to predict, the same must be true about the underlying neuronal activity. At the same time, it should be acknowledged that there are some avenues of research where testing constrained behaviors is still the best or even the only option. Studies on sensation in which scientists strive to test the limits of perception are a notable example. Such experiments oftentimes require highly controlled environments and might make experimentation in multiple freely moving animals difficult to implement, despite all the progress in the field.

Moreover, it cannot be overlooked that modifying existing experimental protocols to comply with the need for addressing the ecological reality would require significant efforts on the part of the scientists and financial support from the funding bodies. The latter might even require lobbying for fund allocation on the part of the scientists. Introducing ethologically sound experimentation calls for additional laboratory space, which also may be a considerable barrier to making a systemic change. In addition, still many behavioral neuroscience labs lack the expertise needed for efficient implementation of automation and digitalization, which makes focusing on ethologically relevant research more of a challenge. To be able to tackle all those issues, broad collaboration among scientists seems indispensable. Community efforts, already undertaken by researchers in the field, such as moving toward more open science, and sharing experimental protocols and scripts for data analysis, might constitute a cornerstone of the paradigm shift. Indeed, to address the problem of behavioral data irreproducibility, standardization of protocols and practices is of paramount importance, especially because researchers will have to face overcoming standards of practice that have been with us for decades. We argue, that there is no better way to do it than by continuing the efforts of openly sharing instructions and algorithms, as has already been done by so many.

Further, we suggest that gradual implementation of the new approach starts by changing the most critical factors influencing well-being and ability to express species-specific behaviors by the laboratory animals. The straightforward first step in this process might be improving the housing conditions by keeping animals

in larger social groups in enriched environments. Another relatively straightforward strategy is the introduction of automated behavioral testing, which has already been shown effective in improving reproducibility, in no small part due to cutting human interference (Krackow et al., 2010; Puścian et al., 2016). Application of those solutions would open new avenues for studying more naturalistic, unconstrained actions and including sex-, genotype-, and strain balancing into the mix. Even more importantly, it would constitute a great improvement in and of its own.

Nevertheless, we argue that studying behavior under more naturalistic settings consistently engaging well-defined, evolutionarily conserved neural circuits is the best way to address the reproducibility crisis in behavioral neuroscience and beyond. The need for more ecologically relevant research has been expressed by many scientists, most notably the ones focusing on applying computational approaches to ethology, neuroethology, and human neuroscience (Anderson and Perona, 2014; Eitan et al., 2022; Liberti et al., 2022; Nastase et al., 2020; Sonkusare et al., 2019; Zhang et al., 2022; Zigelman et al., 2022; Rose et al., 2021). Although it is still a challenge, combining the ecologically relevant behavioral research with advanced methods of brain imaging, measuring neuronal activity, and manipulation of specific circuits has become more and more feasible over the last years. Even though such methodologies are not without their challenges, we are at the point when newly developing technologies allow wireless recording/manipulating many neurons at a time in group-housed animals (Anpilov et al., 2020; Cai et al., 2022; Caras and Sanes, 2017; Inagaki et al., 2019; Li et al., 2022; Lu et al., 2018; Mayer et al., 2019; Montgomery et al., 2015; Murphy et al., 2016; Pinnell et al., 2015; Yang et al., 2021; Zong et al., 2022). Broader implementation of those methodologies increases our chances of developing experiments, whose results will stand over time and thus enable us to break the impasse in proposing new, effective therapeutic strategies.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.P. & E.K.; writing and editing, A.P. & E.K.; funding acquisition, A.P. & E.K.

DECLARATION OF INTERESTS

The authors declare no competing financial interests in relation to the work described.

INCLUSION AND DIVERSITY

While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list.

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