What is ‘Natural’ about Naturalistic Neuroscience?

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SUMMARY:

A growing number of neuroscientific articles now discuss the revolutionary tools and techniques of naturalistic behavioral studies. Falling under the umbrella of as ‘naturalistic neuroscience’, these studies aim to impart the precision and control of traditional behavioral experiments while also documenting ‘real-world’ animal behavior. The present study examines the tools and techniques used in these studies from both a theoretical and modeling perspective. Results of this paper demonstrate the contradictions generated by ‘naturalistic’ empirical manipulations, as well as cases where the process of experimental comparability is hindered. I show that focusing on competing experimental aims more closely aligns experimental outcomes compared to the coarse-grained use of the ‘naturalistic’ concept, which instead oversimplifies the complexity of methodological drivers in behavioral neuroscience. From this analysis, I recommend the use of a revised framework that imparts greater transparency in the experimental aims of researchers studying animal behavior.

Introduction

A central challenge for Neuroscience has been understanding how nervous systems flexibly and reliably generate complex behaviors. How does an animal distinguish a benign encounter from a threat? How is irrelevant information ignored to satisfy its needs? Since the days of Pavlov’s salivating dogs or Skinner’s bar pressing rats, behavioral neuroscientists have constructed highly constrained lab paradigms to study how experience modifies relatively simple behaviors. These behaviors give scientists the benefit of precision and control: by manipulating the temporal relations between stimulus and response, neural activity can be directly tied to the behavior. However, these behaviors are also seen as highly contrived in the sense that there are no levers or bells in the habitats in which rats’ and dogs’ brains evolved, which presumably shaped the neural circuits that generate most behaviors.

In parallel to simplified behaviors, traditional approaches in behavioral neuroscience have focused on animals with simpler nervous systems. For example, the nematode, *Caenorhabditis elegans* with a total of 302 neurons, and the sea slug, *Aplysia californica*, whose nervous system consists of a series of neuronal clusters (ganglia) containing specific neurons identifiable from one slug to the next, were pressed into service to dissect the complicated processes of learning and memory. Experiments using these simplifications in behavior and in the nervous system – what allowed ready access to genetic manipulations - have long been viewed as essential for meeting the challenge of explaining behavioral complexity. The ruthless drive towards simplicity has ignored the many complex behaviors that animals exhibit in their native environments – behaviors shaped evolutionarily by natural and sexual selection acting on development and mature function of CNS neural circuits (Olton and Samuelson 1976; Morris 1981; Miller et al., 2020). By restricting behavioral expression, simplified experiments can generate experimental artifacts and also ignore key individual differences between animals. Neuronal responses can differ when animals are studied outside of artificial lab conditions (Polley et al. 2004), overtraining animals in fixed conditions affects plasticity (Jahangiri et al. 2019), animals respond differently to artificial and natural stimuli (Cuthill et al. 2000; Fleishman et al. 1998), and stress can shape behavioral responses (Brandl et al. 2022, Gouveia and Hurst 2017; Deacon 2006).

Traditional, standardized paradigms of operant boxes or associative learning tasks imported into behavioral neuroscience long ago have also been seen as inadequate for providing insights into how animals variably navigate, choose, plan, persevere or give up in their complex natural worlds. Instead, to offer a more ecologically valid study of behavior, scientists use novel methods that still offer precision and control for measuring an animal's activity, but now with the added sensitivity of study animal behavior without traditional methods of restraint. This movement, known as 'Naturalistic neuroscience', has been described as a 'revolution' in behavioral research (Anderson and Perona 2014). Naturalistic neuroscientific studies characterize animal behavior as they would occur 'in the wild', albeit combined with the control and precision of a standard conditioning experiment which rely on improved tools for measuring behavioral and neural activity (Dennis et al. 2021, Matusz et al. 2019, Hoffmann et al. 2023; Ulanovsky, forthcoming). In its promise to hit a Goldilocks balance between degrees of freedom and experimental control, naturalistic neuroscience distinguishes itself from pre-existing frameworks for behavioral study. This balance promises capturing an animal's 'true' behavior by expanding the dimensionality of testing while maintaining rigorous experimental standards. Of note within these efforts, 'naturalistic neuroscientific' studies embed the assumption that the privileged way to study behavior is to get as close as possible to an animal's true, unaltered behavior.

However, the coarse-grained concept of 'naturalistic' oversimplifies the complexity of issues that methodologically drive behavioral neuroscience. 'Naturalistic' acts as a placeholder for different conceptual and technological experimental goals, as well as various experimental traditions that range from the use of ethological to computational theories. By outlining these experimental aims, I provide evidence against the view that there is an epistemically privileged way to study behavior, and I caution future empirical studies from using the term 'naturalistic' to describe their behavioral studies. The present study provides evidence of empirical harm when scientists use the underspecified description of 'naturalistic' behavior, focusing on how experimental contradictions can get generated within behavioral research, as well as cases where the process of experimental comparability is stifled.

The Many Practices that Make Up Naturalistic Neuroscience

Naturalistic neuroscientific studies are distinct for achieving the control and precision of traditional behavioral experiments while showing sensitivity to animals' complexity of responses. Methods cited in naturalistic studies vary widely, ranging recording freely moving and uncaged animals (Mao et al. 2021, Voloh et al. 2023) to entirely substituting the lab with spaces that mimic natural environments (Yartsev and Ulanovsky 2013) or doing fieldwork studies where the animal is entirely 'in the wild' (Vallet and Wassenhove 2023). Many of these efforts take inspiration from ethology, neuroethology, behavioral ecology, and related fields,

where there is a longstanding emphasis on environmental considerations and evolutionary perspectives (Krakauer et al. 2017; Miller et al. 2023; Testard et al. 2021; Datta et al. 2019).
At the heart of naturalistic neuroscience are the novel technologies that can now capture individual and collective behaviors of freely moving animals (Berdahl et al. 2013; Soria et al. 2021). These tools are thought to confer advantages over both highly contrived research, as well as studies that might have studied animal behavior in the wild, but lacked the tools that could still give scientists control.

For example, one important innovation in naturalistic neuroscience has been developing tools that no longer confine animal movements when engaging in neural recording. Novel techniques, such as wireless head-mounted optogenetic systems (Qazi et al. 2018, Hashimoto et al. 2014, Montgomery et al. 2015) and ultrathin multifunctional optoelectronic devices (Kim et al. 2013) now allow animals to run around freely without close proximity to the computer that is recording neural activity. Advances in materials science have created more flexible electronics, allowing researchers to use optogenetic constructs with a single implanted device without the need for batteries (McCall et al. 2013); developments in wirelessly rechargeable batteries mean that this information can be recorded for days at a time (Kim et al. 2021). Wirelessly networked microchips allow for neural recording and microstimulation (Lee et al. 2021), and the development of miniature wireless fluorescence microscopes (miniScopes) has allowed researchers to record neural activities of freely moving animals at the resolution of single cells (Zong et al. 2021; Dong et al. 2024). These techniques have even expanded the scope of studies to include both the central and peripheral nervous systems (Park et al. 2015).

Behavioral paradigms have evolved in parallel with neural approaches and now include virtual reality environments that test animals' responses to rich sensory stimuli (Brown and de Bivort 2018; Naik et al. 2020). Here too, naturalistic studies benefit from sophisticated technologies, such as improved projectors. Virtual realities and avatars have allowed researchers to better test sophisticated social behaviors or interactions between animals and others (Huang et al. 2020), as well as simple navigation (Jeung et al. 2023). Technological advances also allow for a wider range of experimental subjects including, for examples, small invertebrates, in sophisticated testing conditions (Peckmezian and Taylor 2015, Schultheiss et al. 2017). These new tools also enable novel research using traditional model systems. For example, experimentalists can now do whole brain imaging in freely moving zebrafish (Hasani et al. 2023) and conduct neural recordings in freely moving bats (Ulanovsky and Moss 2007; Yartsev and Ulanovsky 2013). Moreover, new tools have opened possibilities for studying novel and nontraditional animal models that were previously inaccessible to neuroscientific query. Examples include miniature microdrives used to study food caching behavior in freely moving tufted titmice (Payne et al. 2021) and remote monitoring techniques used to document sleep in elephant seals (Kendall-Bar et al. 2023). Labs using these techniques also show signs of the theoretical shifts that emerge from these new tools. For example, neuroscientists increasingly cite incorporating more evolutionary and environmental outlooks in the study of behavior, such as examining animals that are unique from a phylogenetic standpoint. Such studies include how animals transitioned from aquatic mediums to land (salamanders), or those whose behaviors change dramatically depending on environmental demands (shrew, squirrel).

Framing Naturalistic Neuroscience: True Behavior Without Human Intervention

The emphasis on objectivity currently frames the framework for naturalistic neuroscience. Naturalistic studies are viewed as achieving "true" theories of the brain or helping to identify "real" or "real-life" behaviors in the world (Miller et al. 2022, p. 13; Mobbs et al. 2021; Shamay-Tsoory and Mendelsohn, 2019). Studying naturalistic behaviors includes studying species in their natural habitats (Vallet and Wassenhove 2023), as well as modifying the lab environment to resemble the animal's natural habitat.

The technological advances of naturalistic neuroscience – what sits at the heart of these efforts to capture the truth about behavior – also rest on a promise to objectively measure behavior. One way to do this is to record more information, as it is regularly assumed that greater amounts of data used to capture behavior yield greater accuracy about animal activities. Within this framework, 'naturalistic' is a concept that linearly and hierarchically 'scales' (Fan et al. 2021), with the practices of naturalistic behavior best adopted in "modest steps" (Cisek 2024). Such language about naturalistic neuroscience assumes 'improvement' to pre-existing behavioral experiments, once studied in impoverished ways and measured less accurately.

Relatedly, the technologies that drive naturalistic studies of behavior are also thought to improve accuracy by *changing* and even *reconceptualizing* categories of behavior. Here, AI tools have seemingly improved experimentation by refining behavioral categories previously recognized by humans. For example, by using machine learning video-based tracking, researchers are able to rethink associations with behaviors, such as the relationship between sleep and survival (Geissmann et al. 2019). In other cases, the use of these techniques has identified novel behaviors (Hoyer et al. 2008). The objectivity of these tools is tied to the sheer amount of data captured by them, which use sophisticated analyses to identify which functional categories are significant.

Generally, the strategy of naturalistic neuroscience tools has consistently aimed to remove the subjectivity and bias of human observers from behavioral experiments. For instance, better vision recognition technologies allow tracking and uninterrupted recording of hours of animal behavior, and algorithms can now parse those recordings to find behavioral patterns that may not be detected by humans manually tracking behavior. Training on multiple animal model datasets and breaking the animal's movements down into behavioral components can enable deep learning algorithms to identify patterns of meaningful activity. These tools – software such as Bonsai, SLEAP, DeepLabCut, Lightning Pose, and others – can even be tailored to specific animals, such *Drosophila* (BonFly Neurogears 2023) and macaques (Bala et al. 2020, Labuguen et al. 2021; OpenMonkeyStudio and MacaquePose). By automating behavioral analysis (Datta et al. 2019), these techniques can move past human constraints to test multiple animals at once and test more animals overall.

The position that *naturalistic studies are privileged views on behavior* sets up a tension between 'wild' behavior and laboratory 'controlled' behavior (see Figure 1). However, this tension alone fails to distinguish the various experiments, as many studies may both impart control and capture wild behavior without there being any meaningful way of distinguishing which one is more 'naturalistic' than the other (Fig. 1). A framework that simply juxtaposes 'wild' and 'controlled' behavior also assumes the concept of naturalistic behavior to be fixed, even in the face of the rapid technological progress that shapes what 'naturalistic' means. For instance, what is 'naturalistic' today may no longer qualify as naturalistic once a new and more improved technique for studying wild behavior in a controlled setting arrives (Fig. 1). This demonstrates the reliance of 'naturalistic' on the different kinds of techniques that exist in relation to one another.

The current framework for naturalistic neuroscience relies on a concept of 'natural' that is both intuitive, and yet vacuous upon investigation. Consider two widely used animals in neuroscientific research: the rat and the fruit fly. One of the reasons the rat is used as a behavioral model is due to its high adaptability, but this fact also blurs where to designate its natural habitat. Rats may have originated from specific parts of the world, but they have long populated every continent thanks to human migration and now live among humans in urban environments. Which of these histories count when one tries to measure rat behavior 'in the wild'? As I will discuss with the fruit fly, these histories become even more complicated as the domestication of animals continue both inside and outside of the lab.

The overly broad category of 'naturalistic' precludes answering many crucial questions, including: Does naturalistic behavior only apply to traditional animal models (Aplysia, flies, worms, rodents)? How does one study naturalistic behavior in 'wild types' that no longer share behaviors with their own species? Is viewing behavior from an evolutionary standpoint more naturalistic than from an ecological one? How much is too much data for a behavioral study? What is the relationship between the 'observer' of behavior and the behavioral subject? What defines behavior in experiments?

The intuition of 'naturalistic' rests on its use as a marker to distinguish what is 'good' from what is artificial and 'bad'. Moreover, the associations of 'nature' as pure and unbiased, and what keeps this category distinctly separate from humans, is both socially and politically reinforced (Uggla 2010). This invokes the core issue that 'natural' is a normative placeholder for different values and experimental aims. In the next section, I shift the discussion to these aims in effort to elucidate some of the methodological complexities that have been overlooked by mainstream discussions of naturalistic neuroscientific studies.

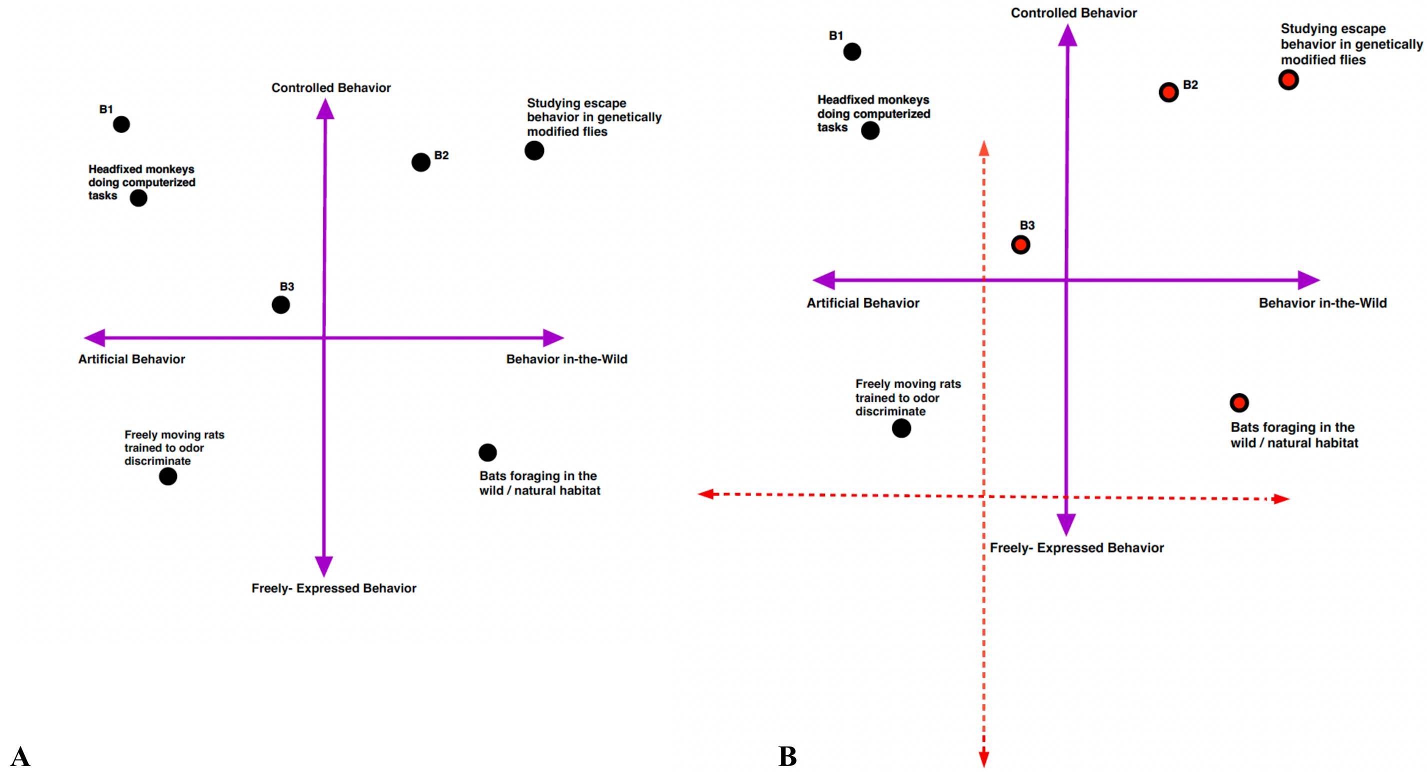

Figure 1: (a) **Naturalistic is Too Broad** Descriptions of naturalistic neuroscience practices are conceptually bound by attempts to get at a more objective characterization of behavior, but they also demonstrate the broadness of the term ‘naturalistic’. There may be two behaviors (B1 and B2) that appear separate on the naturalistic scale but are in fact conceptually similar to one another. (b) **Naturalistic is not a Fixed Concept** The conceptual landscape of naturalistic behavior can easily shift depending on technological innovation. Consider that two naturalistic experiments may, to varying degrees, capture ‘in the wild’ animal behavior while also using technology that offers those researchers fine grained control over their subject’s movements. For example, head fixed flies walking in 2D virtual landscapes vs. freely moving flies using virtual landscapes. The first experiment may be considered less naturalistic and more controlled than the second because, even if the fly is moving in a simulated reality, it seemingly has a more authentic experience compared to a head fixed fly. But now imagine that even a minor development in the technological capacities for measurement – such as more robust tools for measuring untethered flies in 3D environments – can change the understanding of ‘naturalistic’ between these two studies (represented by the red dotted line). What, then, becomes the dividing line on what ‘counts’ as naturalistic? If the tools and models were to change in the future, ‘naturalistic’ changes as well.

Experimental Aims and Epistemic Commitments

To better explain why a study is considered naturalistic and how various naturalistic studies compare to one another, I propose examining the various ways that scientists impart 'control' in the study of behavior. What is 'naturalistic' changes depending on the various experimental aims of scientists, as these indicate differing epistemic commitments. Epistemic commitments reveal what the concept of naturalistic is *relative to* by clarifying one's theoretical positioning, or what principles one is justifying in believing. For example, a neuroscientist may want naturalistic studies because of their systems-level experimental aim of identifying an important circuit, showing an epistemic commitment to a principle of reductionism. Another researcher, having the experimental aim of using a nontraditional animal model for behavior, may latch onto an epistemic commitment about development and the scope of behavioral flexibility.

Some clarity could be added here by identifying at least some of the differing epistemic commitments imparted within naturalistic studies. Doing so can align researchers' interests to make the 'naturalistic' concept more meaningful. It can also support non-intuitive positions, such as defending the use of traditional models for many naturalistic behavioral experiments, as well as tempering the hype around new techniques for naturalistic studies of behavior. In this respect, any discussion of naturalistic neuroscience demands a follow up question: 'naturalistic relative to what?'

Below, I illustrate how, in comparison to the conventional framework of naturalistic neuroscience that simply juxtaposes 'wild' and 'controlled' behavior, a focus on epistemic aims provides a better way forward for discussing naturalistic studies. Although it is beyond the scope of this review to discuss many naturalistic neuroscience studies currently proliferating in the neurosciences, I show the complicated sides to the story of studying naturalistic behavior by centering nonhuman animal modeling research. Moreover, this case alone, exhibiting the range of epistemic issues that can emerge for those working in similar areas, reveals the complexity of the problem as it scales to comparing research across domains.

Models are Mediated: Myth of the 'Wild' Model (overlooking the complexity of experimentation)

A common point of discussion within the conventional characterization of naturalistic neuroscience, particularly as it relates to nonhuman animal models, is a concern with repeatable behaviors and the use of overtrained, traditional models. Traditionally, repeatable behaviors have been key to experimental research in that they allow stable correlations between behavior and brain activity. Repeatability helps scientists compare behavior between animals, control contextual variables that may influence behaviors, and even identify when meaningful changes occur in the behavior of a single animal. Researchers have long identified ways to exploit systems that exhibit repeatable behaviors (e.g. bar pressing or birdsong) or create conditions to make it more likely that an animal will exhibit such behaviors.

Yet, those appealing to naturalistic neuroscience criticize the highly contrived situations that enable repeatability. For example, 'captivity effects', or the behavioral and physiological changes generated by housing animals in confined spaces, have been identified in numerous ways, ranging from genetic expression across the brain (Bedoya Duque et al. 2023) to changes in hippocampal (LaDage et al. 2009) and cortical volume (Bedoya Duque et al. 2023). Simply changing the environment that animals are normally housed in leads to behavioral changes that relate directly to health and fitness of the animal (Vogt et al. 2024), including relieving animals of stress that can affect experimental outcomes. These documented individual differences between animals, such as in reward sensitivity, can also affect how one designs and uses behavioral assays. In an extensive review on this matter, researchers document changes in brain morphology and function in many animals – from chickadees and sparrows to mice and rats – being kept in laboratory conditions as opposed to more enriched environments (Calisi and Bentley 2009). Even simple engagement with laboratory animals can influence an animal's behavior. Familiarity with a researcher can affect the performance of an animal in particular cognitive tasks, as shown in ravens and crows (Cibulski et al. 2014).

Repeatable behaviors can be artifacts of overly controlled conditions and are often cited by naturalistic neuroscientists as a reason for enriching an animals' environment during behavioral testing (Kentner et al. 2021). Under the conventional conceptual framework, housing in a more enriched condition eliminates the distortions experimental control brings to bear on a study, again assuming that there is a privileged view on behavior. For a model to achieve phenomenal access, or access to a behavioral phenomenon of interest (Dietrich et al. 2020), it is assumed that the scientist simply ought to remove the barriers of experimentation that can produce false behaviors, while still maintaining the standards of laboratory control that can help measure them.

Unfortunately, such assumptions often overlook the complexities of experimental methods and the different histories of lab animals. Specifically, standardized and repeatable behaviors are often generated from research models that are engineered as laboratory *tools*, whose status as a tool enables phenomenal access. A canonical example of this is studying naturalistic behavior in *Drosophila melanogaster*, or the common fruit fly, whose transformation to a 'standardized lab model' was famously documented in research (Kohler 1994). Wild-type controls were gradually and systematically modified over time, becoming a laboratory domesticated wild-type. These changes are so well-known among fly researchers that those who use the model admit to how little is known about variable behavior in the many strains of 'wild type' flies (Soto-Yéber et al. 2018, Kaun Lab) and surprisingly little is even known about *Drosophila melanogaster's* natural environment at all (Asinof and Card 2024).

The current characterization of naturalistic neuroscience would perhaps warn us that the failure to enact a model that accurately captures wild behavior is precisely the problem. And yet, as naturalistic neuroscientists put emphasis on phenomenal access, they overlook the process of how phenomenal access is achieved in the first place. Returning to artificially contrived behavioral studies may give us an answer, as we attend to the various experimental aims for using the model, as well as researchers' epistemic commitments.

Consider the following quote from a group engaged in fruit fly research:

> *Often the wild-type strain we use is dependent on the lab we were trained in. My recommendation is to test a bunch of the common ones in your assay and pick the one that produces the most consistent behavior. Use this for your background strain (i.e. the strain to which you backcross / outcross all of your transgenic / mutant lines). If you're not sure where to start, get the most commonly used line from a lab that does the type of behavior you are interested in* (Kaun Lab)

Through the conventional naturalistic framework, one might characterize this advice as a failure of naturalistic experimentation since each 'control' animal seemingly distances the researcher from understanding 'true' or 'real' *Drosophila* behavior. Note, however, that inconsistency in the model would be just as useless as an animal that is *only* consistent, as the former would make a study intractable, and the latter would no longer impart new information. All neuroscientists, naturalistic ones included, instead control their models in ways that support their many experimental aims and epistemic commitments, even when their models are highly mediated. Here, the 'real' behavior does not precede the methods used to control behavior, but rather comes *through* it, as it enables researchers to observe variability in the first place. It is not only futile to attempt to capture behavior 'in the wild' with a model organism, but the way observation of behavior is mediated also makes it the case that any effort to capture 'real' behavior ignores the process of mediation.

Why specific changes in a behavioral study qualify as 'Naturalistic'

Scientists constantly gain knowledge by using misbehaving models. Moreover, they continue to gain meaningful information about naturalistic behavior through canonical models notoriously removed from the 'real' world, such as the fruit fly, zebrafish, and rodents (Orger and Polavieja 2017; Dennis et al. 2021). Many 'naturalistic' studies use *Drosophila melanogaster* (Vanin et al. 2012; Asinof and Card 2024) even though there is little guarantee that the fly in the wild would even exhibit similar behavior. There are well-described pattern generating circuits that elicit different behaviors across contexts, variable signaling processes that are involved when flies engage in cooperative behavior, and distinct escape responses when stimuli presented to flies vary in speed. What is notably prioritized in such cases is the stability of behavior over changing contexts; thus, all of these discoveries, naturalistic in their own right, were made by making choices about which variables could be ignored or focused on.

Here, the organism's history, including the ways experimenters themselves made choices about which wild type to choose, help render reliable results. Researchers simply use models and engage in naturalistic studies to fit their aims in certain dimensions over others, such as prioritizing predicting behaviors versus the discovery of new patterns associated with behaviors. One non-intuitive outcome of this observation of experimentation is that greater experience with the animal and experimental conditions imparts knowledge over time that is in fact relevant for modeling decisions in naturalistic neuroscientific studies.

In neuroscience, experimental aims can range from caring about naturalistic studies for the discovery of neural circuits to furthering a technological end (see Box 1). Studying deviations from a standardized system – an animal that is an engineered tool – can give generalizable information. This might be relevant to the organism, but it might also go beyond knowing about the specific animal itself, since the experimental ends can vary widely: testing the behavioral flexibility of a model, trying to further standardize the model, or knowing what generalizable principles can be extracted. Such is often the case for naturalistic studies in *Drosophila*, where the stated goal is better knowing what the animal would do 'in the wild' while the researchers test the animal's behavioral flexibility to get more generalizable principles. One recent example of this this strategy has been coined 'reverse neuroethology' (Asinof and Card 2024). Here, researchers intentionally choose a highly modeled and controlled system, as this grants them better ways of leveraging the study of its natural behavior. In such cases, naturalistic behavior is paired with methodological development to become a vehicle for capturing criteria that can be applied across species, as opposed to being of interest in an undirected manner.

Two studies of social behavior can direct the idea of 'control' in very different ways, such that both are labeled 'naturalistic' but are not considered naturalistic neuroscience to the different groups studying them. Consider the simple change of moving rodents out of confined environments and into significantly larger arenas. Placing rats in large arenas can still fail to meet the naturalistic 'ideal' because these animals are behaviorally modified due to their selective breeding (Kondrakiewicz et al. 2018). Even so, not all naturalistic experiments are directed similarly. While a larger arena certainly matters for a range of experimental goals – for example, mechanistically examining system-wide brain activity and knowing how different brain regions may interact or behaviorally understanding the social transmission of fear – expanding the parameters of activity too much can generate worse results if one wants to know how odor cues are socially relayed (Datta et al. 2019) or whether or not exposure to predator odors in early life can affect later behaviors (St-Cyr et al. 2018). Having more data in the latter cases will not improving behavioral knowledge. Thus, naturalistic neuroscientists consistently make choices on what kinds of control is appropriate to leverage for one's experimental goals, given they are always navigating the limitations of their models.

To put it simply, there can be different experimental goals under the same heading of naturalistic studies. Is the researcher trying to better understand the general mechanisms of a behavior (i.e., systems involved in survival-critical decisions or what kinds of neurons generate courtship behaviors) or is she trying to understand the ability for the animal to adapt in various conditions (to better understand sensory cue integration or changes in neuronal sensitization)? Is she trying to model the animal into a tool that is comparable to another for future neuroscientific study? Is the researcher trying to study repeatable behavior to link their results to another kind of experiment or another species? Although all of these aims might be considered 'naturalistic' relative to a previous practice, they share very little in common beyond the label.

Replacing Traditional Models with Krogh Animals (Negative Models)

In discussions of naturalistic neuroscience, one can improve a behavioral assay for a more canonical model or re- theorize an experimental approach to modeling altogether by shifting away from the use of canonical animal *models* – such as flies, rats, and mice – more generally. In this vein, researchers have warned against conflating model organisms with natural, unmodified organisms (Katz 2016), and many doing naturalistic studies have rethought model choice, encouraging the use of nontraditional animals instead of lab-mediated ones (Mathuru 2020; Stevenson et al. 2017; Testard et al. 2021; Yartsev 2017; Jourjine and Hokstra 2021). This point of view recapitulates conventional framework which assumes that attempts to control and model animals pushes in the opposite direction of what is 'wild' behavior, since it remains to be seen that behaviors from generations of genetically engineered animals would replicate in the 'real world' (Vanin 2012, Crabbe et al. 1999).

In such discussions, Krogh's principle is often popularly invoked by neuroscientists seeking more naturalistic behaviors (Stevenson 2018; Katz 2016). This principle states that for any biological question, there is an organism whose biology is uniquely suited to answering that question.

Examples of Krogh's principle include using a squid to study potentiation because they have a giant axon (Yartsev 2017) or using mice to study olfaction because that is the animals' preferred means of sensory navigation, as they learn with smell better than auditory and visual stimuli (Nigrosh et al. 1975). It can also involve using other species of traditional models, such as fish and mice that are more specialized to certain tasks – such as aggression in Siamese fighting fish, parental care in deer mice (Bendesky et al. 2017; Jourjine and Hoekstra 2021) – to leveraging the loss of an ability, such as sine song, in *Drosophila yakuba* (Kelley 2024; Ye et al. 2024). In all these cases, a distinct feature of the animal makes it experimentally useful in a way that advantages it in comparison to others.

In contrast to Krogh's principle, animals may be chosen for study on the basis of 'practical' reasons, such as the availability of experimental tools for that model, as well as the logistical conveniences specific models themselves afford. For example, there are many species of fruit flies, but a vast majority of neuroscience research focuses on *melanogaster* because of the availability of lines and reporters. Scientists may also choose this model because of low costs, ease of supply, husbandry, established communities (conferences centered on specific models), databases (FlyBase, Xenbase, WormBook), ease of replicability, and more (Leonelli and Ankeny, Dietrich et al. 2020; Ding et al. 2024; Zilkha et al. 2016). One cause for concern is that these reasons can often trump others when deciding which model system to use for an experiment. For example, there is currently an overwhelming use of mouse and rat models in neuroscientific research, which has limited the range of the kinds of nervous systems studied (Yartsev 2017, Juntti 2019). However, in its criticism of practical models, the traditional conceptual framework for naturalistic behavior continues to fall short by suggesting that the use of nonconventional models somehow leads to a more accurate behavioral readout. Even in the case of Krogh organisms, different epistemic aims can arise that need to be specified.

To help with categorization and comparison, neuroscientists may appeal to naturalistic studies of behavior to study biological diversity, *splitting* organisms into their differences, or they can identify the common mechanisms and patterns by lumping animals together. In a historical review of Krogh's principle, researchers instead show that Krogh organisms do not depend on their generalizability (Green et al. 2020, 4). Unlike standard models whose similarities and differences to other systems are known, the representational scope of the Krogh animal is itself an empirical question. Because of this, it is difficult to know if the identified traits are generalizable or even relevant to other species.

When using Krogh systems, researchers may be less concerned with control for generalization and more interested in control with respect to behavioral flexibility. For example, by having extreme adaptations, Krogh animals often serve other experimental goals, such as helping scientists explore variation over identified physiological features (Green et al. 2020, 8), or by as serving as 'negative' models where animals *lack* the specific features or behaviors that scientists are interested in studying (Green et al. 2020). An octopus, an asocial creature, could be used to study sociality (Edsinger and Dölen 2018), or a naked mole rat, that is cancer resistant, to study anticancer mechanisms (Tian et al. 2013). Serving as comparison cases with positive models, such animals can impart invaluable information about the scope of behavioral flexibility, the importance of environment, energy expenditure, and more. Knowing why certain physiological limitations are *not* observed in selected species can offer invaluable insights.

Box 1: Epistemic Commitments underlying Various Experimental Aims

Various Experimental Goals with Respect to Behavior
Engineering aims: Knowing how context plays a role with respect to behavior and using that to elicit certain responses. Examples of this include leveraging the methodological development and manipulability of model organisms (genetic or otherwise). Example: This approach includes strategies for 'reverse neuro-ethology' (Asinof and Card 2024).
Technological aims: may not be directly tied to understanding the organism itself, but rather finding common motifs that can be exploited in other contexts (such as computational principles); here, naturalistic behaviors are leveraged as reasons for creating models in the first place so they can be meaningfully compared to other models. Example: Recognizing animal models as tools and as systems that are represented, some scientists interested in naturalistic behaviors insist on developing nontraditional animals into animal *models*. Examples of this can include taking individualized approaches to increase genetic accessibility in specific animals, such as using adeno-associated viral tools in amphibians (Jaeger et al. 2024), to large-scale, community building efforts, such as "cephalopod-omics," which tries to apply a conglomerate of techniques normally used on invertebrates, such as sequencing, imaging, and genetic manipulations, to invertebrates (Baden et al. 2024). These studies fall under the scope of naturalistic in their commitment to expanding the use of animals for research, even though the idea is to use these tools to make behaviors more meaningfully comparable, such studies introduce an intermediary for comparison as opposed to directly observing behavioral similarities or differences. It is thought that by doing so, experimentalists open the range of models that can be studied in neuroscience as well (Juntti 2019). Doing so may generate models of abstraction that make the models comparable in the first place.
Aim of exploiting variability: Finding out about behavior in order to derive principles that can be applied to a different system; this is not the same as finding generalizable principles, but instead exploiting knowledge about adaptation, aspects of variability, or extreme behavior. Example: Krogh's principle; researchers can look to naturalistic behaviors that are both extreme behaviors, or even the lack of behaviors (what is not there) to better understand the conceptual potentials and limits of behavior.

Consequences of Adopting a Conventional Framework:

Finally, failing to characterize what is 'naturalistic' without attention to experimental aims is a disregard to an experimenter's conceptual commitments. These can range from methodological commitments - such as determining if one should be in a field or the lab – to decisions about what scientific tradition one should draw from.

For instance, naturalistic neuroscience appeals to a large range of fields for inspiration, including neuroethology, ethology, behavioral ecology, comparative neuroscience, evolutionary neuroscience, and evolutionary biology. Here, naturalistic neuroscientists claim it is important to recognize a range of views: recognizing behavior as evolved (Krakauer et al. 2017; Miller et al. 2023; Testard et al. 2021; Datta et al. 2019), acknowledging the role of non-neuronal processes in supporting the complexity of behavior (MacIver 2009), the importance of fewer constraints on experiments, such as letting an animal run around freely (Gomez-Marin et al. 2014; Brown and de Bivort 2018; Parker et al. 2022), identifying innate behaviors

(Gomez-Marin et al. 2014), and identifying adaptation and selection pressures (Mobbs et al. 2018; Miller et al. 2023; Testard et al. 2021; Mobbs et al. 2018).

The traditions that inspire these activities tend to be cited interchangeably when naturalistic behavior is invoked. Some appeal to evolutionary biology to think about evolved behavior; others look to ethology for methodological inspiration, such as conducting experiments within an open field and uncontrolled environment. However, one challenge to understanding naturalistic behavior from various traditions is that they crucially differ in conceptual commitments, research cultures, and topics of investigation, varying also in their sensitivity to both context and evolutionary and developmental concerns. From traditions like ethology, evolutionary biology, and behavioral ecology alone, a spectrum of views arises that are bookended by two extremes. On one end are propositions to ignore all preceding paradigms used to study behavior; for example, those keen on an ecological tradition may not see value in studying nonhuman systems. Here, they can recommend abolishing all nonhuman animal studies when making attempts to study human behavior. On the other end, scientists see naturalistic studies of behavior as having natural continuity with traditional behavioral paradigms. Although they draw inspiration from ecological experiments, they can propose the status quo with minor adjustments here and there to make a study appear more naturalistic.

This picture gets even more complicated given that, even within a *single* tradition, there have been major historical disagreements about what behaviors are relevant to investigate and complicated discussions about how one should investigate them. This has certainly been the case in ethology and neuroethology (Dhein 2022). A second challenge, then, involves the difficulty of knowing which practices one should prioritize if the experimental aims differ by discipline or research traditions.

Ethologists, for example, have traditionally taken interest in the behaviors they observe in animals' respective ecologies and habitats. This means studying escape, food seeking, recognizing prey, and other practices that are relevant to the day-to-day challenges and survival of the animal. This contrasts with the vast repertoire of behaviors studied in neuroscience, such as drug-seeking behavior, play behavior, sociality, novelty-seeking, binge-eating, compulsive-like, anxiety-like or depression-like behaviors, and so on, that are set by the interests of humans and therefore been imposed on animals that have been highly manipulated and controlled. How can one meaningfully draw from ethology in neuroscience when their goals for studying behavior differ?

Similar to the challenges associated with animal models and behavioral testing, there will be disagreement depending on which tradition one draws from. However, in addition to having various experimental aims, there can be epistemic differences in theoretical commitments as well. This generates conflict with the concept of 'naturalistic' insofar as two groups could be opposing each other's understanding of naturalistic.

Another salient example of when differences in 'tradition' matter is with respect to representationalist commitments. There are dramatic consequences if one ascribes to a literal interpretation of brain patterns representing the 'real' behavior or if one is simply using such language heuristically. These differences separate how research can link across different practices with researchers not even recognizing when this is the case. Consider a naturalistic researcher who uses context to re-conceptualize olfactory behavior as extended and environmentally embedded (Jacobs 2023), whereas another sees olfaction as represented or mapped 'in the brain' (Brann and Datta 2023). While both may be committed to more naturalistic studies, their attitudes about what is in fact going on neuronally may be radically different, with one putting more of an emphasis on environment and embodied behavioral approaches, while the other tries to articulate the 'olfactory code' from a purely computational point-of-view.

Box 2: Animal Behavior Under Various 'Traditions' and Explanations

Ethological: A behavior-based science that is in the business of observing animal behavior and explaining what the animal is doing for the animal itself - Preferred Explanations: ethologists had strong commitments to 'innate' mechanisms, acquired releasing mechanisms, imprinting, drive intensity, fixed action patterns (escape response from Mollusk *Tritonia*), and more. - Ethological explanations made use of physiological information but did not reduce behavior to them, nor localize function to specific areas of the body - Especially interested in reproductive behavior - Self-described "animal watchers" (Tinbergen) - Field-based research
Neuroethology: Study of how animal behavior is realized by the central nervous system. - Preferred Explanations: Delineating what capacities a brain should have to realize the complexity of animal behaviors. Some neuroethologists claim that the nervous system evolved to produce behavior (Camhi 1984). - Interested in the comparative physiology of behavior (principles of neural function are studied in various animals) - How does the nervous system solve specific problems; these can include mating (such as a female sparrow or frog detecting, discriminating, and orienting toward a male call), escape responses, how animals use light to seek food, shelter, detect predators, or orient for navigation, and prey catching (frogs). - Going between 'field' and 'lab' sites
Behavioral Ecology: Studies that try to delineate the ecological factors that can drive behavioral adaptations. - Preferred explanations are rooted in evolutionary principles; seen as a 'successor' to ethology with less demand for theoretical coherence - Population dynamics and models; examining the genetic basis of behavior, behavioral syndromes, - Topics can include parental care - Field-based research
Evolutionary Biology (of Behavior): - Preferred explanation: Ultimate source of explanation is natural selection - Interested in fitness, selection (sexual selection), variation, and retention - Looking at behavior in groups, not just individual behavior - Interested in genomic changes associated with behavioral differences - Field or lab-based
Computational analysis of behavior (Computational ethology/ computational ecology): - Preferred explanation is in the language of computation and conceiving of behavior as information processing - Engaged in simulations of behavior; prioritizes prediction - Interested in substituting human decisions on behavioral motifs with computerized detections - Lab-based research; drawing from datasets

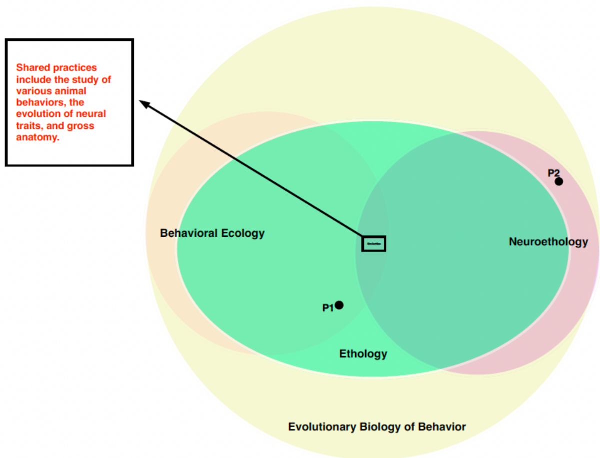

Figure 2: Although many of these traditions share overlap, their explanatory aims can differ in important respects. For example, while all these traditions notably study natural behaviors such as threat detection or foraging, the representationalist commitments of neuroethologists who subscribe to innate behaviors are going to look radically different from those of neuroethologists. The scale of explanation will also differ between them, such as an explanation that meets the criteria for overlapping traditions (P1) but fails to satisfy assumptions within another (P2).

Keeping Mediated Models

The animal models, tools, technologies, and traditions that make up the landscape of naturalistic neuroscience have generated conversations that overshadow the theoretical commitments and goals that inform how those technologies are supposed to meet one's modeling aims.

This piece tempers enthusiasm for the conventional framework of naturalistic studies by challenging the idea that capturing 'real' behavior is hindered by the traditional strategies for experimental control. The conventional framework that describes naturalistic behavior reduces away the many theoretical or epistemic commitments that underlie behavioral studies. One might try to understand how an animal's behavior changes in new contexts and meet other demands, such as knowing how animals compare to one another, or how to make animals comparable in the first place. Many of these strategies leverage control to fit specific goals, undermining the idea that there is any single *privileged* approach to study behavior.

However, recognizing how 'natural' is used as a stand-in term for various experimental aims corrects perspectives on experimentation in more ways than one. For example, neglecting assumptions of objectivity have historically led researchers to harmful outcomes. The term 'natural' has uniquely been a social signifier of exclusion with roots in scientific studies, whether in antiquated discussions about assertive, ambitious women, or more recent history of homosexual behavior. These studies may seem unrelated to the current topic because naturalistic studies are trying to dispense with bias to reveal 'true' behavioral patterns. And yet, the patterned use of 'un/natural' is the same: In the same way that labeling homosexuality as 'unnatural' because of biological differences was about hidden values, labeling a bar pressing rats 'unnatural' because of its differences to wild type rats is about hidden epistemic aims. Failing to acknowledge these aims and the role of the experimenters behind them irresponsibly masks the differences with the word 'natural'.

However, even if there *were* an option to do naturalistic neuroscience in a way that abolished the scientist as a mediator of observation, no one should want that. For one, it would be experimentally intractable and generate unintelligible results. Although it is true that humans do not use sonar or electroreception to locate their food, use smell or magnetoreception to navigate, or see with polarization or infrared, we came to know most of these differences in other animals from previous empirical research. This demonstrates an irony about the study of naturalistic behavior, where much of the reason for knowing that studying 'naturalistic' behavior is preferable to behavior confined and controlled environments precisely emerges from the fact that those behaviors were first studied in controlled settings (Clarkson et al. 2018). However, a second reason we should not eliminate the human observer is that it makes empirical observations of the world intelligible *to us*. It is our experiences that give us the capacities for modeling decisions in neuroscientific experiments (Nemati 2024). Part of the reason for this predicament is that neuroscience is a science that inherently relies on modeling and abstracting from complexity to proffer appropriate explanations (Chirimuuta 2024). Unlike some classic ethological practices that were simply in the business of documenting animal activities, there are different requirements for the kinds of mechanistic and causal explanations neuroscience should be giving us. Moving forward, it would benefit us to take a historical lens to the technological improvements that have made naturalistic studies of behavior possible, as well as the theoretical assumptions embedded in them. We now raise many questions about behavior because it is now possible to capture the dynamic and multi-dimensional features of an environment and of brain activity. Shifts from traditional views of brain modularity (Anderson 2021) have allowed scientists to favor probabilistic distributions of neural and behavioral activity that rely on more neural data and population-level activity over linear statistical models of discrete variables (Brown et al. 2004; Cunningham and Yu 2014; Pang et al. 2016). Dynamical and adaptive thinking (Fairhall et al. 2001) emphasizes the changing brain, encouraging the study of the brain's robust plasticity (Gomez-Marin 2014). Neuroscientists can also now simultaneously record the activity of very large numbers of neurons, from many different brain regions, as the animal engages in a specific task (Neuropixels), enabling analyses of neural networks (Bassett and Sporns 2017; Bassett et al. 2018) while powering dynamical explanations and use of dynamical systems theory itself (Izhikevich 2007; McClelland et al. 2010; Ross 2022). Finally, improved devices, such as better GPS technology, microphone arrays, motion sensors, and sophisticated cameras, now capture complex behavior with more storage space to do it.

Twenty years in the making, these shifts share a heightened regard for behaviorally relevant naturalistic and environmental factors. Yet, understanding how these tools embed their own assumptions is important for knowing how certain studies of behavior are privileged over others.

While much work remains to be done to show how modeling aims link to experimental outcomes, talking past one another can have more serious epistemic consequences for experimentation, such as when experimenters put focus on experimental choices when their underlying assumptions do in fact differ. Not recognizing that we are asking different questions may generate miscommunication and the illusion of reproducibility errors, as was the case in two similar odor studies that got varied mechanistic explanations on the basis of different tasks (Federick et al. 2017). It also bears consequences by distracting researchers when there are quick explanations for why an experiment fails. Rather than adopt a seemingly better model or task, or using a more advanced tool to measure behavior, as has often been suggested, it may do behavioral neuroscience good to accept that not all experimental goals require such approaches.

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