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Article

Naturally Occurring Nonhuman Primate Models of Psychosocial Processes

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Abstract

Human research into psychological processes such as anxiety, depression, or loneliness typically involves accruing cases in which the phenomenon of interest is naturally occurring, and then comparing such a sample with control cases. In contrast, animal research designed to model similar processes to test mechanistic hypotheses typically involves inducing the phenomenon of interest via some exogenously (i.e., human) administered procedure. In the present review, the author proposes that naturally occurring animal models can complement induced models in understanding complex psychological phenomena. Advantages and disadvantages of naturally occurring versus induced models are described, and detailed examples of three naturally occurring models—for loneliness and health, behavioral inhibition and asthma, and social functioning and autism—are described, along with a formal program (the BioBehavioral Assessment program) at the California National Primate Research Center, that is designed to quantify variation in biobehavioral processes in infant rhesus macaques to facilitate development of naturally occurring models. It is argued that, because of the similarity in complex behavioral and psychological processes between macaques and humans, naturally occurring primate models provide a bridge between human studies and induced primate models and have the potential to identify new models for translational research.

Key words: animal models; asthma; autism; behavioral inhibition; loneliness; social behavior; temperament

Introduction

If we were interested in examining the role that an inhibited temperament style in young children plays in the later development of social anxiety disorders, we might survey a day care facility, and ask the staff to fill out a questionnaire assessing each child's tendency to exhibit distress, especially in response to novel people or unfamiliar situations. Alternatively (or in addition), we might also survey the parents of the children and ask them the same questions. Or we might simply observe the children and record their behavior, either during free-behavior periods, or in response to some simple social situations that we construct. Once we have collected the data, analysis would reveal which children show evidence of behavioral inhibition, and which do not. We might then conduct periodic follow-ups to determine if the inhibited children are still inhibited, or if they are developing social anxiety. If our interest is in the biological mechanisms through which inhibition affects later anxiety, we might combine our questionnaire/behavioral data collection with physiological data collection (e.g., heart rate recordings, or collection of saliva) to test hypotheses about physiological correlates/biomarkers of inhibition and later social anxiety. Once we have identified our expected link between inhibited temperament and later social anxiety, we might then conduct a study in which we intervene (either behaviorally or pharmacologically) to prevent the development of social anxiety in a new cohort of inhibited children. Studies such as those

we have described have been conducted, and have generated important results (see review in Fox et al., 2005).

Now suppose we were interested in developing a nonhuman primate model to study the same thing. This would enable us to take advantage of the many benefits of animal models. For example, we can experimentally manipulate the animals in ways that would be unethical with humans; we could collect tissue samples that would be unavailable from human subjects; we would have careful control over many aspects of the lives of our subjects; we would benefit from the more rapid pace of development of nonhuman primates, so that a single study of inhibited temperament in infant monkeys and later social anxiety in the juvenile stage could all be accomplished within a single five-year grant period. And because we know that exposure of a pregnant female to a noise stressor during mid- to late gestation results in offspring that show characteristics of behavioral inhibition (Clarke and Schneider, 1997), we could randomly assign pregnant females to a stressor or a control condition to generate inhibited infants or their controls, respectively.

The description of these two approaches is meant to illustrate that human and nonhuman research often proceeds along different, but usually quite complementary, lines. Human studies often involve surveying large numbers of individuals to find those that have the phenotype of interest, or waiting until cases show up at the clinic with that phenotype, after which we might identify some case-controls for comparison. We would consider these "naturally occurring" cases, in the sense that the scientist did nothing to generate the individual differences that are of interest to her. In contrast, animal studies often involve "inducing" the condition of interest, and then comparing those individuals with controls that did not experience the inducing event. In the human case, we do not have a clear idea of why the inhibited children are the way they are—perhaps the mothers of some of these children were prenatally stressed, for example—and the possibility exists (and actually is quite likely) that the outcome of interest, namely inhibited temperament, shows equifinality: "the same end state may be reached from a variety of different initial conditions and through different processes" (Cicchetti and Rogosch, 1996, p. 597). Prenatal stress is one such initial condition, but there are likely others. In contrast, we know exactly why our monkeys are showing behavioral inhibition—they experienced stress as fetuses at specific times during gestation. Are the results of the monkey study generalizable to all behaviorally inhibited children, or only those whose inhibited temperament originated via prenatal stress? We do not know the answer, but the question itself illustrates an important point about animal models, namely that they are "models" of what we are interested in knowing about in humans. A model is, by definition, a simplified representation of a phenomenon, and the very process of simplification means that some of the complex reality is lost.

We propose a second approach to development of animal models, one that more closely parallels the situation in humans: identification of naturally occurring variants for further study. Such an approach has benefits and costs. To continue the example of behavioral inhibition, as with humans, we would not know the origins of the inhibited phenotype and in fact, in both species, the inhibited sample would almost certainly represent individuals whose inhibition reflects a heterogeneous set of precipitating conditions (i.e., in both species, inhibited temperament shows equifinality). In this sense, one could argue that the external validity of this model might be higher than that of an induced model. On the other hand, it is possible that this heterogeneity may require larger samples than might be needed for a sample in which inhibition was induced by a single event, prenatal stress. In fact, given the detailed records kept at modern primate facilities, study of the multiple origins of a naturally occurring phenotype may facilitate the development of new induced models; for example, we have demonstrated that, for monkeys with the low transcriptional variant of the *monoamine oxidase-A* gene, greater exposure to ketamine in the first trimester of gestation is associated with an inhibited temperament style in infancy (Capitanio et al., 2012). A major cost in studying naturally-occurring models, particularly of psychological phenomena, however, is in identifying the phenotype in the first place. Behavioral processes typically require well-trained humans to spend considerable time quantifying the behavior of large numbers of animals in order to find the relatively small number of individuals displaying the phenotype of interest.

We recognize, of course, that the phenomenon of naturally occurring animal models of human disorders is not new; this is particularly true in the area of nonhuman primate research, where, unlike for rodents, the species are genetically diverse and outbred, which can contribute greatly to the presence of variation. The field of veterinary medicine contains examples of spontaneously occurring health problems in primates that eventually became an animal model of a human phenomenon. In terms of behavior, others have also examined naturally occurring variation. Some investigators, for example, have mined colony databases to identify variation in birth weight, and then gone on to examine contributors to this variation, as well as biobehavioral consequences of low birth weight (e.g., Burbacher et al., 2013). Others have examined naturally occurring variation in maternal style as a contributor to later developmental outcomes, as well as the consequences of abusive maternal behavior which, in colonies of rhesus monkeys, is fortunately rare (Parker and Maestripieri, 2011). In my laboratory, we have been developing naturally occurring models of psychological phenomena and exploring the associated physiological- and disease-related outcomes, since the early 1990s (Capitanio et al., 1994). In this report, we provide three recent examples of this approach, the second and third of which originated from a colony-wide assessment program that identifies a variety of phenotypes for further study by investigators both within and outside our own facility. For all examples, we provide some details about methods used to identify the phenotypes of interest.

A Rhesus Monkey Model of Loneliness

In humans, loneliness has been shown to be a significant risk factor for mortality, with effect sizes similar to what one sees for the risk of obesity (Holt-Lunstad et al., 2015 and references therein). Loneliness has also been shown to be significantly related to a variety of poor health outcomes, including high blood pressure, coronary heart disease, cognitive decline, depression, and dementia (Green et al., 1992; Hawkley et al., 2010; Holwerda et al., 2014; James et al., 2011; Valtorta et al., 2016). Given that loneliness tends to increase with age, and that the aged population is growing, some have referred to loneliness as an epidemic that is happening now and is likely to get worse (Cacioppo and Cacioppo, 2014; Linehan et al., 2014). Unfortunately, the mechanisms by which loneliness can impact health are largely unknown, and recently, a call has been made for more animal studies to address this very issue (Cacioppo et al., 2015).

Does loneliness exist in nonhuman animals? An argument has been made that loneliness may actually be biologically adaptive, and so may very well be present in other taxa (Cacioppo et al., 2014). Humans are a highly social species, and our sociality is embedded deeply in our biology. From this perspective, loneliness signals that something is amiss in our social world, and could motivate us toward greater social connection. In this sense, loneliness is like other aversive states that we experience (e.g., hunger, pain) that motivate behavior change. Viewed in this way, there is nothing necessarily species-specific about loneliness, and it may well be a psychological feature of any highly social species (Cacioppo et al., 2015). Loneliness is, however, distinct from merely having a lack of social connections; that is, loneliness does not reflect *objective* social isolation, but rather *perceived* social isolation. Perlman and Peplau (1981) suggested that the essence of loneliness is a discrepancy between one's actual and one's desired social network. Thus, one could have a small network and be perfectly satisfied with it, whereas someone else might have a large network, but feel that that network does not meet her psychological needs. One might think that someone who is lonely could simply make more satisfying social connections, and the problem would be solved. Unfortunately, loneliness involves an extreme vigilance for social threat, which can manifest as a fear that one's social overtures might be rejected. This fear of rejection means that one can be lonely despite being in a crowd, or being surrounded by family.

How might one go about studying loneliness in a nonhuman species? An easy answer might be to simply isolate the animal from its companions and observe changes in behavior and physiology. This would be an induced model of loneliness. In fact, a substantial animal literature exists that involves randomly assigning animals to social versus isolated housing conditions. These studies have not been about loneliness per se, but rather about the effects of environmental enrichment on brain and behavior, investigation of behavioral disorders and potential treatments, and so on. Recent reviews on the neurology and endocrinology of loneliness (Cacioppo et al., 2015; Cacioppo et al., 2015) have relied on such studies to provide insights into mechanisms by which loneliness might affect health in humans. But are these studies modeling loneliness (and we reiterate that the authors of such studies are typically not framing their studies as models of loneliness)? As models of loneliness, per se, we would argue that they are inadequate, as they confuse the subjective experience that is at the heart of loneliness with the objective alteration of the animal's social environment.

Loneliness, whether in humans or rhesus monkeys, occurs in a social context. An important psychological component of loneliness is that social opportunities exist—the individual simply cannot make use of those opportunities effectively. Equally important, however, is that within that same social context, there are other individuals that *can* make use of the available social opportunities—the lonely and nonlonely group members live side by side, but differ in their perceptions of the same situation. Viewed in this way, then, loneliness is not so much about the structure of an individual's social environment, but rather reflects psychological constraints affecting the individual's view (and use) of that environment. To understand how loneliness affects health, we need to know what characterizes—in psychological, neuroendocrine, autonomic, and immune terms—those individuals that are lonely and compare them to individuals that are not lonely. Simply isolating an animal brings a variety of other factors into play (e.g., a total absence of companions; a change in the physical environment), which could themselves affect the processes of interest. Moreover, employing a manipulation such as social isolation to model loneliness presupposes that all animals will respond similarly to the manipulation; compelling evidence indicates, however, that lonely and nonlonely individuals are different from each other in multiple ways, both psychological and physiological. Depending on how the isolation situation is constructed, lonely and nonlonely individuals may respond in the same way (though perhaps for different underlying reasons, demonstrating equifinality) or they may respond differently.

As described earlier, identifying naturally occurring rare phenotypes in animals requires considerable effort, and, in collaboration with colleagues Dr. John Cacioppo at University of Chicago and Dr. Steve Cole at UCLA, we have employed just such a strategy in our studies of loneliness in adult male rhesus monkeys (Capitanio et al., 2014; Cole et al., 2015). This work grew out of earlier work that examined variation in the personality trait sociability (which reflects a tendency to affiliate), and how that variation was related to differences in physiological functioning and disease progression in the simian immunodeficiency virus (SIV) model of AIDS (Capitanio et al., 2008; Sloan et al., 2008). In those studies, our procedures were simple: a sample of animals was identified (e.g., 5- to 8-year-old adult male rhesus monkeys, living in their natal, outdoor, 0.2-ha field cages at the California National Primate Research Center [CNPRC]), trained behavioral observers recorded quantitative data on each animal using focal animal sampling twice per day, four or five days per week for two to four weeks (depending on the study), and at the end of the observation period, rated each animal on a series of personality traits. Factor analyses of the traits identified the factor sociability, which reflected high ratings on the traits warm and affiliative, and a low rating on the trait solitary. In these early studies, our interests were primarily in the rating data; the focal observations were to be used to validate those measures, but more importantly, served to ensure that the observers who would do the ratings had equal opportunity to watch each animal (instead of having their attention drawn disproportionately by the most vocal or boisterous or aggressive animals). For some of the SIV studies, animals were selected without regard to personality status, but for other studies, we selected animals that were high or low on the distribution of sociability. Animals were then relocated for the specific study in which they were enrolled.

Our interest in loneliness arose because it appeared that there were two types of animals that had low scores for sociability (low-sociable monkeys were defined as those that had a z-score of −0.5, which reflects the lower 30% of the distribution). Our assumption was that putatively lonely animals would show, overall, a low level of social attainment, yet might want more social interaction than they currently have. This would be manifested in some low-sociable animals having higher scores for social initiations—approaches and walkbys—and that they might be more likely to make those social overtures to "safe" interaction partners, such as juveniles or adult females. In contrast, the other group of low-sociable animals (which we refer to as putatively introverted) would show low levels of social initiations to all. Cluster analyses revealed evidence of this pattern; in fact, levels of social initiations by putatively lonely animals were comparable to those seen among high-sociable animals (Figure 1A), and the lonely animals distributed their initiation behaviors principally to adult females and juveniles, whereas the distribution of initiations by introverted animals was more balanced (Capitanio et al., 2014;

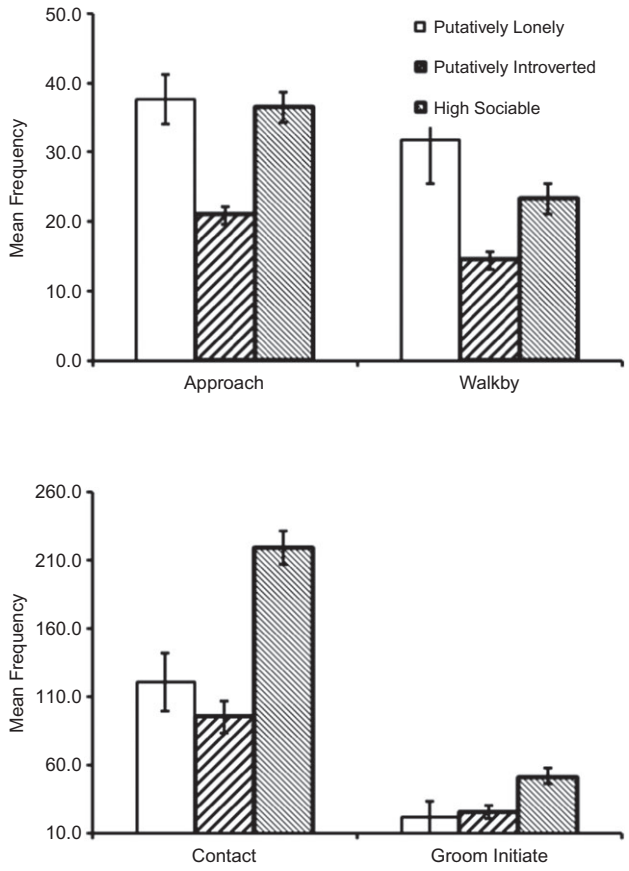

Figure 1 Frequencies of social behaviors for putatively lonely (open bars), putatively introverted (wide, upward diagonal), and high-sociable (light, downward diagonal) adult male rhesus monkeys. (A) Simple initiation behaviors. (B) Complex social behaviors.

Table 1). When we examined the frequencies of more complex social behaviors, however, such as contact and grooming, we found that lonely animals' frequencies were comparable to those of the introverted animals; both groups had lower frequencies than did high-sociable animals (Figure 1B). In short, for more tentative behaviors involved in social initiations, lonely animals (which comprise 15–20% of our low-sociable samples), like high-sociable animals, had high frequencies (with introverts showing the lowest frequencies), but for more complex behaviors, the lonely and introverted animals were similar, showing lower frequencies than the high-sociable monkeys. We believe these results capture the essence of loneliness—a discrepancy between one's social desires and one's social attainment.

The translational strength of this naturally occurring model of loneliness was highlighted by the fact that we were simultaneously studying humans, and both papers (Capitanio et al., 2014; Cole et al., 2015) also presented data on humans. When we probed the psychological differences between our lonely and introverted monkeys, we found evidence that lonely monkeys, like lonely humans, were sensitive to social threat. For example, on three occasions, one week apart, we showed high-sociable, lonely, and introverted monkeys two videotapes of unfamiliar animals displaying viewer-directed affiliative behaviors. One video depicted a juvenile male monkey, and the other showed an adult male. As expected, lonely animals showed greater social interest in the "safe" juvenile target compared to the "risky" adult target, whereas no such preferences were seen for the high-sociable or introverted monkeys. Moreover, this group difference waned across the three sessions for the lonely monkeys, suggesting that their initial response preference stemmed from social novelty, and not just an inherent preference for juveniles. This parallels what is seen among lonely humans—the fear of rejection is most evident with novel social partners. This and other behavioral studies described in our papers (Capitanio et al., 2014; Cole et al., 2015) suggest close parallels with the phenomenon of loneliness in humans. But what about physiological data? Leukocytes from both lonely humans and lonely adult male rhesus monkeys showed many parallels: a pattern of gene expression associated with increased inflammation and reduced antiviral responses; downregulated functional activity of the glucocorticoid receptor, and upregulation of a transcription factor that is proinflammatory; high urinary levels of norepinephrine, and elevated monocyte numbers. The monkey studies extended the human results, however, by suggesting that it is a specific subset of monocytes that was increased in the lonely animals. In addition, analysis of archival data from an SIV study demonstrated that monkeys identified as lonely showed poorer control of the virus: reduced interferon gene expression, elevated SIV viral set-point, and reduced anti-SIV antibody titers. Together, the full set of results from the human and monkey studies suggests that lonely individuals' chronic perceptions of social threat stimulate the sympathetic nervous system, which affects the type and number of leukocytes (especially monocytes) produced in the bone marrow. These cells are primed to create an inflammatory response even when such a response may not be needed. The cells also seem to be less responsive to the action of glucocorticoids, one function of which is to "turn off" a proinflammatory signal. These cells are also less effective in fighting off viruses and in the presence of a viral infection, lonely individuals show poorer control of viral replication.

As the loneliness example indicates, we believe that the many parallels—at both the psychological and physiological levels—between rhesus monkeys and humans demonstrate the translational value of our approach. This approach involves broadly surveying natural populations of animals to identify the phenotype of interest, then utilizing the benefits of animal model research—experimental manipulation, tight control of conditions, greater access to tissue—to better understand the mechanisms by which this psychological condition might affect health. Our next steps in this research program are to delve deeper into primary and secondary lymphoid tissue to further understand how the sympathetic nervous system affects development of leukocytes (and especially monocytes) and immune function in lonely versus nonlonely individuals—studies that are impossible to do with humans. Moreover, our current studies aim to determine whether a specific psychosocial intervention can improve behavioral functioning in lonely monkeys, and whether this behavioral improvement is accompanied by beneficial changes in primary and secondary lymphoid tissue.

Surveying the Psychological Landscape—The Biobehavioral Assessment (BBA) Program

As suggested above, one of the drawbacks to developing naturally occurring models is the effort required to survey large numbers of individuals to identify the rare phenotypes of interest. In 2001, we established a 25-hour-long assessment program at the CNPRC that was designed to quantify variation among

3- to 4-month-old rhesus monkeys in a number of measures that we refer to as "biobehavioral organization" (see also Capitanio, 2017, which provides more extensive information on this program). Behavior-related measures include assessments of temperament, behavioral responsiveness to social and nonsocial stimuli, and activity. Because of the role of the hypothalamic-pituitary-adrenal (HPA) axis in a variety of psychological processes, as well as its role in countering inflammation, we also included a thorough assessment of HPA regulation. In addition, we obtained a complete blood count for each individual, and genotyped the animals for two genes of neuropsychiatric interest, the promoter for the serotonin transporter gene (*5HTTLPR*) and the promoter for the monoamine oxidase-A gene (*MAOA-LPR*). In addition to these biobehavioral data, we also recorded information on the dam (e.g., age, number of prior conceptions), the infant's rearing history up until the time of our assessment at 3 to 4 months of age (e.g., outdoor field cages, nursery), SPF status, whether the infant was foster-reared, and degree of Chinese ancestry (Kanthaswamy et al., 2011).

Our goals for the BBA program from the outset were to provide this information to both colony management staff who might use the data to help make management decisions, and to principal investigators to select animals with extreme phenotypes for study (which can thereby reduce the number of animals needed for a study), for stratifying experimental groups (to spread the naturally occurring variation evenly across treatment groups), and even to mine the database itself to test hypotheses of interest. At a more basic level, we were interested simply in identifying how much variation is present in our colony, and what both the causes and the consequences of this variation might be. As the program developed, however, we found that a number of principal investigators were interested in our assessment program to provide them with a set of biobehavioral outcome measures that could be used to test their own hypotheses about the consequences of prenatal and perinatal manipulations that were of interest to them (e.g., maternal immune activation during pregnancy [Bauman et al., 2014]; iron deficiency [Golub et al., 2006]; neonatal hippocampal and amygdala lesions [Goursaud et al., 2006]; early rearing experience [Rommeck et al., 2011]). One unintended consequence of the development of this program, then, was that the assessment program itself, and not just the data from the assessment, became a secondary resource for investigators.

In the next section, we describe two examples in which variation in measures obtained from the BBA program has been used to identify correlates of physical and mental health outcomes. In each example, data were obtained from the monkeys months to years after they had participated in the BBA program, and the goal was to identify BBA measures that were statistically related to those outcome measures. Because retrospective studies like this can lead to spurious results, ideally after this initial analysis the next step would be to use the BBA data in a prospective fashion to confirm the suspected relationships, and then to try to better understand the mechanisms by which the BBA measures might affect the health outcomes of interest. Ultimately, once the mechanisms are more clearly understood, interventions can be implemented to try to forestall the development of adverse health outcomes. As each example is discussed, we will briefly describe the specific BBA assessments that produced the data of interest; more extensive details of all assessments can be found in Capitanio (2017). Space limitations prevent us from describing other naturally occurring models that have been developed with the BBA database; interested readers can find discussions elsewhere, however (e.g., depression [Hennessy et al., 2014]; anemia [Golub et al., 2009]; diarrhea [Elfenbein et al., 2016]; stable social relationships [Weinstein and Capitanio, 2008; 2012]).

Behavioral Inhibition and Asthma

In the United States, asthma is a significant health concern, with recent estimates indicating that 7.7% of the population self-report asthma (CDC, 2016). Numerous studies, dating back to the 1970s have demonstrated a significant psychosocial component to asthma, with evidence that children with asthma are more likely to also display an inhibited temperament style, and adults that show anxiety and depression (which are associated with inhibited temperament in childhood) are at greater risk for developing asthma (see Capitanio et al., 2011). One problem with many of these studies is that the data (particularly data from national surveys) are usually in the form of a self-report, in which participants are asked questions like "Have you ever been told by a doctor or other health professional that you had asthma?" It is the rare human study that employs physiological measures of lung function.

Asthma has been a subject of interest to the Respiratory Diseases group at CNPRC for many years, with studies typically assessing lung function objectively via pulmonary function testing. In this procedure, increasing doses of methacholine are administered to anesthetized monkeys until such time as they demonstrate 150% or 200% (depending on the study) of baseline airway resistance. Animals that achieve this level of resistance at low concentrations of methacholine are said to show airway hyperresponsiveness (AHR), which is a critical feature of asthma. One study of AHR in rhesus monkeys that had been conducted for other purposes was found to include 21 animals, ranging in age from 19 to 35 months, that had participated in the BBA program at 3 to 4 months of age. Given that the psychological construct most relevant to young children with asthma is inhibited temperament, we tested the hypotheses that inhibition might predict AHR in these animals. We selected two measures that reflect an inhibited temperament style. The first measure was obtained from the very first assessment that animals in the BBA program undergo—approximately 15 minutes after the animals are moved into the holding cages in our test area where they will spend the next 25 hours, their behavior is recorded for a 5-minute period. The frequency and duration behaviors from this assessment were subjected to an exploratory and confirmatory factor analysis (Golub et al., 2009), and two factors emerged: activity and emotionality. We selected the emotionality measurement because one feature of an inhibited temperament is withdrawal from novel situations, which can manifest as reducing the amount of affective behavior displayed. The second measure we selected is vigilance, which is a temperament dimension that is assessed in the BBA program at the very end of the 25-hour assessment period: once all formal testing is concluded, the technician that had tested the animals rates all animals on a list of 20 traits (e.g., confident, aggressive). Temperament dimensions were derived by performing exploratory and confirmatory factor analyses on the raw rating data (Golub et al., 2009). Finally, we also looked at a measure of HPA regulation. While the relationship of asthma to HPA function is somewhat unclear, some studies have reported that blunted basal or stress levels of cortisol are associated with asthma (Buske-Kirschbaum et al., 2003; Kauffmann et al., 1999). Moreover, there is some evidence that inhibited temperament may be associated with blunted cortisol responsiveness (Gunnar et al., 2009). We averaged

two samples taken at approximately 2 and 7 hours after the animals arrived in our test area.

The results of our retrospective analysis (Capitanio et al., 2011) showed that the three measures together—emotionality, vigilance, and plasma cortisol concentrations—predicted which animals showed AHR and which did not. The logistic regression with these three measures entered as predictors correctly classified 95.2% of the animals (i.e., 20 of the 21 subjects). Armed with these results, we performed a prospective study by selecting 24 animals from the BBA database that had low emotionality, high vigilance, and blunted cortisol, and contrasted them with 25 noninhibited controls on our pulmonary function test. Again, behaviorally inhibited animals were significantly more likely to show AHR compared to the controls (Chun et al., 2013). Our asthma work provides a clear example of the initial steps of the analytic strategy described above: an outcome of interest is identified, the biobehavioral database is queried to identify potential correlates of that outcome, and then a new sample of animals is drawn to prospectively test the relationships seen in the retrospective study.

One unexpected outcome of this line of research was that the inhibition-AHR result appears to be a model of nonatopic asthma. About two-thirds of asthma cases involve allergic (i.e., atopic) responses, whereas the other one-third do not. People with nonatopic (also called "intrinsic") asthma are typically older than those with atopic asthma, and the clinical course is generally considered more severe (Humbert et al., 1999). Respiratory biologists at CNPRC have focused almost exclusively on atopic asthma. In fact, the animals in our retrospective analysis (Capitanio et al., 2011) had all been skin-tested to determine their allergic responsiveness. While inhibited temperament was associated with AHR in that study, it was not associated with atopy. In our prospective study, we tested additional hypotheses concerning whether inhibition is associated with immune responses that are characteristic of allergic asthma. Again, these results were nonsignificant. Thus, behavioral inhibition was associated, in both studies, with the airway response, which characterizes both atopic and nonatopic asthma, but was not associated with the atopic and immune responses that characterize atopic asthma. These results suggest that the behavioral inhibition/AHR link may actually be a model of nonatopic asthma, a condition for which no satisfactory primate model has existed up to this point. We suspect that the AHR response may be a manifestation of an alteration in autonomic nervous system activity, which can be triggered by aeroallergens (in the case of atopic asthma) or by other, still unknown factors (in the case of non-atopic asthma). Two ongoing studies are aimed at better understanding the mechanisms by which behavioral inhibition is associated with AHR—and both are utilizing the strategy of identifying subjects from the BBA database as subjects.

Visual Attention and Autism-Related Social Deficits

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects 1 in 68 children in the United States (Christensen et al., 2016) and is characterized by deficits in social perception and socio-emotional reciprocity (DSM, 2013). Unfortunately, there are still no treatments available to treat the core social deficits in autism, and a principal reason for this is that the underlying biology remains largely unknown. Mouse models are of limited value in that they do not possess the complex social abilities that characterize our species and that would be valuable to model.

In collaboration with Dr. Karen Parker from Stanford, we have been developing a novel rhesus monkey model of the social deficit seen in ASD by studying naturally occurring variation in sociability, as described above in our example of loneliness (Sclafani et al., 2016). Large numbers of young male monkeys (typically 1–4 years of age) that are living in our outdoor field corrals are observed and rated, and we identify low- and high-sociable animals. This research program has multiple goals, including identification of biomarkers associated with variation in sociability as well as understanding better the socio-cognitive deficits, at the psychological level, that distinguish low- from high-sociable animals. (We note that, in our initial studies, we have not made the distinction, among our low-sociable animals, between those that we described above as putatively lonely versus putatively introverted. As indicated earlier, though, the "lonely" phenotype is rare.) One goal, however, was to utilize data from the BBA program to determine if we could identify significant behavioral markers that might enable the BBA program to serve as a high-throughput screening tool to identify animals at-risk of becoming low- or high-sociable later in life. In fact, among children, socio-cognitive deficits related to later development of ASD appear within the first 2 years of life before many of the social deficits are apparent (Merin et al., 2006). If successful in identifying BBA measures in infancy that map onto later social deficits, then the potential would exist to use this animal model to intervene at a very early age in an attempt to forestall the development of these social deficiencies.

We focused on two assessments in the BBA program that tap into some of the most fundamental social processes necessary for successful life in a social group—the ability to recognize faces, and the ability to respond appropriately to social signals. These processes are assessed in two procedures in the BBA program: a test of face recognition (FR), and a video playback (VP) test, respectively. The FR task is administered after the behavioral observations in the holding cage (described above) have been performed, and after the first blood sample has been drawn, about 2.5 hours into the 25-hour program. Facial recognition is determined using a procedure that assesses a visual preference for one stimulus over another, and capitalizes on the fact that macaques, like humans, have a general preference for novel stimuli in such a test (Gunderson et al., 1987). The FR test comprises seven problems, each involving color photographs of unfamiliar rhesus monkey faces showing a neutral expression. For each problem, a monkey is presented with a pair of identical pictures for a 20-sec familiarization trial, followed by a 5-sec intertrial interval. Next, an 8-sec recognition trial is presented in which the now-familiar picture is paired with a novel one. After another 5-sec intertrial interval, a second recognition trial is conducted but with the positions of the familiar and novel stimuli reversed. A camera is positioned between the two stimuli and records the gaze of the viewing monkey for later coding by a skilled behavioral observer. Face recognition memory is inferred if subjects looked longer at the novel, than at the familiar, face. After all animals experience the FR task, each animal is tested in the VP task, in which a color videotape, 10 min in length and depicting an unfamiliar adult male rhesus monkey, is played and the animals' responses recorded. The 10-minute-long tape contains seven segments, four of which show the stimulus monkey displaying nonsocial behavior—manual and oral exploration of the cage, visual exploration of the cage and surrounding area, etc.—and three segments in which the stimulus monkey displays viewer-directed aggressive behavior such as threats,

tooth-grinds, lunges, and cage shakes. The principal measures of interest in the VP test are the rates at which the monkeys looked at, and gaze averted to, the aggressive and neutral segments.

As expected, the 25 LS and the 25 HS monkeys differed in their preferences for the novel stimuli in the FR test—the HS monkeys showed a statistical preference for the novel faces, but the LS animals did not. During the VP, both sets of animals looked longer at the aggressive segments than at the neutral segments, and for the neutral segments the rates of looking and gaze aversion did not differ between the two groups of animals. In contrast, the HS monkeys looked at, and looked away from, the aggressive segments at a higher rate than did the LS monkeys. A logistic regression analysis utilizing three measures from these two tests—the mean preference for a novel face across the seven FR problems; the ratio of looking at the aggressive/neutral segments; and the same ratio for gaze aversion—demonstrated 100% classification of these 50 monkeys into low- versus high-sociable categories (Sclafani et al., 2016). These data indicate that differences in naturally occurring social interest at 1 to 4 years of age can be completely predicted by BBA measures of social information processing at 3 to 4 months of age. The next steps for this research program involve follow-up testing under carefully controlled conditions to better understand the social deficits of LS juveniles, and to see if the BBA measures predict *specific* types of social-cognitive deficits. Once we have a better sense of the relationships between social functioning and the BBA measures, we will have a screening tool, as originally hoped for, to use in identifying animals early in development and then intervening, either behaviorally or pharmacologically, to reverse the deficits and restore normal social function. We believe this naturally occurring model has enormous translational potential for understanding and treating the social deficits associated with ASD.

Conclusions

There is a fundamental difference in the way much human and nonhuman research is conducted, which focuses on whether one's sample of interest (lonely individuals, inhibited individuals, socially challenged individuals) is identified through survey of the natural population (human research), or whether the sample is selected following induction of the process of interest (animal research). It is our position that development of naturally occurring animal models of disease, whether physical or psychological, is a valuable approach to translational research, and in this report, we have provided three detailed examples of how this approach has been fruitfully used.

We have also acknowledged that the use of such models involves costs. Rare phenotypes necessitate the surveying of large numbers of individuals. Unlike humans who can be surveyed using paper and pencil methods, animal studies involve considerably more time, money, and effort to survey thoroughly in order to identify the important phenotypes. Obviously, we are of the opinion that formal phenotyping programs, such as the BBA program, benefit the cause of developing naturally occurring models. The BBA program is an extensive one, and not surprisingly, produces a wealth of data for each animal that can be used to develop new models in a variety of domains. Because the assessment program is so thorough, however, it is also time-intensive, and it may not be practical for other facilities. In fact, a second approach to broad-based phenotyping in nonhuman primates is underway at this time, through the National Primate Research Centers Consortium's Phenotype Mining and New Model Development initiative. One of the phenotypes that is of interest at many NPRCs—from both scientific and animal welfare perspectives—is anxiety, and a working group was formed in 2015 to develop a common assessment tool to measure anxiety quickly and reliably in monkeys at all of the seven NPRCs. This would provide a pool of animals at each center, which had already been prescreened as "anxious" or "nonanxious," for further study by principal investigators.

There are some questions regarding naturally occurring models that remain unanswered. One issue, discussed earlier, is that the origins of the variation that one sees in the phenotype of interest are unknown. Induced models clearly show what the origin of the variation is—it is the procedures that were employed to produce the phenotype. Of course, once one has surveyed a large number of individuals—human or nonhuman—the question of origins of the variation can itself be examined. In our work with the BBA program, for example, we have studied how genotype, rearing history, prenatal experience, rearing history of the animal's sire, or constituents of mother's milk (to name but a few influences) might contribute to that variation (see references in Capitanio, 2017). A second issue concerns the comparability of data from induced versus natural models. For example, we described above our studies of naturally occurring behavioral inhibition and its relationship to the airway response that is a critical component of asthma, and earlier we indicated that behavioral inhibition can be induced through exposing the pregnant female to stressful circumstances (Clarke and Schneider, 1997). Would such animals also show the hyperresponsive airway response that we have seen in our naturally occurring model? It is possible, of course, that some of our naturally occurring inhibited animals are inhibited because their mothers experienced stress prenatally, but that is certainly not the only cause for developing an inhibited temperament style, as our previously described study of *MAOA* genotype and prenatal ketamine exposure demonstrated (Capitanio et al., 2012).

In the past decade or two, it has become increasingly important to know about the genetics of our captive primate colonies and the provenance of the animals (i.e., of Indian or Chinese origin). Similarly, knowing the health histories of animals in our colonies is important for insuring that animals that are selected for studies do not have any compromising preexisting conditions. The need to know about genetics and about health have prompted primate facilities to develop databases that can be mined for information by veterinary staff, health technicians, and scientists. We propose that having systematically collected biobehavioral data that index major psychological constructs that have been associated with health and disease in humans can also be valuable. While other scientists have conducted studies utilizing naturally occurring variation, as we described above, we believe that our principal contribution to the further development and utilization of naturally occurring models is the development of a formalized testing program that can provide this type of basic, biobehavioral data on large numbers of animals for use in translational science.

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