Behavioral Inhibition in Rodents: A Model to Study Causes and Health Consequences of Temperament

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Abstract Behavioral inhibition (BI), a trait related to fearful temperament and withdrawal/avoidance of novelty, is an important predictor of adult health trajectories. However, specific mechanisms underlying this temperament-health relation are poorly understood. In order to model underlying physiological and developmental processes associated with behavioral inhibition to identify causal mechanisms for specific health trajectories, we developed a rodent model of early emerging behavioral inhibition. This behavioral trait of low exploration has been documented in many species and represents a relatively basic behavioral phenotype, which supports the goal of developing a non-human animal model. In this chapter, I review the behavioral and physiological characteristics of the rodent behavioral inhibition model, with an eye toward identifying biological mechanisms that may bias behaviorally inhibited individuals toward certain health trajectories. In addition, I review information on developmental correlates and influences on behavioral inhibition, with an eye toward identifying and testing interesting social and environmental interventions that could minimize health biases. I complete the chapter with a discussion of areas of future research with a rodent behavioral inhibition model.

Temperament, personality, and individual differences are of interest in multiple disciplines. A key question of intrigue for a long time has been: "What is it that makes one individual respond to a set of circumstances in one way while another responds to the same situation quite differently?" This question has been posed by psychologists and philosophers trying to understand the development and function of behavioral diversity, by medical professionals trying to understand variability in disease progression and outcomes, and by biologists trying to understand evolutionary and ecological processes underlying variance. Variance is present in all systems and often regarded as meaningless noise. However, when this variance is systematically

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characterized, quantified, and understood, the potential implications are large, particularly in a clinical setting (e.g., Cavigelli, 2005; Forster, Finn, & Brown, 2017; Gatt et al., 2007; Gurevitz, Geva, Varon, & Leitner, 2014; O'Leary-Barrett et al., 2017; Reeb-Sutherland et al., 2009).

For example, in the case of behavioral inhibition, if we can identify specific physiological or cognitive processes associated with this trait, and predispose an individual to specific health outcomes, then we can better identify interventions to minimize specific negative health outcomes associated with the behavioral trait (e.g., Cavigelli, Michael, & Ragan, 2013; Morales, Pérez-Edgar, & Buss, 2015; Pérez-Edgar et al., 2011). To better understand the origins and the health implications of behavioral inhibition, we examined the validity of a potential rodent model of this trait. Such a model would allow for experimental and longitudinal research on the causal mechanisms leading to trait development and on mechanisms involved in trait-specific health outcomes. Specifically, a rodent model of this relatively basic trait allows for complementary experimental, developmental, functional, and mechanistic studies on the causes and consequences of behavioral inhibition (see the chapter "Behavioral Inhibition in Nonhuman Primates: The Elephant in the Room" by Capitanio). Here, we present current knowledge on this rodent model of human behavioral inhibition.

Behavioral Inhibition as a Basic Trait

Human behavioral inhibition (BI) refers to a behavioral predisposition that indicates an initial, general fear response to unfamiliar situations, for example, slower approach and faster retreat from novel objects or situations (García-Coll, Kagan, & Reznick, 1984; Kagan, Reznick, & Snidman, 1987). This is a relatively basic behavioral response pattern, and there is a wealth of historical animal and human research to indicate that an approach-withdrawal spectrum of individual differences is likely universal across species and cultures (Blanchard, Flannelly, & Blanchard, 1986; Rothbart, Ahadi, Hershey, & Fisher, 2001; Schneirla, 1965; Stevenson-Hinde, Stillwell-Barnes, & Zunz, 1980; Suomi, 1987; Thomas & Chess, 1977).

In the field of animal personality research, investigators have identified five broad behavioral dimensions that involve stable within-species individual variance: boldness, exploration, activity, sociability, and aggressiveness (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Interestingly, these five broad dimensions identified from a review of the literature are similar to broad personality dimensions identified in humans using a data-driven approach—i.e. the "Big Five" dimensions of neuroticism, extraversion, openness to new experiences, agreeableness, and conscientiousness (Costa & McCrae, 1992; Gosling & John, 1999). Within these two broad personality frameworks, behavioral inhibition likely lies along the dimensions of "exploration" (willingness to engage novelty) and "openness to new experience" or "emotional stability/neuroticism" (e.g., curiosity/creativity or calmness/balance). The field of personality and temperament research is very complex, but for

the purpose of this chapter, I highlight that behavioral inhibition is one basic characteristic that defines reliable differences among individuals within a species and that this trait has been identified both in humans and animals.

Developing a Rodent Model of Behavioral Inhibition

In the United States, early laboratory-based tests indicated that 15–20% of children displayed signs of generalized behavioral inhibition (García-Coll et al., 1984; Kagan et al., 1987; Kagan & Snidman, 1999). These tests consisted of exposing children to a battery of conditions that involved different forms of novel or unfamiliar stimuli (e.g., novel toys, unfamiliar people, etc.). The trait was relatively stable across age and could be characterized as early as infancy (Kagan & Snidman, 1991; Reznick et al., 1986). Follow-up studies have identified physiological correlates of behavioral inhibition, including increased autonomic nervous system activity, greater basal (i.e., "unstimulated") hypothalamic-pituitary-adrenal (HPA) axis activity, greater HPA reactivity to novelty in certain cases, and increased activation of the amygdala in response to novel stimuli (e.g., Buss, Davidson, Kalin, & Goldsmith, 2004; Kagan et al., 1987; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Schwartz, Wright, Shin, Kagan, & Rauch, 2003). All of these physiological response biases, and others to be identified yet, may "set the stage" for the development of specific health outcomes.

Children that display signs of behavioral inhibition are more prone to allergies and anxiety disorders (e.g., Hirshfeld et al., 1992; Kagan & Snidman, 1991, 1999; Turner, Beidel, & Wolff, 1996). Capitanio (see the chapter "Behavioral Inhibition in Nonhuman Primates: The Elephant in the Room") notes in his work a link between behavioral inhibition in nonhuman primates and airway hyperresponsiveness, a marker of allergic asthma. In particular, there is long-standing evidence that behavioral inhibition is one of the best predictors of later social anxiety (Biederman et al., 1990; Chronis-Tuscano et al., 2009; Clauss & Blackford, 2012; Hirshfeld et al., 1992; Hirshfeld-Becker et al., 2007; Kagan, Reznick, Clarke, Snidman, & García-Coll, 1984; Pérez-Edgar & Fox, 2005; Reeb-Sutherland et al., 2009; Schwartz, Snidman, & Kagan, 1999). Finally, older community-dwelling adults that self-identified as low in curiosity (information/stimulation seekers) had higher mortality rates than more curious adults (Swan & Carmelli, 1996). The physiological correlates of behavioral inhibition (i.e., enhanced limbic activation/physiological stress activation) could be one mechanism that predisposes individuals to develop allergies and anxiety. For example, cChronically elevated physiological stress (e.g., allostatic load) can have long-term consequences on neuronal, immune, and metabolic function associated with allergies and anxiety (Brindley & Rolland, 1989; Dhabhar & McEwen, 1999; Wilckens & De Rijk, 1997).

Because behavioral inhibition emerges early in life, can be a stable trait over years, and is associated with elevated physiological responses associated with emotion and stress regulation and health consequences, it would be beneficial to determine the relative causal relations among temperament, developmental experiences, physiological response biases, and health outcomes. If a certain temperament is

causally related to specific developmental experiences and/or with a physiological response bias that is detrimental to a particular health outcome, then "interventions" that target these specific mechanisms may be particularly effective in maintaining quality of life. (In this case, "interventions" may involve social or environmental alterations.) The relative benefit of interventions could be assessed in the short-term by capturing the amount of change observed in the physiological response bias that is associated with poor health outcomes.

These motivations drove us to develop a rodent model of behavioral inhibition. Our goals in developing a short-lived animal model was to provide a means to (1) identify BI-associated physiological response biases that may have important health consequences for humans; (2) test causal relations among temperament, peripheral physiology, and health using experimental methods that are not possible in human research; and (3) conduct reasonable lifelong longitudinal studies to document the development and stability of temperament, associated physiological biases, and health outcomes. Results from a short-lived rodent model can provide preclinical insights into physiological and developmental mechanisms that bias health trajectories in fearful or inhibited children and adults.

In the early 2000s, we began to test the viability of a rodent model of behavioral inhibition. For several reasons, we chose rats as an ideal rodent model species to study the causes and long-term consequences of behavioral inhibition. Rats are a relatively complex social species, with a great deal of background literature on behavior, development, and physiology. They are also relatively short-lived which allows for life-span studies on the influence of early life factors on adult health and physiology. In addition, rats are a relatively large rodent which allows for ample and repeated physiological sampling over the life span. Finally, prior studies had documented a trait similar to behavioral inhibition in many species (e.g., "neophobia," "emotionality," or "shyness"), including rats, suggesting that the trait is relatively well-conserved across species, even those that undergo experimental breeding (Buss & Plomin, 1984; Einon & Morgan, 1976; Gosling & John, 1999; Takahashi & Kim, 1994; Wilson, Clark, Coleman, & Dearstyne, 1994). These preliminary studies supported the notion that the equivalent of behavioral inhibition in humans could be identified in other species and that an animal model with naturally occurring variance in this trait was theoretically viable.

To develop a valid rodent model of behavioral inhibition, we had several goals. First, we wanted to use behavioral tests with rodents that were similar to those used to test behavioral inhibition in humans. Second, we wanted to determine the relative stability of the trait within an individual across development and begin at the earliest point at which the trait could be reliably identified. Last, we wanted to verify that the physiological response biases identified in human behavioral inhibition, which may be responsible for specific health trajectories, were present in rodent behavioral inhibition. We conducted the bulk of this work with outbred rats (Sprague-Dawley) in order to test these questions in a genetically heterogeneous population that would maximize behavioral variance.

Given the above goals, we conducted initial studies to quantify infant, juvenile, and adult rat behavioral and physiological responses to test arenas that were made to be comparable to laboratory behavioral assessment conditions in early studies of

childhood behavioral inhibition (e.g., novel toys and novel social partners; García-Coll et al., 1984; Kagan et al., 1984; Reznick et al., 1986). We tested whether fearful responses generalized across different environmental conditions (both social and nonsocial unfamiliar environments) and whether the inhibited phenotype was seen in a similar percentage of rats as had been documented in humans (i.e., ~15% of those tested). To mimic human laboratory test conditions, we studied rat responses to controlled novel conditions in which threatening stimuli were minimized—i.e., low light and noise, well-protected and simple arenas, and tests conducted during waking hours (Cavigelli et al., 2007; Cavigelli & McClintock, 2003).

Rats were tested in two different novel conditions on separate occasions—one that included a novel social partner and one that included only novel objects (i.e., novel social vs. nonsocial environments). To estimate behavioral inhibition or fear-related responses, we documented latency to approach novelty, overall locomotion, and frequency to inspect novelty. Latency to approach a novel social partner is one of the behavioral responses most linearly associated with basal and reactive corticosterone production, although many of the behavioral responses to novelty are closely associated with one another and with glucocorticoid production (Cavigelli et al., 2007, 2009).

Given these characteristics of behavioral responses, we defined rodent behavioral inhibition as a longer-than-median latency to approach novelty in both a social and nonsocial arena. Using this definition, behaviorally inhibited rats make up approximately 30% of any tested group (Cavigelli et al., 2007). We have found that defining a rat's temperament relative to others within a specific cohort is an important method to control for slight variations among cohorts and testing conditions among studies. Importantly, a rat's response to one arena (e.g., social novelty) did not necessarily predict its behavioral response to the other arena (e.g., nonsocial novelty; correlation of individual approach latencies in each arena: $r_{58} = 0.194$, ns) suggesting the importance of varied behavioral testing to identify generalized inhibition to multiple forms of novelty (Cavigelli et al., 2007; Kagan, Snidman, McManis, Woodward, & Hardway, 2002). The lack of correlation also reflects Kagan's (see the chapter "The History and Theory of Behavioral Inhibition") argument that behavioral inhibition reflects a specific category of temperament, rather than a constellation of continuous traits.

To test trait stability over time, we documented behavioral responses to the social and nonsocial novel arenas across multiple test ages and also tested trait stability over relatively long stretches of the rat's life span. In one case, we were able to test stability in the nonsocial arena over the life span, and in another case we tested stability in the two different arenas over a shorter span of 4 months (~20% of the median rat life span in laboratory conditions; Cavigelli & McClintock, 2003, Cavigelli et al., 2007, Caruso, McClintock, & Cavigelli, 2014). Behavioral responses to the nonsocial arena were linearly stable over time ($r_{28–51} = 0.32–0.75$, $p < 0.05–0.001$) with higher stability when shorter test-retest intervals were used (i.e., 4 vs. 10 months; Cavigelli & McClintock, 2003, Cavigelli et al., 2007, Caruso et al., 2014). In addition, in both arenas, males were slower to approach novelty and moved less than females (Cavigelli, Michael, West, & Klein, 2011). *Mean* response to both arenas was also relatively stable over time. For rats retested in both arenas at two test ages, 4 months apart, the mean approach latency in the novel social and nonsocial

arenas at test age 1 was linearly related to mean latency at test age 2 ($r_{59} = 0.39$, $p < 0.01$). Lastly, 65% of rats classified as "inhibited" at time point one continued to display inhibition 4 months later (again, with behavioral inhibition defined as longer than median latency to interact with novelty in both a nonsocial and social test situation; Cavigelli et al., 2007, 2009). Overall, repeat testing on both arenas at two ages led to an identification of stable inhibition in 17% of rats tested, and a similar percentage of stably non-inhibited rats (i.e., shorter than median approach latency on both arenas at both test ages). This behavioral inhibition percentage is comparable to the percentage of behavioral inhibition in humans (Cavigelli et al., 2007).

In a complementary model of rodent behavioral inhibition, Kalin and colleagues have experimentally elicited similar behavioral characteristics in male and female Sprague-Dawley rats by exposing them to predator cues (Campeau, Nyhuis, Sasse, Day, & Masini, 2008; Nanda, Qi, Roseboom, & Kalin, 2008). This model allows for greater experimental control of behavioral inhibition (here defined as freezing and decreased locomotion/hypervigilance) and allows for repeated testing/elicitation of the phenotype since rats do not habituate to predator cues (Blanchard et al., 1998). With this model, individual differences in BI-related responses to acute predator exposure were stable across repeat testing and with greater stability when repeat tests were conducted closer together in time (e.g., test-retest interval of 2 days, in adolescence, $r_{22} = 0.538$, $p < 0.01$, or adulthood, $r_{22} = 0.723$, $p < 0.001$; test-retest interval of 28 days, from adolescence to young adulthood, $r_{35} = 0.475$, $p < 0.01$; Qi et al. 2010). Further, individuals could be characterized as either high or low behavioral inhibition based on the stability of their behavioral response to predator exposure tested 2 days apart. Rats with high stability during this short test-retest interval also showed much more stability in behavior over the longer test-retest interval (28 days, $r_{94} = 0.902$, $p < 0.001$; Qi et al. 2010). Finally, similar to behavioral inhibition elicited by exposure to a more benign arena with a novel rat or objects, adult males showed more inhibited behavior in response to a predator than adult females (Cavigelli et al., 2011; Qi et al., 2010).

In this complementary model of rodent behavioral inhibition, the inhibited behavior (but not other behaviors, such as grooming and rearing) was decreased by an injection of anxiolytic drug (diazepam) just prior to predator exposure (Qi et al., 2010). Thus, based on results from two models of rodent behavioral inhibition (predator vs. benign novelty exposure), trait stability in Sprague-Dawley rats was relatively high, with greater stability with shorter test-retest intervals and a greater number of repeat tests, and males displaying more behavioral inhibition than females.

The above work has been conducted with rats. However, there would be some advantages to extending this work to mice. Laboratory mice present an attractive complementary rodent species because they cost about half as much to maintain in the laboratory (because of smaller body size), and because of this, there has been more extensive work conducted to modify their genetic makeup. To minimize genetic variance and maximize certain traits in a rodent population, selective breeding has been conducted with both rats and mice, but in mice, there is a longer history of conducting more refined genetic modifications compared to rats. For example, there are more established "knockout" and transgenic mouse vs. rat models, and these models,

which involve targeted modification of specific genes, allow for more targeted experimental tests of how a specific physiological mechanism affects phenotype.

Given some of these benefits of mouse models, we have tested whether the results described above for outbred rats translate to mice. With several mouse strains (in- and outbred; e.g., C57BL/6, Balb/c, CD-1), we have conducted pilot studies to determine the relative stability of behavioral inhibition within individuals. To date, we have no good evidence of stable behavioral inhibition across time or any evidence of a reliable relation between fear-related behavior and physiology in these mouse strains. This lack of relation may result from methodological or species differences between mice and rats. For example, behavioral tests and measures with mice may require additional modifications to identify subtle behavioral differences in a species that is one-tenth the size of rats. In addition, in a smaller prey species, there may be less variance in exploratory behavior since high exploration is particularly detrimental in a small species that is easily consumed by predators. Last, by chance or design, mouse breeding histories may have led to less behavioral variance among individuals, which would minimize power to identify reliable individual behavioral differences.

Developing models in mice may require different behavioral tests; however, movement to a viable mouse population could be beneficial, both economically and scientifically. A mouse model may provide more genetic tools to study underlying physiological mechanisms involved in behavioral inhibition and associated health outcomes. However, there are also distinct advantages to a rat model. For example, refined genetic modifications are being used with rats now, and rats carry certain physiological advantages such as a larger body size that allows for more feasible and accurate physiological manipulations and measures. Additionally, many behavioral and physiological processes are more similar between humans and rats as compared to between humans and mice (reviewed in Ellenbroek & Youn, 2016). Thus, additional refinement of a mouse model could provide a specific complement to the rat model, primarily in providing a means to study behavioral inhibition in two rodent species to strengthen the ability to identify a range of mechanisms that support the presentation and maintenance of behavioral inhibition.

Physiological Processes Underlying Rodent Behavioral Inhibition

Based on arguments made at the beginning of this chapter, we are interested in modest physiological response biases associated with behavioral inhibition. Even slight modifications in physiological responses associated with behavioral inhibition could lead to significant cumulative impacts on health over the life span. In a rodent model, the physiological measures that can be most accurately collected are slightly different from those in humans. For example, in humans, minimally invasive fMRI provides an excellent measure of central neurobiological function, and noninvasive methods can be used to measure cardiovascular function (blood pressure, heart rate variability, etc.). In rodents, these technologies are retrofit for a small species, and

involve involuntary and relatively long-term restraint, which may lead to less reliable indices of basal neurobiological or cardiovascular function. However, collecting peripheral blood samples to measure metabolic, endocrine, and immunological responses, and conducting controlled experimental manipulations, can be easily, accurately, and humanely collected in a rodent as compared with children. Given these slight differences in procedural efficacy, we have focused primarily on documenting peripheral physiological correlates of rodent behavioral inhibition, although we have also documented more static alterations in neurobiological function (e.g., receptor binding, receptor gene expression).

Before reviewing physiological response biases present in inhibited rats, we touch on a brief but important issue about different cross-disciplinary use of the term "behavioral inhibition" (see also the chapter "Behavioral Inhibition in Nonhuman Primates: The Elephant in the Room" by Capitanio). Across research domains, the term "behavioral inhibition" refers to slightly different concepts. In classic preclinical biomedical research (i.e., mechanistic/molecular level research with nonhuman animals), "behavioral inhibition" is used to refer to an acute behavioral response to potentially rewarding conditions and is thought to involve behavioral control and clamped impulsivity associated with resilience to drug addiction. Neuronal mechanisms underlying this behavioral response have been studied in an acute fashion, and there is ample information to indicate that this acute behavioral response is related to prefrontal cortex and hippocampus function. This definition of behavioral inhibition is not used in the current chapter.

In the current chapter, I focus on the temperamental aspect of behavioral inhibition, that is, a stable trait associated with chronically altered physiological regulation. Specifically, I review physiological response biases that exist in individuals that regularly show inhibited behavioral responses to novel conditions as opposed to reviewing acute neuronal responses/mechanisms involved in the acute display of behavioral inhibition. Given our focus on developing a model to understand cumulative influences of altered physiological regulation on long-term health conditions, we have focused our research on peripheral rather than central physiological processes that are associated with behavioral inhibition. These peripheral responses influence long-term health trajectories and may help us understand the processes that bias health outcomes in inhibited individuals. In addition, these peripheral physiological processes can be measured in a minimally invasive fashion and therefore documented over time to determine relative stability of physiological response biases associated with behavioral inhibition.

Life Span

In the rodent model, we have documented several physiological correlates of behavioral inhibition. One of the more striking and well-supported results is the shorter life span: stable behaviorally inhibited rats die, on average, 7–8 months earlier than non-BI rats (Cavigelli & McClintock, 2003, Cavigelli et al., 2009). Contextualized

as a percentage of overall median life span, this difference corresponds to a ~15-year difference for humans. We have replicated this shortened life span in two separate cohorts, studied at two different institutions. Importantly, the better predictor of this shortened life span was stable inhibition in a social (as opposed to nonsocial) setting ($\chi^2 = 12.80$ vs. 3.89, $p < 0.01$ vs. $p = 0.14$, Cavigelli et al., 2009), although nonsocial inhibition was also related to life span (e.g., Cavigelli & McClintock, 2003). The greater predictive value of social vs. nonsocial inhibition presents a consistent theme in other domains of the rodent model and is important because this aspect of human behavioral inhibition also seems to be of greater clinical and physiological significance (e.g., Buss et al., 2004, Kertes et al., 2009; see the chapter "The Neural Mechanisms of Behavioral Inhibition" by Jarcho and Guyer).

To understand underlying physiological processes that are associated with behavioral inhibition and that may predict a shorter life span, we have measured several different physiological responses. Based on human studies, two physiological responses that may predict a shortened life span include consistently elevated glucocorticoid levels and increased autonomic activity (e.g., Gilad & Gilad, 1995; McEwen & Seeman, 1999). For example, in a study of human biomarkers of health and longevity, both elevated cortisol production and systolic and diastolic blood pressure were significant predictors of increased mortality rates in older adults adults (Gruenewald, Seeman, Ryff, Karlamangla, & Singer, 2006). In addition, glucocorticoid production in early life may be an important predictor of later childhood maladaptive behaviors (e.g., Pérez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008), and in rodents, individuals with elevated glucocorticoid responses are shorter-lived than lower glucocorticoid-producing individuals (Cavigelli et al., 2006; Gilad & Gilad, 1995, Cavigelli & McClintock, 2003, Pérez-Álvarez et al., 2005).

Glucocorticoid Production and Associated Physiology

Based on these suggestions from the literature, and associated health implications of elevated physiological stress, we documented basal and reactive glucocorticoid production in behaviorally inhibited vs. non-BI rats. Consistently across multiple studies, we have found that behaviorally inhibited rats have elevated basal and novelty-induced glucocorticoid production relative to non-BI rats (Cavigelli et al., 2007; Cavigelli & McClintock, 2003). Behaviorally inhibited rats have 20–30% more glucocorticoids in circulation than non-BI rats, and as was seen with behavior, glucocorticoid production within an individual was linearly consistent over time ($r_{53} = 0.37$–0.66, $p < 0.01$ with a test-retest interval of 2–4 months; Cavigelli et al., 2009). Further, elevated basal GC production was better predicted by social inhibition rather than nonsocial inhibition (Cavigelli et al., 2007). Importantly, in the rat model of behavioral inhibition, the magnitude of difference in GC production between behavioral inhibition and non-BI rats is comparable to the difference between behaviorally inhibited and non-BI children GC levels (Kagan et al., 1987; Nachmias et al., 1996).

Similar GC elevations in low-exploration individuals have been documented by others, although there is variance in these results. Variance in results relates to the behavioral tests used and whether the modeled trait is thought to reflect behavioral inhibition or novelty-seeking, two traits that seem related but that do not necessarily represent two ends of the same spectrum (e.g., Cavigelli, 2005; Dellu, Piazza, Mayo, Le Moal, & Simon, 1996; Gentsch, Lichtsteiner, Driscoll, & Feer, 1982; Kabbaj, Devin, Savage, & Akil, 2000; Pérez-Álvarez et al., 2005; Ray & Hansen, 2004). Specifically, when a simple test arena is used (e.g., without rat-sized objects or partners present), variance in exploratory behavior likely reflects variance in novelty-seeking or escape motivation rather than variance in fear. In these simple testing conditions, higher exploration tends to be associated with higher circulating glucocorticoids (e.g., Dellu et al., 1996). However, in more complex test arenas, variance in exploratory behavior likely reflects variance in fear or behavioral inhibition, and in this context, higher exploration is usually associated with lower circulating glucocorticoids (e.g., Cavigelli et al., 2007; Ray & Hansen, 2004).

To determine the relative association between BI-related glucocorticoid (GC) production and health outcomes, we conducted pilot studies to compare variations in GC production to important health outcomes. In a longitudinal study, we found that basal GC production in young adulthood (4–8 months of age) linearly predicted basal metabolic rate in late adulthood (18 months), with greater basal GC production predicting diminished basal metabolic rate in old age ($r_9 = -0.698$, $p < 0.05$; McCarter & Cavigelli n.d.). Interestingly, young adult GC response to an acute challenge did not strongly predict late adult metabolic rate ($r_7 = 0.354$, $p > 0.35$). Similarly, in another correlational life-span study, basal GC production in young adults (4–8 months) was a better predictor of a shorter life span than was young adult acute GC response to a brief challenge ($\chi^2 = 3.16$ vs. 0.21, $p = 0.076$ vs. 0.65, Cavigelli et al., 2009). In other words, a physiological profile that involves elevated basal (i.e., unstimulated) GC production, rather than elevated peak (stimulated) production, may lead to more chronic biological overexposure to GC throughout the life span. Elevated circulating GC during low-stimulation periods likely persists longer than elevated circulating levels after an acute stressor, and this longer-term, albeit lower-grade, over-exposure to GCs during basal periods may be more detrimental for metabolic function and longevity.

Elevated basal GC production in behavioral inhibition is likely just one of many physiological process that may influence health. When we included both basal GC production and degree of behavioral inhibition (i.e., mean latency to approach two different forms of novelty) in a statistical model of life span, each variable accounted for a unique proportion of life-span variance, and stable behavioral inhibition was a better predictor of life span than was elevated basal GC production (Cavigelli et al., 2009). These results suggest that other variables, beyond elevated basal GC levels, account for a shortened life span in behavioral inhibition. These preliminary results suggest specific experimental follow-up studies that can be uniquely conducted in rodents. For example, manipulations of circulating GC levels could determine if slight but chronic GC elevations are enough to alter basal metabolic rate and life span (discussed in “Future Directions”). In addition, with a rodent model, it is possible to chronically manipulate some of the following physiological processes to test their long-term influence on specific health outcomes.

Cardiovascular Function

In humans, a classic physiological correlate of behavioral inhibition is elevated autonomic nervous system activity in response to novelty compared to non-inhibited children (Heponiemi, Keltikangas-Järvinen, Kettunen, Puttonen, & Ravaja, 2004; Kagan et al., 1987; Kagan & Snidman, 1999; Marshall & Stevenson-Hinde, 1998; Stevenson-Hinde & Marshall, 1999). Interestingly, this physiological trait may be relatively consistent over the life span: older adults that self-identify as shy also have elevated sitting systolic blood pressure compared to less shy individuals (Bell et al., 1993). Follow-up studies specifically point to increased sympathetic activity in behaviorally inhibited children estimated from more refined cardiovascular measures such as heart rate variability and respiratory sinus arrhythmia (Burgess, Marshall, Rubin, & Fox, 2003; Kagan & Snidman, 1991; Stifter & Corey, 2001).

To determine if rodent behavioral inhibition is related to elevated autonomic activity, we documented basal and responsive blood pressure and heart rate in behaviorally inhibited and non-BI rats. This work was conducted with a noninvasive blood pressure cuff similar to that used with humans, although with rats the cardiovascular measures require involuntary restraint to minimize animal movement. Restraint necessarily introduces a significant stressor, and thus cardiovascular measures must be interpreted accordingly. To estimate *basal* cardiovascular function, we measured blood pressure and heart rate immediately after placement into the restrainer affixed with an inflatable tail cuff. We estimated cardiovascular *reactivity* by measuring these variables several minutes after the initial introduction to the restrainer/cuff. At rest (i.e., within the first minute of restraint), behaviorally inhibited rats had heart rates that were 5–10% greater (450 vs. 412 BPM) and blood pressures that were 15–20% greater (systolic mean 164 vs. 138 mmHg; diastolic means 94 vs. 82 mmHg) than non-BI rats. In the longer-term response to restraint, behaviorally inhibited rats maintained elevated heart rates and increased blood pressure, while non-BI rats decreased rate and pressure over time (Fig. 1).

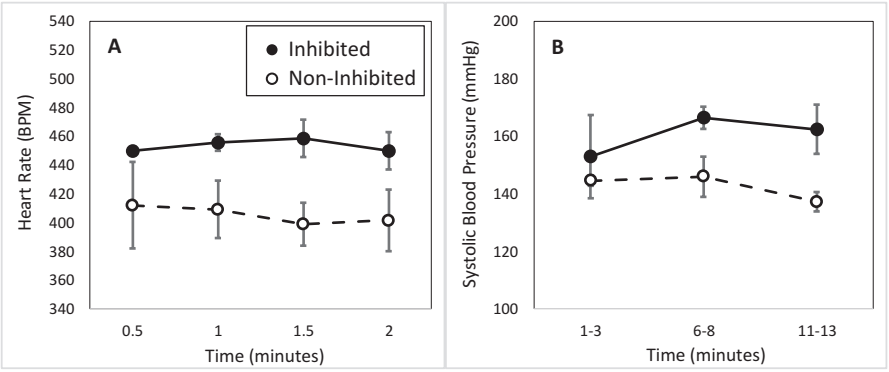

Fig. 1 Mean (**A**) heart rate response, and (**B**) systolic blood pressure response to novelty (restraint) for inhibited (filled circle) and non-inhibited (open circle) adult male Sprague-Dawley rats

Immune Function

Immune function is associated with many of the physiological and health symptoms of BI: altered regulation of cardiovascular function, GC production, life span, allergies, and mental health. We have studied several immune responses in behaviorally inhibited and non-BI rats. Given the low-grade chronic elevation in basal GC production in behaviorally inhibited rats (and potentially in children), we focused on two immune responses that are affected by experimentally induced chronic GC overexposure: innate proinflammatory signaling and localized delayed-type hypersensitivity (DTH) (Dhabhar & McEwen, 1997, 1999; van de Garde et al., 2014).

Given that behaviorally inhibited rats consistently have higher concentration of GC in circulation during basal conditions, we hypothesized that, even in the absence of chronic stress, behaviorally inhibited rat immune cells would experience GC resistance often seen in an individual experiencing chronic stress (i.e., desensitization to the normal anti-inflammatory effects of GC). If this were the case, we would expect an accentuated innate inflammatory response in behaviorally inhibited rats. We also predicted a dampened DTH response as a result of a GC-induced shift toward a T-helper-2-cell-mediated immune bias (Elenkov, 2004). Importantly, this immunological profile could be a risk factor for chronic disorders associated with behavioral inhibition (allergies, asthma, anxiety; Elenkov & Chrousos, 1999; Maggi, 1998).

In support of this hypothesis, in two cohorts of young adult male rats, we found that behaviorally inhibited rats had an elevated acute peripheral interleukin-6 response to a systemic, moderate dose of lipopolysaccharide (an endotoxin) (Michael et al., n.d.). This accentuated response is an index of accentuated innate inflammatory response. Interestingly, in response to this immune challenge, behaviorally inhibited rats also produced more GC than non-BI rats, suggesting that the normal anti-inflammatory action of GC was dampened in behaviorally inhibited rats. This result suggests a testable hypothesis and mechanism for chronic, unregulated peripheral inflammation in behaviorally inhibited children. Behaviorally inhibited rats also had a dampened DTH induration response when re-exposed to a novel non-pyrogenic antigen (keyhole limpet hemocyanin), indicating that not all immune responses are elevated or underregulated. As with GC production, these immune differences between behaviorally inhibited and non-BI rats were more closely associated with a rat's inhibitory response to a novel social rather than nonsocial stimulus (e.g., $r_{25} = 0.40$ vs. 0.23, and $p < 0.05$ vs. ns). In other words, a rat's GC and immune profile were more closely predicted by behavioral response to social novelty as opposed to nonsocial novelty.

Central Neurobiology

Given stable behavioral and physiological differences between behaviorally inhibited and non-BI individuals, we examined evidence of static differences in central neurobiological signaling. Based on prior literature on central mechanisms of stress, anxiety, and fear-related behavior, we focused on GC, corticotropin-releasing hormone, and

serotonergic receptors/transporters in limbic brain areas involved in HPA regulation and stress-related psychopathology (prefrontal cortex, hippocampus, hypothalamus). To examine basal differences in neurotransmitter signaling in behavioral inhibition vs. non-BI (i.e., stable behavioral traits), we focused on static postmortem receptor measures (e.g., receptor mRNA and binding levels), rather than measures of dynamic signaling (e.g., neurotransmitter release, EPSC/IPSC, glucose utilization measures).

Based on receptor gene expression, we found that behaviorally inhibited rats had decreased expression of the glucocorticoid receptor (GR) gene in the hippocampus and serotonin transporter (SERT) gene in the brain stem ($F_{2,26} = 6.33$, $p < 0.01$, $F_{2,21} = 3.37$, $p < 0.01$; Fig. 2). In addition, mean latency to approach both social and nonsocial novelty was a linear predictor of hippocampal and hypothalamic *GR* expression and prefrontal corticotropin-releasing hormone receptor 1 gene (*Crhr1*) expression. Approach latency accounted for 42% and 14% of the variance in *GR* mRNA expression in the hippocampus and hypothalamus, respectively ($\beta = -0.66$, $t_{28} = -4.57$, $p < 0.001$; $\beta = -0.42$, $t_{26} = -2.30$, $p < 0.05$) and 10% of the variance in prefrontal *Crhr1* expression ($\beta = -0.36$, $t_{28} = -2.01$, $p = 0.05$; Caruso, Crouse, & Cavigelli, 2015). In the predator-induced model of rodent behavioral inhibition, hippocampal (CA1) and hypothalamic expression of *homer1a* were linearly and positively associated with a greater behavioral inhibition response (Qi et al. 2010). These differences in gene expression between behaviorally inhibited and non-BI rats are comparable to changes seen in chronically stressed rodents that experience altered HPA axis regulation (Raone et al., 2007; Zhu et al., 2014). Overall, these results suggest that the rat brain in behavioral inhibition functions in a manner that may be comparable to individuals experiencing chronic stress.

In summary, there are several aspects of rat physiology in behavioral inhibition that may suggest important clinical insights about human behavioral inhibition. Briefly, (1) social inhibition, rather than inhibition in nonsocial settings, is a more important predictor of biases in physiological regulation, (2) altered regulation of

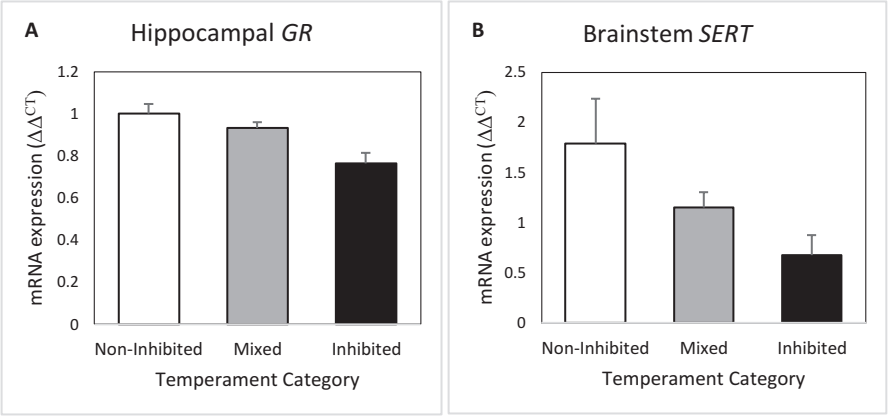

Fig. 2 Central nervous system gene expression. Mean (**A**) hippocampal glucocorticoid receptor and (**B**) brainstem serotonin transporter gene expression in non-inhibited (open square), mixed (gray square), and inhibited (filled square) male Sprague-Dawley rats

basal physiology may be more important than altered regulation of acute physiological responses, and (3) alterations to many physiological processes must be considered when trying to understand underlying mechanisms involved in different health trajectories in behaviorally inhibited vs. non-BI individuals.

Developmental Processes Underlying Rodent Behavioral Inhibition

In humans, a basic trait like behavioral inhibition emerges relatively early in development, but the phenotype can be relatively flexible, with some individuals showing consistent inhibition throughout development and others showing decreased inhibition over time. Clinically, developmental consistency in behavioral inhibition over time appears to be a key predictor of susceptibility to social anxiety (Chronis-Tuscano et al., 2009; Hirshfeld et al., 1992). Given the value of a developmentally predictive behavioral trait that allows for early targeted interventions (Kennedy et al., 2009; Rapee, Kennedy, Ingram, Edwards, & Sweeney, 2005; Rapee, 2013), and greater flexibility of personality traits during childhood/adolescence (e.g. Roberts & Delvecchio, 2000), it is likely that interventions during development rather than adulthood may be more effective (see the chapter "Behavioural Inhibition and the Prevention of Internalising Distress in Early Childhood" by Rapee and Bayer). In addition, attention to early life social contexts (e.g., family dynamics, parenting styles) and their influence on behavioral inhibition physiology and health is key (e.g., Cho & Buss, 2017; Hane & Fox, 2006; Kertes et al., 2009; Kiel & Buss, 2011).

To determine developmental experiences that shape behavioral inhibition in rats, we have examined early and later social predictors of behavioral inhibition (particularly early maternal behavior and later peer social interactions). These studies suggest a certain degree of flexibility in the behavioral inhibition phenotype. Like in humans, there is some evidence that overattentive parenting (in the form of maternal licking/grooming of young pups) is associated with increased behavioral inhibition. In three different studies, we found that differential maternal licking rates among pups in a litter was a good predictor of offspring behavioral inhibition and anxiety-related behavior (Cavigelli, Ragan, Barrett, & Michael, 2010; Ragan, Harding, & Lonstein, 2016; Ragan, Loken, Stifter, & Cavigelli, 2012). Pups that received the most licking were later more inhibited than their littermates and they solicited more maternal responses early in life. That is, they solicited maternal interactions/attention more often than their littermates. These results suggest that increased maternal attention within a litter may not cause increased offspring inhibition but that early life inhibition may be associated with behaviors that solicit increased maternal attention (e.g., Stern, 1997).

These studies are all correlational and thus the causal relation has yet to be determined. In addition, this literature is quite mixed, with abundant historical evidence that mothers that are highly attentive (i.e., maintain high lick/groom rates) produce litters of offspring that are, on the whole, relatively low anxiety (e.g., Caldji et al., 1998; Caldji, Diorio, & Meaney, 2003; Francis, Diorio, Liu, & Meaney, 1999; Meaney, 2001; Pan, Fleming, Lawson, Jenkins, & McGowan, 2014). Thus, in studying early developmental

conditions that enhance or diminish behavioral inhibition, it is important to distinguish variability in maternal behavior among offspring within a family vs. variability among different mothers. In addition, it is important to note whether offspring behavioral and physiological outcomes are more closely related to anxiety or to behavioral inhibition. From the rodent model perspective, there is still much work that could be done to determine the causal role of early offspring-mother interactions in the development of a stable or flexible behavioral inhibition phenotype in offspring. Importantly, with the rodent model, these early developmental questions could be examined experimentally by using creative methods to manipulate maternal licking behavior (e.g., Francis et al., 1999; Lee & Williams, 1974; Lovic & Fleming, 2004).

In addition to early life experiences, we have found that adolescent social experiences can influence adult behavioral inhibition (Caruso et al., 2014). Adolescence represents a final developmental period of behavioral and physiological flexibility, when individuals engage in frequent and diverse extrafamilial social interactions and when both behavior and HPA axis regulation can be shaped by social experiences (Sachser, Kaiser, & Hennessy, 2013). We have found that a lack of novel social experiences during adolescence can cause a transient blunting or shift in behavioral phenotype. Male behaviorally inhibited rats that do not experience novel social partners during adolescence displayed increased exploratory behavior soon after adolescence, compared to those that experienced novel social partners (Caruso et al., 2014).

Importantly, behaviorally inhibited and non-BI rats that showed a transient shift in behavior after exposure to impoverished adolescent social experiences returned to their predictable low or high exploratory phenotypes within a month of their adolescent social experience (Caruso et al., 2014). However, GC production in these animals seemed to be persistently altered into adulthood. Non-BI rats that had been exposed to novel social experiences during adolescence produced expected low-GC production relative to similarly treated behaviorally inhibited rats. However, non-BI rats exposed to impoverished adolescent social experiences had increased GC production in adulthood and no longer showed the expected lower GC production relative to their behaviorally inhibited littermates. These studies suggest that while behaviorally inhibited and non-BI behavior may be transiently altered by adolescent social experiences, physiological processes may be re-regulated in a more permanent fashion by adolescent social experiences. This work carries important significance in terms of understanding how early social interventions may alter underlying physiological biases that may be responsible for health trajectories in behavioral inhibition.

Future Directions and Limitations

Broadly speaking, there are two main areas for future work. The first involves building on the correlational validation studies reviewed here. The major benefits of a preclinical rodent model center on the power provided to conduct experimental and longitudinal studies that are prohibitive with humans. The second area for future work involves increased characterization of sex differences underlying the development, physiology, and health consequences of behavioral inhibition.

Experimental/Longitudinal Studies

Basic studies with outbred rodents, and other species, indicate that behavioral inhibition is a basic trait that can be identified relatively early on in development, is relatively stable in certain individuals, and is associated with predictable physiological response biases, which may also be relatively stable within individuals. Given these similarities between human and rodent behavioral inhibition, future experimental work should test (1) the relative role of different physiological biases in predicting health outcomes and (2) the influence of environmental conditions on the development and health consequences of the trait. In particular, environmental manipulations during later childhood and adolescence may be particularly beneficial since this represents a later period of behavioral and physiological "programming" and an age at which school interventions may be more easily implemented.

In terms of manipulating underlying physiology, experimental rodent studies provide a means to ask whether underlying physiological processes associated with behavioral inhibition are a causal agent in altered health and life-span trajectories in behavioral inhibition. If underlying physiological processes are key mediators of health trajectories associated with behavioral inhibition, then this work would suggest interventions that target physiological processes rather than interventions aimed toward altering behavior per se. A specific example of a possible appropriate physiological manipulation in the rodent model involves chronic manipulation of basal GC production. As a first experimental study, this manipulation would be useful because basal GC production is trait-like and relevant to other physiological processes, such as metabolic and immune function, and is related to life span in the rodent model. Further, basal GC production is relevant to the same health-related processes in humans and other species.

In preliminary tests, we have used a noninvasive method to alter basally circulating GC levels in a physiologically relevant manner by administering corticosterone in drinking water. This method allows for circulating GC manipulations that mimic those seen in behaviorally inhibited rats while also maintaining circadian and pulsatile rhythmicity of normal GC release into circulation (Fig. 3).

This manipulation works elegantly because rats drink more during the active period, which is the time when circulating corticosterone levels are normally highest during the 24-h day. In addition, rats drink in bouts that occur every 60–90 min which mimics the natural frequency of corticosterone pulses in rats (Kakolewski, Deaux, Christensen, & Case, 1971; Lightman et al., 2008; Marwine & Collier, 1979). With this manipulation, we found no evidence of behavioral changes, although this would have to be replicated in a larger study. Other physiological manipulations are also possible, and, like the described GC manipulation, these manipulations can be designed to be minimally invasive so that they can be sustained over time and made to mimic naturally occurring physiological differences between behaviorally inhibited and non-BI individuals. This allows for specific physiological processes to be manipulated while monitoring lifelong health outcomes.

The rodent behavioral inhibition model also provides a means to determine, experimentally, how developmental experiences influence the trajectory of the trait, at both the behavioral and physiological level. Theoretically, it will be of interest to determine if a transient and/or permanent change in behavioral predisposition

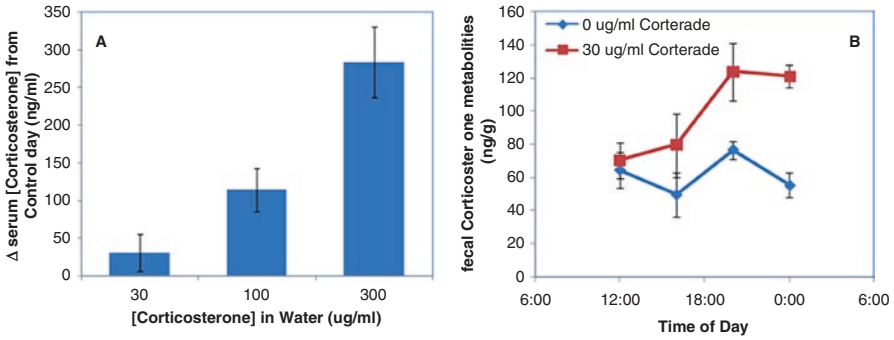

Fig. 3 Experimental manipulation of circulating glucocorticoids. (**A**) Mean relative increase in circulating corticosterone levels in male Sprague-Dawley rats provided one of three corticosterone concentrations in the home cage drinking water. Rats provided with water that had a 30 μg/ml concentration of corticosterone showed a relative increase in circulating concentrations that most closely mimicked the naturally-occurring increase in circulating GCs in BI rats. (**B**) Mean excreted corticosterone metabolite levels across the day in male rats provided *ad lib* home-cage access to pure tap water (blue line: "0 μg/ml Corterade") vs. tap water with low corticosterone concentration (red line: "30 μg/ml Corterade"). Experimentally increased GC levels are most pronounced during the active portion of the day (20:00 and 0:00 h)

induced during development leads to long-term changes in either health outcomes and/or underlying physiological processes that influence health. It is highly likely, as we found previously, that underlying physiological processes may be more open to "permanent reprogramming" during postnatal life than are behavioral processes. Behavioral profiles may involve more complex cognitive/learning processes that are more difficult to shift in a permanent manner. This could be of interest clinically—it may be that behavioral inhibition, defined according to behavioral repertoire, can remain intact while a permanent shift can occur in correlated physiological processes (e.g., basal GC production, innate immune responses, etc.). If we find that the physiology of innate behavioral inhibition presents itself as a major mediator of health, then the ideal goal may be to alter the physiology rather than the behavior of behavioral inhibition. In terms of environmental experimental manipulations, the focus will likely be on early developmental experiences, when behavioral and physiological traits are more flexible, fluid, and less fixed.

Sex Differences

A final area for future study involves a better characterization of the sex differences involved in the development, physiology, and health trajectories of behavioral inhibition. At present, in rodent models, there is strong evidence that behavioral tests to quantify behavioral inhibition are more sensitive or accurate for identifying behavioral inhibition in male rather than female rodents (e.g., Cavigelli et al., 2011; Qi et al., 2010). Some studies suggest that major exploratory motivations in rodents differ for males and females and that these differences may drive differential sensitivity of tests for identifying a reticent exploratory style in males vs. females

(Fernandes, González, Wilson, & File, 1999; Ray & Hansen, 2004). In particular, females are more active and more exploratory than males (Cavigelli et al., 2011; Ray & Hansen, 2004), and based on careful behavioral analyses, Ray and Hansen (2004) concluded that these rodent sex differences reflect a female bias toward more novelty-seeking behaviors vs. a male bias toward more harm-avoidance behaviors. Thus, it is possible that males prefer and need to be tested on a simpler novel environment, while females seek and require more complex stimulation (Pisula & Siegel, 2005).

In prior studies with older male and female Sprague-Dawley rats, we have seen opposite relationships between exploratory behavior and glucocorticoid reactivity for males vs. females. For males, increased locomotion in a novel environment was associated with low glucocorticoid responses to novelty, indicative of low fear (Cavigelli & McClintock, 2003), whereas in females, increased locomotion was associated with increased glucocorticoid production, indicative of elevated sensation-seeking (Cavigelli et al., 2006). These results were documented in relatively old rats where sex steroids in females were declining, and differential aging of the reproductive system among high- and low-active females may have accounted for the differences. Thus, further research is required on the developmental and physiological processes that may underlie sex differences in behavioral inhibition and whether these processes are related between humans and animals.

Conclusions

Studies on rodent behavioral inhibition suggest many parallel links with human behavioral inhibition: behavioral and physiological stability parameters are similar, the trait can be identified early in development and predicts later behavior and physiology, and developmental interventions seem to have some lasting influences on underlying mechanisms associated with behaviorally inhibited health trajectories. These parallels suggest that behavioral inhibition is a fundamental trait that likely can be well-modeled in other species, with the expected limitations of any animal model. The rodent model provides a unique arena to experimentally test causal relationships and to conduct life-span longitudinal studies to understand change in both behavioral and physiological traits, their relative stability over time, and their relationship to health outcomes.

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