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## Curiosity as an approach to ethoexperimental analysis: behavioral neuroscience as seen by students and colleagues of Bob Blanchard

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### Abstract

This review is a synopsis of an *IBNS* symposium which focused on the elements of Behavioral Neuroscience for which Robert J. Blanchard was a Pioneer, Leading Expert, Advocate, Mentor, and Sage. Bob Blanchard's work demonstrably changed our broad understanding of animal behavior, and led the way to experimental design and analysis for studies of animal behavior that helped to clarify the deep complexity and subtleties of behavior. Bob's impact on the field of Behavioral Neuroscience includes the behavior, neurocircuitry, neurochemistry, and pharmacology related to social interactions, aggressive behavior, defensive behaviors, flight, freezing, threat, attack, risk assessment, anxiety disorders, animal models, models of social behavior, and autism. The methods and designs developed by Bob Blanchard over a lifetime have been adopted by scientists around the world, and form a standard of excellence in the field. The article addresses these topics in a way that presents developments in the field, describes the newest research data, and pays tribute to a great scientist and founder of this field of work, Bob Blanchard.

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## Keywords

Blanchard; ethology; ethoexperimental analysis; psychology; antipredator; defensive behavior; ecological validity; autism

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## 1. The scientific setting for a tribute: Bob Blanchard

Behavior is at the heart of all research in neuroscience and biomedical science. This is so because even the most molecular adaptations in subcellular neural systems ultimately regulate behavior. Similarly, although much of biomedical science does not implicate changes in behavior, medical therapies that drastically alter behavior, or have off-target effects that produce maladaptive behaviors, are not acceptable. Thus, elucidation of animal behavior (including that of humans) is ultimately critical to salient progress in the neuro- and biomedical sciences. Tributes often follow scientific contributions that fulfill a substantial need, and thus it is that we pay tribute to the life and science of Robert J. Blanchard. His lab's work demonstrably changed our understanding of animal behavior, and what is more, he led the way to experimental design and analysis for studies of animal behavior that helped to clarify the deep complexity and subtleties of behavior. His methods, adopted by scientists around the world, are a standard of excellence in the field.

Robert J. Blanchard, a former president of IBNS and a long-term friend and mentor to many of us in the Society, died on November 24, 2013, at the age of 76. He had not retired, and in fact supervised two dissertation meetings within a few weeks of his death from liver cancer. Bob was known for many things, including his child-like love of toys – these ran to all types of gadgets but included motorcycles and unusual cars; his fondness for opera; and his huge interest and support for the developing scientific careers of students and young researchers. This last attribute reaped its own reward. The Blanchard lab was incredibly fortunate in the quality of its students and postdocs, including in recent years Mu Yang, Karina Borelli, Eduardo Carvalho-Netto, Nathan Pentkowski, Yoav Litvin, Hiroyuki Arakawa, Roger Pobbe, Catherine Farrokhi, Michael Corley, Brandon Pearson, Erwin Defensor, Ashley Jensen, Ksenia Meyza, and Amy Vasconcellos.

This article is aimed at illustrating how Bob's work and life influenced research in these and allied fields. Although the *IBNS* symposium (June 2015, Victoria, BC) provided many anecdotes – it transpired that several of his students had kept a list of “Bobisms” experienced during their years in the lab, while his wife and collaborator Caroline Blanchard contributed a number of her own “Bob stories”—this article will focus on the science amply outlined in talks from Jacki Crawley; Newton Canteras; David Eilam; and John Rodgers –all of whom shared research interests in these areas, as well as Nathan Pentkowski (former student) and Caroline Blanchard (wife). Many of these speakers also contributed to a memorial issue of *Physiology & Behavior* (Volume 146; Guest Editors, Jaap Koolhaas and Sitse de Boer), which was presented to Dr. D. Caroline Blanchard at the close of Victoria IBNS symposium. This special issue contained a plethora of original research and expert commentary, which is in contrast to the current review focusing on broad concepts and previously published literature – all inspired by or derived from Bob Blanchard.

## 2. An introduction to ethoexperimental analysis

Bob's interest reflected his overwhelming curiosity about behaviors and the evolved mechanisms underlying them. In particular, Bob is known for an approach to analysis of behavior that deviated sharply from the conventions of his generation. He coined the term "ethoexperimental analysis" to indicate that it combines features of the ethological tradition with an emphasis on experimentation as the most efficient way to determine causal relationships among these factors. This ethoexperimental approach, emerging in the context of decades-long work from the Blanchard lab on defensive behaviors (Blanchard et al, 1990) also informs the design and interpretation of research on aggression, and on social behaviors, the other two major foci of the work in that lab. This methodological focus provides one aspect of Bob's influence, while his enormous enthusiasm for behavior as the core output of evolved neural systems in animals and his huge encouragements of those studying these phenomena provide additional dimensions to his legacy.

Ethoexperimental analysis, an approach informally emerging from his early work and casually named sometime later (Blanchard & Blanchard, 1988) combined what Bob Blanchard saw as the core advantages of several disciplines –ethology, experimental psychology, neuroscience– which were, at the beginning of his career, relatively non-overlapping. What was taken from ethology was, first, detailed attention to description and analysis of behavior(s). The 'Ethogram' – a sort of flow-chart of behaviors, often meticulously described and differentiated – could be constructed for the successive actions of a single animal, or, for the interactive actions of two members of a pair; in the latter case, enabling a particularly dynamic analysis of how one animal's actions impacted the other. In considerable contrast, traditional psychological measures at that time ran heavily to bar-press or similar behaviors in which measures are registered through the effect of the action on some type of recording device. Alternatively, locational changes such as entering/leaving a chamber or crossing over the lines of a grid, have always been popular; reflecting an emphasis (well-placed but limiting) on measurement objectivity. From the very first such studies that could be said to have an "ethoexperimental" flavor, such as Blanchard & Blanchard (1969), this emphasis on self-registering or excruciatingly objective measures went out the window, in favor of observation and description of what actually happens under specific circumstances. Caroline Blanchard noted that the graduate student who first observed the effects of a single brief foot shock on a rat came to report that the pilot study was a failure. "He said, 'this is not going to work. That rat's not doing anything. He's still just sitting there, 20 minutes later!'" A quick current search of pubmed provides about 2000 articles indicating that this particular state of immobility, first termed 'crouching' then 'freezing' (Blanchard & Blanchard, 1972) is now a primary measure of fear.

In this respect, behavioral measurement and analysis, ethoexperimental approaches are clearly closer to ethology. In another respect, underlying philosophy, they are closer still to the ethological tradition. A core belief that informed the direction and interpretation of work in the Blanchard lab was that behaviors are systematic and meaningful because they have evolved as adaptive responses to a range of situations that pose greater or lesser threats to reproductive success. Even though this view is identical to that of classic ethology, its importance is specifically emphasized in ethoexperimental analysis to incorporate the

necessity to consider how different contextual or situational features can alter the adaptiveness of behaviors. This was particularly visible in the context of defensive behavior, a pattern of different actions in which the adaptiveness of any specific action in reducing the danger of a threat is substantially dependent on features of the context in which threat occurs, as well as crucial features of the threat itself. Over many decades of animal research varying such features, these analyses resulted in set of predictions that were later evaluated with people, in the form of responses to threat situations encapsulated in a series of scenarios incorporating systematic variation in contextual and threat stimulus characteristics (Blanchard et al, 2001b): Briefly put, the human responses were strikingly similar to those of rodents in parallel situations. These results have been strongly replicated in additional studies from several countries, suggesting that biology, not culture, is more responsible for these relationships (Perkins & Core, 2006; Shuhama et al, 2008).

### **3. Bob Blanchard: The ethoexperimental approach and antipredator defensive behavior (section contributed by Nathan Pentkowski, PhD)**

Bob Blanchard, or as many of his former students and colleagues often refer to him “The Godfather of Behavioral Neuroscience”, pioneered the ethoexperimental approach in experimental psychology. As detailed throughout this review, the influence of Bob and Caroline Blanchard’s research philosophy can be seen across a broad spectrum of animal models of human psychopathology, including aggression, anxiety, autism, fear, panic and stress. While much of this work has been detailed elsewhere, the following is an homage to Bob and his contributions to behavioral neuroscience stemming from his ethological analysis and modeling of defensive behavior. This tribute summarizes Bob’s approach to studying human emotions, in particular fear and anxiety, using the ethoexperimental approach to analyze antipredator defensive behavior. As discussed below, by incorporating semi-natural environments to isolate and maximize species-typical-natural behaviors, Bob’s seminal work provided valuable tools to probe the neurobiological mechanisms of defense, including animal models of human fear and anxiety.

As I reflect on Bob’s contribution to the ethoexperimental approach, I can’t help but think of Charles Darwin. Though many may think that Bob tried to emulate his appearance, it is not the basis for drawing this comparison. Instead, I am staring at my most treasured graduation gift, an original newspaper print of Darwin I received from the Blanchards. Bob’s passion for using the ethological analysis of innate animal behaviors to understand the neurobiology of human emotion can be traced to Darwin. Indeed, the extensive use of traditional learning paradigms to investigate the neurobiology of emotion is somewhat ironic considering Darwin (1872) first suggested that human expressions of emotion evolved from similar expressions in animals, and that they were innate unlearned responses. Darwin supported this view by observing that facial expressions of emotion were similar all over the world, concluding that they were inherited or preprogrammed rather than learned. Research by Ekman and Friesen (1971) investigating isolated tribes in New Guinea provided support for Darwin’s notion, as these tribesmen were able to recognize, differentiate and reproduce facial expressions of emotion seen worldwide. This seminal work culminated in fear being classified as one of six primary emotions or emotional states that occur during the

expectation of, or encounter with, danger, and that is critical to the survival and successful adaptation of most mammalian species (Ekman et al., 1969; Ohman, 1986). This human subjective state of fear can also be thought of as activating a defensive behavioral system that includes unlearned and learned components, which function to protect all species including rodents, primates and humans, against potential environmental threats (Blanchard et al., 2001b; McNaughton and Corr, 2004; Misslin, 2003). For small mammalian prey species living in their natural habitat, such threats include predation and conspecific attack (Blanchard et al., 2005a; Blanchard et al., 1975a; Eilam, 2005; Endler, 1986).

Researchers investigating the neurobiology of fear have extensively relied on traditional Pavlovian fear conditioning techniques. Typically, a painful unconditioned threat stimulus such as footshock is applied to elicit an unconditioned fear-like response (freezing; Blanchard and Blanchard, 1969), which, through repeated pairings can be readily conditioned to environmental cues and/or contexts (Fanselow, 1980). This type of learning is critical for evolutionary success, enabling organisms to form neural representations of the world that are flexible and thus adaptable, providing a means whereby environmental cues can signal or predict danger (Blanchard et al., 2001c; Fendt and Fanselow, 1999). These learning processes are of great biological significance as they have increased the survival of animals in a world where the predictive value of stimuli change. However, while learning associations between fearful stimuli and the environment are critical for survival, a sole focus on the expression of conditioned fear (i.e., freezing) ignores innate, or unconditioned components of defense (i.e., freezing, risk assessment, flight, etc.), resulting in an incomplete description of emotions. Additionally, as Bob would often iterate, the use of an artificially contrived threat stimulus such as footshock is limited, as “it has no ethological relevance for a rat”. Therefore, to gain a more complete understanding of emotion, a focus on unconditioned antipredator defensive behaviors, including responses to actual live predators or potential (predator cues) threatening stimuli, as well as the features of the environment and stimuli that elicit them, need consideration. Furthermore, the use of predators as unconditioned threat sources provides an ethologically relevant model to examine robust conditioned fear-like behavioral responses (Blanchard et al., 2005b).

A major advantage to studying innate defensive behaviors is that they are highly conserved, and thus seen in all higher animals including humans (Blanchard et al., 2001b; Shuhama et al., 2008). As Bob would often point out, these behaviors are not species-specific, but rather can be described as species-typical and situation-specific; they are consistently expressed in a given species under similar circumstances, as well as being similar across species in form and function (Blanchard and Blanchard, 2003; Blanchard et al., 1975b). In small prey species such as rats and mice, antipredator defensive behaviors include flight, freezing, defensive threat, defensive attack, and risk assessment (Blanchard and Blanchard, 1988). These defensive behaviors are situation-specific in that they are influenced by specific features of the environment as well as stimulus characteristics, with predators tending to elicit flight if escape is possible (Griebel et al., 1996) or freezing if escape is not (Blanchard and Blanchard, 1969). This pattern of freezing to an inescapable predator dramatically changes as the threat stimulus approaches the animal (Blanchard et al., 1976a, b). As defensive distance is decreased, (the actual distance between the animal and the threat source), defensive threat (sonic vocalizations, upright posture, weapon display) becomes the

predominant behavior, which gives way to defensive attack (jump attacks, biting) when direct contact is made by the threat source or when in very close proximity (within approximately 0.5 meters for wild rats). When animals are exposed to predators in semi-natural environments, such as a visible burrow system, where escape is possible, their initial behavior is flight to a side chamber/tunnel where they avoid the surface and freeze (Blanchard et al., 2001a). After this initial period of immobility, a pattern of risk assessment emerges with orientation toward the threat source, including cautious approaches to the surface, and visual and olfactory scanning of the surface from the tunnel openings. After the potential for risk is assessed, and no further signs of the predator are detected, this period of risk assessment ceases and the animals engage in non-defensive behaviors (grooming, playing, eating and copulation); this whole process occurs over a period of hours.

In contrast to live predators, exposure to potential threats or cues that signal possible environmental threat such as predator odors, elicit strikingly different patterns of defensive behavior. These behavioral responses also depend on stimulus and environmental factors, such as level of intensity (Takahashi et al., 2005) and the ability to escape or hide (Dielenberg and McGregor, 2001). These species-typical antipredator defensive behaviors include risk assessment responses designed to facilitate predator detection by orienting toward and investigating the source of potential threat (Blanchard et al., 2011; Blanchard et al., 1990). From an evolutionary perspective, avoiding predators via detection of predatory cues is adaptive in that it decreases the chances of coming into direct contact with a live predator, thus promoting species survival (Blanchard and Blanchard, 1988). These behaviors include visual, auditory and olfactory investigation from a distance, as well as cautious flat back approach (stretched behaviors), often culminating in contact or manipulation of the odor stimulus; behaviors all designed to identify and determine the level of threat. Risk assessment behaviors may also manifest as crouch sniffing (Blanchard et al., 2001c), vigilant sensory scanning (Blanchard et al., 2005a), and rearing (Lever et al., 2006). When threat risk is assessed as low or not imminent, risk assessment usually culminates in a return to non-defensive behaviors; however, when the threat source is identified as an imminent threat, defensive behaviors that are more intense such as flight or freezing occur (Blanchard and Blanchard, 1988; Blanchard et al., 2003). If potential predatory threat sources such as cat odor are presented in a semi-natural environment (e.g., visible burrow system), the defensive pattern involves initial flight to – and freezing in – the side chambers; however this period is much more brief than in response to live predator exposure, and the animal more quickly engages in risk assessment behavior (Blanchard and Blanchard, 1989). This again consists of stretched behaviors, culminating in contact with the threat source, as well as visual and olfactory scanning of the area where the odor is located. After the animal assesses the potential for risk, their behavior returns to a non-defensive state; this process occurs much more quickly than during live predator exposure. Thus, in both instances, risk assessment serves as a method for evaluating the level of potential or real danger that results from exposure to potentially dangerous stimuli, leading to the selection of the appropriate behavior (non-defensive or defensive) in response to the threat stimulus; freezing functions to reduce the chances of predator detection. Thus, risk assessment provides environmental feedback regarding potential risk, which eventually results in a decrease in defensiveness over time.

One of Bob's greatest scientific achievements was isolating and maximizing the aforementioned antipredator defensive behaviors to create behavioral assays for investigating the neurobiological mechanisms of human neuropsychiatric disorders. Over the past few decades Bob and various colleagues conducted numerous pharmacological studies that provided predictive validity for these behaviors, as drugs effective in treating certain human psychiatric disorders reliably alter specific defensive behaviors (Blanchard et al., 2001a). For instance, drugs used clinically to treat anxiety (i.e. generalized anxiety disorder) selectively affect risk assessment and defensive threat/attack, while drugs used clinically to treat panic (i.e., panic disorder) selectively affect flight (Blanchard et al., 2003). Thus, during exposure to cat odor anxiolytics lower defense by reducing risk assessment and producing a faster return to a non-defensive state. In contrast, during live cat exposure anxiolytics lower defense by facilitating the transition from freezing to risk assessment (decreasing latency), which eventually results in a more rapid return to a non-defensive state (Blanchard et al., 1997). These effects suggest that anxiolytics such as benzodiazepines (e.g., diazepam and chlordiazepoxide) and 5-HT agonists (e.g., buspirone) lower defense, with the specific behavioral manifestation dependent on the level of environmental threat. Thus, against a background of high threat (predator exposure; high levels of freezing), anxiolytics reduce freezing and increase risk assessment, while against a background of lower threat (cat odor; high levels of risk assessment) anxiolytics lower risk assessment and increase non-defensive behaviors. This pharmacological support, along with studies reporting that humans also display basic defensive reactions, and that these responses share with their nonhuman equivalents many factors that determine the form of the response, support the existence of homology of defense across mammalian species (Blanchard et al., 2001b; Perkins and Corr, 2006; Shuhama et al., 2008) and the use of antipredator defensive behaviors to screen novel therapeutics for anxiety and panic.

#### **4. Ecological validity and the studies by Bob and Caroline Blanchard (chapter contributed by David Eilam, PhD)**

A fundamental question is whether impoverished testing laboratory environments appropriately approximate the behavior of wild species in their natural environment. This parameter, usually referred to as '**ecological validity**', asks whether behavior observed in the laboratory can be generalized to natural behavior (Schmuckler, 2001). The question is usually circumvented by appropriate controls that highlight a specific facet of the behavior via basic measurements that focus on specific behaviors (startle response, freezing, aggression, etc.) and provide some external validity (generalization). Nevertheless, the problem of the impoverished environment and measurements becomes especially critical in defensive behavior, where in nature, predator-prey interactions are derived from a continuous arms race in which both opponents incessantly adopt new means by which to neutralize the behavior of one another. From among the numerous studies by Bob and Caroline Blanchard, I have chosen their works on the visible burrow system (VBS) (Blanchard and Blanchard, 1989; Blanchard et al., 2001d), as an elegant example of a laboratory study with ecological validity.



The VBS habitat was designed by the Blanchards to mimic the burrow systems of rats. In nature, hierarchical groups of rats inhabit a burrow system, from which they depart to foraging roundtrips outside. The VBS apparatus is a system of chambers interconnected by short and long tunnels, some of which open to a surface area with no ceiling (Figure 1). Behavior is recorded on video to capture the movements of the animals within the tunnels and burrows as well as on the surface area during both light and dark circadian phases. Laboratory rats adapted to the VBS, sleeping during the diurnal phase and were active on the surface during the nocturnal phase (Blanchard et al., 1985). They established dominance hierarchies, with the dominant and subordinate males and females displaying differential use of the surface and burrows (Blanchard and Blanchard, 1989). The VBS apparatus has been extensively used in numerous studies, while slightly modified versions of it have also been used in other studies, especially in the context of studying social stress (Melhorn et al., 2010; Tamashiro et al., 2007). Additionally, new animal models, which also strive for ecological and ethological validity, have been inspired by the elegant design of the VBS (Robertson et al., 2015). Originally, the VBS environment was used to study defensive behavior, but the apparatus also supports a variety of non-defensive behaviors, including social, sexual, and consummatory activities, thereby enabling a scrutiny of the interaction of defensive and non-defensive behaviors.

In the context of the VBS, we engaged in scientific inquiry, addressing the question of ecological validity in studies of wild species. Specifically, some of the results obtained by Bob and Caroline Blanchard by introducing a domestic cat on top of the VBS and examining the response of individual laboratory rats that were inside it, were also reflected in our study with barn owls (*Tyto alba*) attacking groups of social voles (*Microtus socialis guentheri*) (Bodek and Eilam, 2015). The findings of the Blanchards most relevant to our work:

- i. Dominant male rats were least affected by the cat compared to subordinate males. For example, compared with subordinates, dominant rats displayed shorter latencies to move, groom and cross open areas.
- ii. Following exposure to the cat, a convergence to a similar behavior characterized the 12 groups of rats that were tested in the VBS. In other words, the differences in behavior between females, subordinate males, and dominant males, as noted during the pre-cat period, diminished following their exposure to the cat.

As in other studies by the Blanchards, these results were also derived while paying attention to the sensitivity and specificity of measurements such as latency to move, latency to groom, latency to enter the surface area, etc., as displayed by animals of different social rank and gender. Instead of the laboratory rats and the cat in the VBS studies by the Blanchards, in our studies we used voles, which are heavily predated upon by owls, and a barn owl, which is the voles' natural enemy, as the threat (Eilam et al., 2011; Eilam et al., 2012; Izhar and Eilam, 2010; Kleiman et al., 2014). We used body mass as a proxy for social rank. Specifically, social animals usually establish a social hierarchy, and it is commonly the larger and stronger individuals that are ranked higher than the smaller and weaker ones. Indeed, body mass is a reliable predictor that positively correlates with social rank in voles (Sokolov et al., 1990) as well as in other species (for example: reindeer – Holand et al., 2004; monkeys – Morgan et al., 2000).

Figure 2 depicts the behavior of grouped individual voles in an elevated plus maze before and after they were attacked by a barn owl (via a wire mesh that prevented physical contact between owl and voles) (Kleiman et al., 2014). It adds to descriptions of behaviors in home cage situations in direct response to threat presented there, by examining changes after predator exposure in animals' responses in a task commonly used to evaluate anxiety. As shown, voles with above-median body mass (red circles), presumably of higher social rank, hardly changed their behavior when exposed to the owl (●) compared to their behavior when the owl was absent (○). In contrast, voles with below-median body mass (squares) and presumably of lower social rank, displayed large changes in their behavior after the attack (■) compared to their behavior before it (□). Moreover, the behavior of most of the high-mass voles was in the mid-range (center of the abscissa; encircled in dashed green circle), whereas the low-mass voles were at the extremes (outside the dashed circle). As in the VBS experiments by the Blanchards (Blanchard and Blanchard, 1989; Blanchard et al., 2001d), our results also indicate that dominant individuals respond less variably to predatory threat compared with subordinate individuals. Another similarity between the studies was the finding that the differences between individuals diminished following exposure to live threat.

A strength of many of the studies by the Blanchards is that behavior was usually also controlled by variables at additional levels of analysis, generally that of the hormonal or neurotransmitter systems. In the same vein, we measured the levels of corticosteroids in grouped voles that were attacked by an owl compared with voles that were not attacked, and assessed their response while emphasizing the impact of gender and social rank. We found that females had higher blood corticosterone than males, and that corticosterone levels for both increased after owl attack compared to the level before the owl attack (Bodek and Eilam, 2015). In terms of social rank, the moderate behavioral change noted in males with higher social rank under owl attack (Figure 2) was also reflected in their steady level of corticosterone (Figure 3, arrow). Similarly, the greater behavioral change in low-mass voles (Figure 2) was reflected in a significant increase in their blood corticosterone (Figure 3). In low-mass females, corticosterone levels without the owl were near the top sensitivity of our corticosterone measuring kit (900 ng/kg), while a somewhat lower level was measured in high-mass females that were not exposed to the owls. This ceiling of our measuring kit was reached by both the low-mass and high-mass females that were exposed to the owl (Figure 3).

Altogether, our study with predator and prey in wild species reconfirms and adds ecological validity to the seminal findings by Bob and Caroline Blanchard with laboratory rats and domesticated cats in a semi-natural testing environment. This illustrates the scholarly approach of the Blanchards to research, in which attention to the sensitivity and specificity of measures and innovative testing apparatuses facilitated results with strong external and ecological validity.

## 5. The focus of attention

A final major overlap with ethology can be found in an emphasis on particular species differences. Specific differences in characteristics are associated with the modulation or organization of a wide range of behaviors. One of these is sociality. Social nature becomes

of particular importance when correspondence between animal models and behavior disorders of humans (an incredibly social species) is the focus of attention. Following a Bethesda visit several years ago with Dr. Jacki Crawley about social abnormalities in autism and in mouse models of autism, Bob reported to his wife that the conversation prompted him to “study something important, for a change” (Bob’s fondness for dramatic effect was frequently in evidence; Caroline Blanchard, personal report). The result was an abrupt switch, aimed at the construction of new and more selective tests to evaluate autism-relevant behaviors in mice. Bob, Caroline and their students proceeded to investigate social behaviors in several mouse models of autism. Between 2007 and 2015 their team published 18 papers on social behaviors in BTBR, *Mecp2*, and oxytocin receptor knockout mice.

Although there were a number of components to this effort, one particularly interesting feature that emerged was a “zoom out – zoom in” approach. This approach captures both the overall time/behavior-budget for different mouse strains in a semi-natural situation, the VBS (“zoom-out”). Which can then be compared this with the detailed dynamics of some of the same behavior patterns, observed in a much more restrictive and focused situation, the Social Proximity Test (“zoom-in). These comparisons indicated that, while the BTBR inbred strain (BTBR *T+ Itpr3tf1*), the most commonly used idiopathic mouse model of autism, which displays social deficits in many standard assays (McFarlane et al., 2008), did tend to show deficiencies in social behaviors in the VBS that could be explained in terms of enhanced avoidance of conspecifics (Pobbe et al, 2010). Importantly, the behavior of this strain when avoidance was not possible (Social Proximity Test) showed other, and very specific, parallels to the autism behavior phenotype. In particular, frontal orientation with nose tip to nose tip behavior was strikingly reduced in BTBR mice in the Social Proximity test, (Defensor et al, 2011). Notably, this specific behavior provides a possible parallel to gaze aversion, a strong component of the autistic behavior phenotype and a potential early predictor of this phenotype in young children (Clifford et al, 2007). Importantly, this behavior showed no trace of normalization in response to diazepam, an anxiolytic that did, in fact, reduce measures of avoidance in both the “zoom out” and the “zoom in” situations, providing a potential dissociation of autistic-phenotype-relevant behaviors that are, or are not, dependent on social anxiety (Pobbe et al, 2011).

The additional component of the ethoexperimental approach, experimentation, while clearly associated with the psychological tradition, is equally characteristic of behavioral neurosciences, a field emerging over much the same period: IBNS was itself founded in 1992. And, it was a toe into the water of molecular biology that constituted the last major research focus of Bob’s life. While behavior was, for him, fascinating for its own sake, he acknowledged a primary role for animal models of enabling experimentation to understand the mechanisms underlying behavior; particularly important in the context of treatment for behavioral disorders (Blanchard et al, 2013). The consistency of findings of an autism-relevant behavior phenotype for BTBR mice suggested a potential value in investigating some of the mechanisms involved in forebrain connectivity in these mice. This fostered interest in a site, the lateral ventricle subventricular zone, in which a significant proportion of forebrain cells are generated, differentiate into neurons and glia, and from which they migrate to their appropriate cortical and subcortical destinations. A major molecular modulator of all of these activities, heparan sulfate, proved to be reduced by about 50% in

the LV-SVZ of adult BTBR mice compared to C57BL/6J controls (Meyza et al, 2012). Subsequent studies in the LV-SVZ of individuals with autism vs. typically developing (TD) individuals, provided an even more striking difference: Young children with autism showed a reduction of about 75% in heparan sulfate in this area. By age 60 little difference between autism and TD tissue was seen, as heparan sulfate declined dramatically with age, perhaps in consequence of being less necessary or functional in older brains (Pearson et al, 2013). This work continues, currently focusing on genetic and epigenetic differences in this area with autism, but alas without Bob.

## 6. The future of the ethoexperimental approach

Translational biomedical research utilizing rodent models will continue to benefit from using ethoexperimental designs. Optogenetics is a prime example of an emerging molecular technology allowing analysis and modulation of complex brain functions including an emphasis on behaviors and underlying emotions carefully analyzed in the 5 decade history of the Blanchard lab. For example aggression (Lin et al, 2011 Nature), anxiety (Tye et al, 2011), conditioned fear (Namburi et al, 2015) and numerous other behavioral expressions have all been cleverly and precisely manipulated optogenetically. Furthermore, the development of CRISPR-cas9 mutational techniques permits the rapid generation of mutant models pertinent to human somatic and neuropsychiatric conditions. This rapid proliferation of molecular tools advances the ability of behavioral neuroscience to assess neural mechanisms, test hypotheses about disease and to evaluate novel therapeutic approaches. However, these approaches are even more valuable in the context of behavioral methods such as zoom-in, zoom-out techniques as described above for autism models. As the precision of molecular tools advances, so too should the subtlety of behavioral analyses, especially under conditions requiring stricter translational validity. Thus advanced molecular tools, together with the classic ethological approach to careful observation and ethoexperimental tenets of context, form and function, permit rapid discovery in environment-gene-brain-behavior-disease relationships. The natural curiosity of Bob Blanchard, his collaborators, students and friends, and other like-minded behavioral neuroscientists will continue to contribute greatly to advances in modern medicine and basic knowledge, equipped with relevant and valid tools.

While scientific acceptability is increasingly centered on rapid high-volume results, Bob Blanchard reminded us that the methods by which we approach behavioral experiments are just as significant. As Pierre Flourens would have suggested from early critical experimental approaches to brain and behavior (Flourens, 1842), methods create results, and determine how significant those results may be. The Blanchard ethoexperimental approach captures the dynamism of a natural environment and the mechanisms that are specific to highly nuanced social behaviors. He recognized the value of ecologically and ethologically relevant models for elucidating psychological disorders, and called for the creation of other new models which incorporated similar dynamics (Blanchard and Blanchard, 1989; Carpenter et al., 2009; Robertson et al., 2015; Smith et al., 2015). As we continue to investigate the neural mechanisms involved in producing clinically relevant disorders, Bob Blanchard's influence on how we design our model systems and behavioral paradigms, specifically, using ethoexperimental approach to explore translational interactions of physiology, genetics, and

environment on social adaptations will continue to inspire better experimental design and promote a greater understanding of disorders of the brain.

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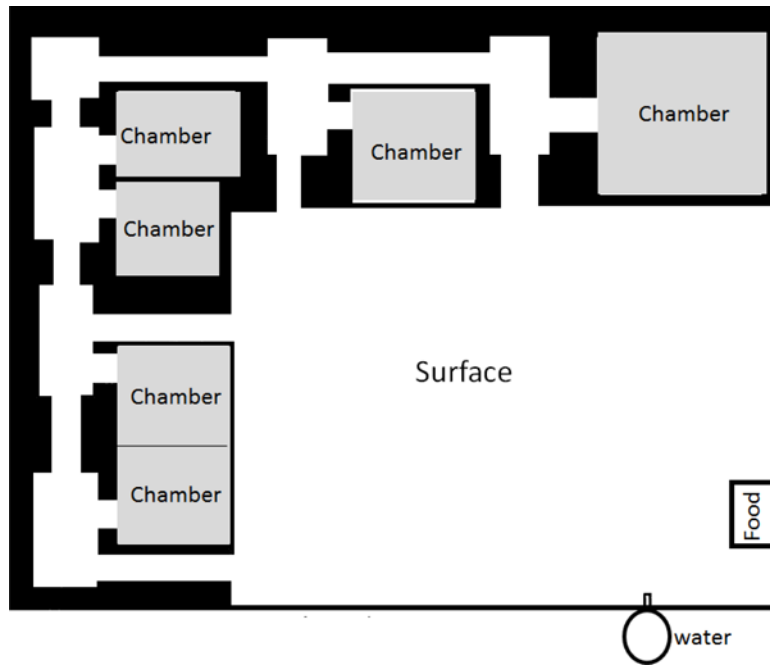
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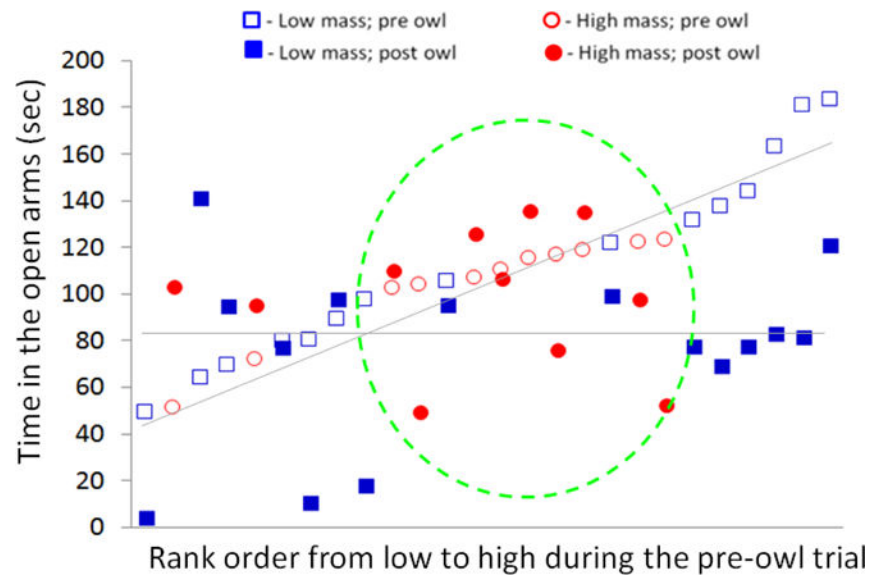
### Highlights

- Bob Blanchard's work demonstrably changed our broad understanding of animal behavior
- Ethoexperimental approach informs the design and interpretation of research
- Neural representations of the world are flexible and thus adaptable
- The visible burrow system represents an animal model with ecological validity
- A zoom out-zoom in approach captures both general sociality with detailed dynamics
- Blanchard called for the creation of new ecologically and ethologically relevant models

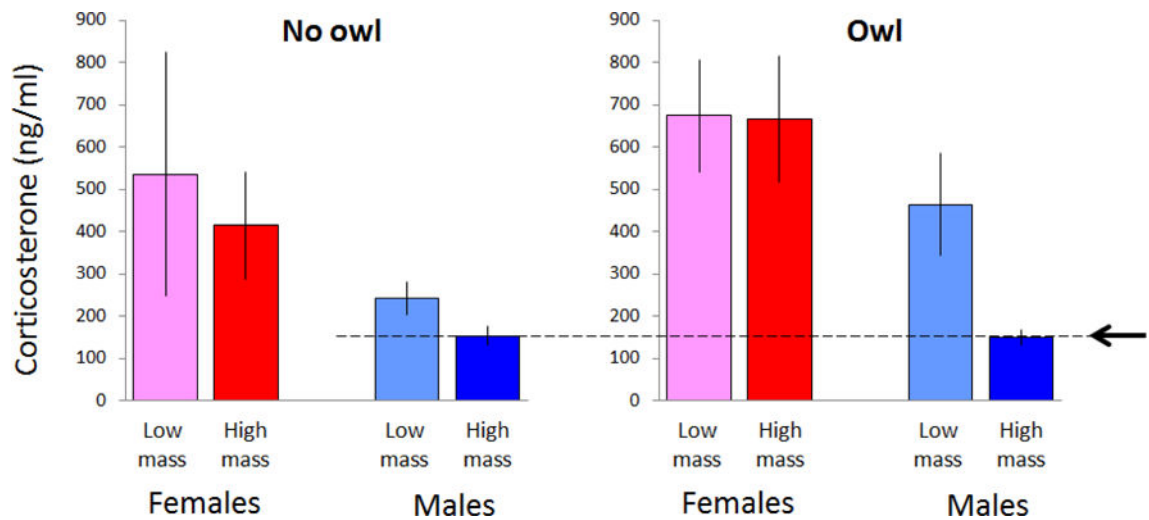




**Figure 1.** (replotted after Blanchard and Blanchard, 1989): The visible burrow system, comprising a set of chambers connected by tunnels to an open surface area.



**Figure 2.** (Data from Kleiman et al., 2014). The impact of exposure to the owl on the time that individual voles spent in the open arms of the elevated plus-maze. Each vole was individually tested in the maze twice: 24 hrs before exposure of its group to the owls, and two hours after the owl had attacked the voles' cage. The voles are ordered along the abscissa from low to high according to their behavior during the pre-owl trial (open symbols), resulting in an inclined sequence (diagonal gray trend line). The respective post-owl scores for each individual vole (closed symbols) are depicted in the same order as those of the pre-owl scores, with the behavior of the same vole aligned vertically for the pre- and post-owl trials. A trend line of all voles in the post-owl trial is depicted by the horizontal gray line. Voles with above-median body mass are depicted by circle symbols and voles with below-median body mass by square symbols. Voles that spent a long time in the open arms of the elevated plus maze during the pre-owl trial (ordered at the right of the figure) spent less time in them during the post-owl trial. Voles in the mid-range (mostly high-mass voles) spent about the same amount of time in the open arms in both pre-and post-owl trials. Voles that spent little time in the open arms during the pre-owl trial (left ones) dichotomized to an increase or decrease in time spent in them during the post-owl trial. Overall, these changes result in an horizontal trend line for the entire group.



**Figure 3.**

Corticosterone level (ng/ml; mean  $\pm$  SEM); as measured by Immuchem Double antibody  $^{125}\text{I}$  RIA by MP Biomedicals, Orangeburg, New York kit with sensitivity range of up to 900 ng/ml). Data are depicted for voles that were exposed to the owl (right) compared with voles that were not exposed to it (left - see Bodek and Eilam, 2015 for detailed analysis of these results). Note that the corticosterone levels are relatively stable in high mass voles (arrow and dotted line).