**ILAR J**

Volume 55, Number 2, 2014

***Behavioral Assessment in Animal Models: Relevance for Human Psychopathology***

**Novak and Meyer. Introduction, pp. 217-220**

SUMMARY: This article is an introduction to the November ILAR issue, which centers on animal models of neuropsychiatric disorders and the methods that can be employed to study these disorders.  Some examples include but are not limited to, experimentally induced PTSD or idiopathic self-injurious behavior (SID) in rhesus macaques, induced abnormal mood states in rodents such as forced swim and tail suspension tests, learned helplessness (placing animals in an inescapable aversive situation), olfactory bulbectomy, or chronic mild stress paradigms.

There are several challenges in the development of animal models of neuropsychiatric disorders.  For example, mental health disorders include symptoms that are highly subjective (e.g., feelings of anxiety) or involve impairments in higher cognitive processing (e.g., language) making them difficult to model in animals.  Due to this, feelings cannot be directly interrogated in nonhuman animals. Nevertheless, animal model testing routinely uses behavioral measures that are assumed to be indicative of the animal’ s subjective state, as demonstrated by the terminology of “ depressive-like” or “ anxiety-like” behavior. It is unlikely that any animal model will replicate all relevant symptoms associated with a particular disorder. Instead, the model may attempt to replicate specific symptom clusters rather than the full spectrum of symptoms.

The following paragraphs briefly describe a few of the articles mentioned in the introduction: Fiona Hollis and Mohamed Kabbaj summarize the social defeat model of depression in which an adult male rat or mouse is repeatedly introduced into the home cage of dominant male resident animal. Each time, the intruder is rapidly attacked and defeated by the resident eliciting a powerful acute stress response in the intruder and leads to depressive-like behaviors such as decreased activity, social avoidance, increased fear and anxiety, anhedonia, and altered food intake and body weight gain.

Hagit Cohen and colleagues describe the current status of rodent models of PTSD, such as exposure of the animals to predator stimuli such as cat or fox odor.  They also discuss their “cut-off behavioral criteria” model in which rats are exposed to predator scent and subsequently tested for anxiety-like behavior on the elevated plus maze and auditory startle response. Only those animals that exhibit an “extreme behavioral response” on these tests are considered to have developed a PTSD-like syndrome. This paradigm has the benefit of recapitulating the observation that only a subset of trauma-exposed humans develops sufficiently extreme symptoms to reach a clinical diagnosis of PTSD.

Julie Worlein focuses on depression in nonhuman primates in which exposure to early-life stress in concert with genetic predispositions prime the central nervous system to respond to subsequent stressors with pathological behavior.

The strongest model to date in nonhuman primates involves the development of depression in infants as a result of prolonged or repetitive separation from the mother.

QUESTIONS

1. True or False? Individuals with serious mental health disorders not only have poor quality lives, they also are at increased risk for premature death.

2. Match the term with the definition:

1. Face validity

2. Construct validity

3. Predictive validity

A. The neurobiological mechanism underlying the mental health disorder and the proposed animal model are as similar as possible

B. Refers to concordance of the model with behavioral and/or neurobiological/physiological symptomatology observed in the human disorder.

C. Proposed animal model responds appropriately to treatments (usually pharmacological) that offer therapeutic benefit to patients with the disorder being modeled.

3. True or False? Approximately 90% of all suicides per year across the globe are associated with some form of mental health disorder.

ANSWERS

1. True

2. 1. B; 2. A; 3. C

3. True

**Hollis and Kabaj. Social Defeat as an Animal Model for Depression, pp. 221-232**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (Mus musculus) and Rat (Rattus norvegicus)

SUMMARY

Social Defeat Paradigm:  Social defeat (also referred to as the resident-intruder test) uses social conflict between members of the same species to generate emotional and psychological stress.  Social defeat is initiated when a male rodent is introduced into the home cage of an older, aggressive, dominant male.  The intruder is quickly attacked and forced into subordination for the remainder of the physical interaction.  When flight is barred, the intruder will assume a submissive, supine posture, emitting frequent calls of distress and illustrating freezing behavior.  Repeated exposure to social defeat generates persistent emotional stress without habituation.  The lack of habituation is in stark contrast with other common stressors, particularly restraint stress, where animals quickly adapt by the fourth or fifth presentation.  Even a single defeat experience can elicit a number of profound physiologic changes, such as changes in daily body temperature rhythms, retarded growth, sensitivity to other stressors, and increased anxiety.

Effects of Chronic Social Defeat on Behavior:  After two to four consecutive days of social defeat, rats exhibit significantly decreased locomotor and exploratory activity, reduced aggression and sexual behavior, and increased submissive behavior and anxiety.  In humans, a diagnosis of depression can be made after the display of a number of symptoms, including self-reported depressed mood, guilt, suicidal thoughts, loss of pleasure (anhedonia), social avoidance behavior, hopelessness, sleep disturbances, weight changes, and psychomotor alterations.  Of these symptoms, a subset can be studied in animals, including anhedonia, social avoidance, locomotor changes, and metabolic changes.

Neurobiology of Defeat-Induced Behaviors: Rodents repeatedly exposed to social defeat have decreased volume and cell proliferation in the hippocampus and medial prefrontal cortex that is reversible by chronic antidepressant treatment.  Social defeat also has strong effects on dendritic morphology.  Another major link in the pathogenesis of major depression is altered serotonergic neurotransmission.  Attention has also been given to the role of neurotrophic factors in the causation of depression.  Neurotrophic factors are growth factors that induce the survival, development, and function of neurons.  One neurotrophin in particular has been the focus of numerous studies- namely, brain-derived neurotrophic factor (BDNF).

Social Defeat as a Model for Depression:  Animal models of psychiatric disease face a number of challenges, from poor construct validation to poor predictive power.  Although a single etiology for depression remains unlikely, exposure to stress appears to be a significant contributing factor, providing some measure of construct validity for social defeat.  One serious concern is the difficulty in studying female subjects (models of social defeat typically rely on male aggression not readily displayed in female subjects).  Another potential drawback of social defeat is the age during exposure.  Social defeat is performed nearly exclusively in adult animals.

QUESTIONS

1.   Anhedonia is:

a. Reduced pain perception

b.  Reduced sense of smell

c. Reduced tactile sensation

d.  Reduced ability to experience pleasure

2.  Models of social defeat are more commonly studied in:

a.   Females

b.   Males

c.   Juveniles

d. Neonates

ANSWERS

1.  d.  Reduced ability to experience pleasure

2.  b.  Males

**Cohen et al. Maintaining the Clinical Relevance of Animal Models in Translational Studies of Post-Traumatic Stress Disorder, pp. 233-245**

Domain 3: Research

Primary Species: Rat (Rattus norvegicus)

SUMMARY:  A diagnosis of PTSD requires 4 types of symptoms; 1) re-experiencing like flashbacks or dreams, 2) avoidance and emotional numbing, 3) negative mood, thoughts, and 4) exaggerated responses to normal stimuli.  These symptoms must still be present 1 month after the traumatic event.  People who suffer from PTSD also may develop permanent changes in the hippocampus and disturbances to the HPA-axis.  Animal models for this condition are based on observable behavioral responses to adverse stimuli and histopathological changes in the brain.  The authors describe cutoff behavioral criteria (CBC) to model PTSD in rats.  First, the rats must be exposed to a traumatic event.  The CBC model uses 10-15 minute exposure to cat urine (predator scent stress, PSS).  They argue this has ecological validity as “it mimics short, intense, threatening experiences with lasting affective consequences.”  The rats are then observed on an elevated plus maze (EPM) to measure anxiety, fear, avoidance, and hyper-alert behaviors.  Their acoustic startle response (ASR) is also tested.  Based on these tests rats are classified as having an extreme, partial, or minimal behavioral response.  The model accurately reflects prevalence of PTSD in the population after trauma.  Some of the rats also developed metabolic syndrome later in the course of study, consistent with long-term disruption of the HPA-axis.  Interestingly, a large dose of corticosteroid or sleep deprivation in the immediate aftermath of the traumatic event seemed to prevent this long-term HPA dysfunction and lessen the severity of PTSD signs.

QUESTIONS

1.   Which of the following tests are used to measure anxiety?

a.  Elevated plus maze

b.   Morris water maze

c.  Porsolt swim test

d.   Tail flick response

2.  Animal models of PTSD can accurately reflect:

a.  Hyper-responsiveness

b.  Numbed affect

c.  Intrusive dreams

d.  Dissociative aspects

3. What is a possible long-term sequela of PTSD?

a. Hypothyroidism

b.  Metabolic syndrome

c.  High blood pressure

d.  Stroke

ANSWERS

1.  a

2.  a

3.  b

**Goode and Maren. Animal Models of Fear Relapse, pp. 246-258**

Domain 3

Primary Species: Mouse (Mus musculus) and Rats (Rattus norvegicus)

SUMMARY: There are many similarities between humans and animals in the neurobiological systems that underlie emotional memories, and animal models have been and continue to be important in the search for new therapies for anxiety.  Fear memories are generally formed rapidly and last over time, making it challenging to erase fear memories.  Behavioral therapies directed at erasing fear memories-known as extinction learning- typically suppress rather than fully erase these memories, and as a result, fear can overcome extinction and can return under a variety of conditions.  The study of emotional learning in lab animals relies heavily on Pavlovian fear conditioning, where a neutral conditioned stimulus (CS), such as a tone or light, is paired with an aversive unconditioned stimulus (US), such as a foot shock.  During this experience, animals form an association between the CS and US, as well as between the US and the context in which it occurs.  This includes the physical environment as well as the animals’ internal state of being.  Pavlovian fear conditioning serves as a translational model, as fear acquisition in rodents is very similar to that of humans.  Extinction of fear involves degrading the link between the CS and US by exposing the subject to the CS alone repeatedly without the US, or in the case of context fear, exposing the subject to the conditioned context without the US.  Extinction has been shown to engage neural circuits that act on and interact with those involved in conditioning, and these same neural circuits are active in humans during the suppression of fear; thus fear extinction in rodents may model exposure therapy in humans, which is used to treat many anxiety disorders, such as PTSD.  However, certain fear-relevant cues in humans have been shown to be difficult to extinguish.

Extinction Retention: Pavlov was the first to note that extinction procedures produce only a temporary loss of the conditioned fear response (CR).  Thus, extinction procedures do not completely erase fear memories but rather lead to a new memory that inhibits the representation of the US.  There are four fundamental fear relapse phenomena: renewal, spontaneous recovery, reacquisition, and reinstatement.

Renewal: Renewal of the fear CR can occur when the CS is encountered outside of the extinction context.  Although fear memories readily generalize across contexts, extinction learning is characteristically limited to the context in which the extinction memory was formed.  Renewal appears to be mediated by an unexpected occurrence of the CS in any given context; since contextual information is so important in fear responding, it is thought that renewal interacts with other forms of fear relapse.

Spontaneous Recovery: This is when a CS returns merely with the passage of time.  In animal models of renewal or spontaneous recovery, the presentation of a reminder cue of the extinction context alleviates relapse of fear.

Reacquisition: Reacquisition occurs when the CS and US are paired again after extinction has occurred; the restoration of the fear CR is often quite rapid.  The reacquisition in some cases can be much slower, and may depend upon the presence of contextual cues for extinction; when the CS and US are re-paired in the presence of conditioning context cues (and the absence of extinction cues), the reacquisition of fear is much more rapid.  There are ways to deepen extinction and make reacquisition of fear less likely through intermittent pairings of CS and US alongside CS-alone presentations; however some reacquisition of fear is likely to occur.

Reinstatement: Reinstatement occurs when the US is encountered in the absence of the CS after extinction and reinstates the fear response to the CS.  Reinstatement is generally context-specific but not always, and reinstatement may be observed outside of the context in which the US is presented.  Reinstatement may be influenced by the simple aversiveness of the US, such that the fear state induced by an unsignaled US reminds the animal of the state of fear at the time of conditioning, thereby causing fear conditioned response.  Stress may also play a role in reinstatement and fear relapse, as stress symptoms in the brain are known to overlap and interact with fear circuitry, but the exact relationship between stress and fear relapse is unknown.

Stress and Fear Relapse: Stress can be caused by many different things and has a variety of physiological effects; it can also strengthen or inhibit fear memory formation depending on the nature of the stressor.  There are many factors that contribute to fear relapse, including type of stress (physical vs. psychological; controllable vs. uncontrollable), time course (acute vs. chronic), and physiological effect on the animal.  In rodent models, physical stressors are ones which expose the animal to a physical strain, such as foot shock, loud noise, and forced swim.  Psychological stressors include those that do not place a physical strain on the animal e.g., predator odor exposure, prolonged elevation, and social isolation.  Psychosocial stress can be induced through the introduction or removal of conspecifics, such as social defeat, maternal deprivation, and chronic subordinate colony housing.  Electrical stimulation of anxiogenic regions in the brain and certain drugs can also induce a stress response.  All of the stressors used in rodent animal models are analogs of stressors used in human research, and rodent research has highlighted the potential role of psychological stress in fear relapse.  In several rat studies, a relapse of previously extinguished fear response was induced by exposure to unsignaled physical or psychological stress, suggesting that any aversive experience may result in reinstatement of extinguished fear.  Other rodent studies have demonstrated that stress prior to conditioning facilitates fear learning, while stress prior to extinction can impair acquisition of extinction; this is similar to what is seen in humans.

Individual Differences and Susceptibility to Relapse: Many factors may influence an individual animal or human’s susceptibility to anxiety, fear relapse, and fear extinction.  These include sex, developmental stage/age, genetic predisposition, and epigenetic factors.  Rodent and human studies have demonstrated that these variables likely play a role in determining the nature of an individual’s fear acquisition and expression of extinguished fear, but results are not consistent across all studies.  Further research into these areas is needed, and may provide insight to develop strategies of relapse prevention that can be targeted to susceptible groups.

Strategies to Prevent Relapse

*Optimizing Behavioral Therapy*: Extinction training is widely used in humans and rodent models to prevent fear relapse.  Extinction learning is slowly acquired, and the duration and timing of the training can influence the magnitude and duration of the fear reduction.  Spacing fear extinction trials and training over numerous time points (as opposed to massed extinction trials with short inter-trial intervals) may lead to stronger fear extinction memories.  Extinction in multiple contexts may also help facilitate fear suppression in future encounters with the CS, and pairing massed extinction with this strategy may be more effective at preventing relapse than either strategy alone, but results are not consistent.  Enriched environments may offer protection against stress-induced fear relapse, and voluntary exercise may foster resilience in animals.  In a rodent model, those animals that spent more time on an exercise wheel were less negatively impacted by uncontrollable stress.  The time of day that fear extinction training occurs may be a factor in the strength and efficiency of the training as well, with work in humans demonstrating better extinction when training occurred in the morning.  The time of initiation of extinction training is also debated; in humans with PTSD it is common to institute behavioral therapy immediately following the trauma, and in some cases have been effective in preventing relapse.  However in rodent models, immediate extinction after conditioning is often unsuccessful at creating long-term fear suppression.  In human studies, delayed extinction has been shown to be generally more effective at preventing relapse in renewal and spontaneous recovery paradigms.  There are many ways to approach extinction training and more research is needed to determine the best way to apply these techniques to enhance fear extinguishing training and increase chances of long-term success.

*Pharmacotherapeutic Interventions*: Treatment of anxiety disorders often includes medications in combination with behavioral interventions.  Researchers have identified many different drug strategies that have shown to facilitate extinction learning in animals in different contexts, including L-dopa, propranolol, cannabinoids, scopolamine, D-Cycloserine, and some antidepressants.  Research into other, more unconventional molecular targets has yielded some interesting results, e.g. increased magnesium levels in the brain improved extinction retention and injection of fibroblast growth factor 2 in rats helped prevent renewal.

*Deep Brain Stimulation*: Regions of the prefrontal cortex (PFC) in rodents and humans have been implicated in fear suppression and extinction, and stress may disturb these same brain areas.  Functional activation of the PFC through high-frequency stimulation may facilitate extinction and may even help prevent relapse of fear.  The combination of brain stimulation and behavioral training may prove to be more successful.  There is also evidence that low-frequency stimulation can also reduce spontaneous recovery and that peripheral vagus nerve stimulation paired with extinction training can produce rapid fear suppression.  Further research in these areas is needed to fully elucidate the influence of drugs, behavioral therapy, and deep brain stimulation on fear extinction.

Conclusions: Fear relapse after exposure therapy remains a major hurdle to successful treatment of fear and anxiety.  There are a plethora of factors that can influence the development of fear and facilitate successful fear extinction.  The study of Pavlovian fear conditioning and extinction in rodents has provided many insights into the understanding of fear relapse phenomena, although further work is needed to more clearly understand why and how fear returns in some patients.

QUESTIONS

1.  Which of the following is an example of a conditioned stimulus used in rodent models of fear conditioning?

a.  Foot shock

b.  Brief tone

c.  Toe pinch

d.  Forced swim

2.  Which of the following is NOT one of the four fear relapse phenomena mentioned in the article?

a.  Renewal

b.  Reinstatement

c.  Reacquisition

d.   Spontaneous recovery

e.  Regeneration

3.  What learning model is typically used in animal models of fear and anxiety?

a.   Pavlovian fear conditioning

b.  Stress-induced fear

c.   Exposure therapy

d.  Positive reinforcement training

ANSWERS

1.  b. Brief tone

2.  e. Regeneration

3. a. Pavlovian fear conditioning

**Worlein. Nonhuman Primate Models of Depression: Effects of Early Experience and Stress, pp. 259-273**

Domain 3

SUMMARY: Depression affects over 350 million people in the world and has been ranked as the fifth leading cause of disability.  In the US, nearly 2 in 10 people is affected by major depressive disorder one or more times in their lifetime, and this insidious disease has repercussions for not just the individual but also creates an economic burden.  There are many confounding factors that make studying depression in the human population difficult; animal models provide a way to eliminate some of these variables and study the disease in a more controlled setting.  While NHPs are not a perfect model for depression, they have been very useful in studying the effects of early life experience on later behavioral and physiologic development in relation to depression. The availability of genetic determination in NHPs and the relative ease with which their life history experiences can be varied makes them useful for evaluating gene-environment interactions and epigenetic effects.  It is also helpful that NHPs live in complex social groups with extended childhood dependent on parental care even after weaning, and that their brains more closely resemble human brains than rodent species.

The Diathesis-Stress, or Two-Hit, model of depression (and other psychopathology) hypothesizes that events occurring during brain development (the first “hit”) produce a CNS that is primed to express psychopathology; later in life this primed CNS encounters a second “hit” in the form of stress or another triggering event which leads to the emergence of the psychopathologic symptoms.  Human data suggests that there is a genetic vulnerability for depression, and the heritability of major depression is estimated to be 31-50% in human population.  Several human studies have demonstrated that early life neglect, abuse or parental loss results in an increased propensity for depression in adults.  Although the Two Hit model may be useful, it is only one of many models of depression.  Depression has been shown to develop in humans and NHPs after overwhelming physiological trigger events in an otherwise non-primed CNS (examples include Cushing’s syndrome and administration of certain medications, hormones, and proinflammatory cytokines).

Experimental NHP models of early adversity fall into three categories: (1) rearing without a mother but with contact peers during development (peer rearing) (2) separating and reuniting mother-infant pairs at variable time points during development (maternal separation) and (3) subjecting the mother to stress-inducing experimental manipulations that affect the mother-infant relationship and alter infant developmental trajectories.  Peer rearing produces significant behavioral and physiological changes in NHPs that persist into adulthood, and peer-reared infants are more likely to develop affective responses that are seen as indices of depression-like states.  Behavioral changes include increased rate of self-directed stereotypic and self-injurious behavior as well as lower levels of play.  There have also been reports of alterations of levels of oxytocin, dopamine, serotonin, and norepinephrine in the cerebrospinal fluid of these NHPs, and peer-rearing produces a dysfunction of the hypothalamic-pituitary-adrenal axis, although there are conflicting reports on the degree and specific changes in the basal and stress response cortisol and ACTH levels in these infants.  This model has been used to assess the effects of early experience on later development of anxiety and depressive symptoms in response to stress.  Studies in both humans and NHPs have demonstrated that lack of maternal care during development can lead to impaired developmental outcomes, including depression.  The maternal separation model has shown that disruption in the form of maternal separation in early life causes infant distress that results in temporary and permanent alterations in infant behavior and physiology.  The typical macaque response to maternal separation is quite similar to the protest/despair responses seen in children who are separated from their mothers, and is characterized by initial agitation characterized by distress behaviors and increased heart, rate, body temperature, and cortisol levels followed 24-48 hours later by a depression phase during which the infants have disruptive sleep and circadian rhythm patterns, decreased heart rate and appetite, and lose interest in play and social interactions.  Pharmacologic antidepressive therapies have shown to reduce separation responses.

Further investigation of this model has shown that there are long-term behavioral and physiologic effects of maternal separation, including relatively permanent alterations in immune response and juveniles that were less social and more timid, with an increased negative response to novelty.  The final paradigm involves creating maternal stress, thereby altering the mother-infant relationship.  Mothers exposed to unpredictable foraging opportunities had infants with alterations in behavior, CNS structure, and CSF biogenic amines.  In addition to the above described models, macaque mothers occasionally demonstrate maternal abandonment and abusive behaviors towards their infants in natural free-ranging, group-living settings.  Abused infants exhibit elevated basal cortisol levels during their first month of life (when abuse is most frequent), and data suggests that there is an enduring effect on the HPA axis up to 36 months of age.  NHP models have consistently shown that stress during development can have deleterious behavioral effects and altered physiology into adulthood that is consistent with those seen in humans.

The hippocampus is associated with regulation of emotional functioning and memory in humans, and also plays an important role in regulating corticosteroid activity.  Depressed human patients can exhibit HPA axis dysregulation, and postmortem analysis of the brains of suicide victims have shown they have significantly lower levels of glucocorticoid receptors in the hippocampus.  Hippocampal atrophy may be directly related to major depressive disorder; treatment with antidepressants resulted in hippocampal neurogenesis in both humans and NHPs.  The hippocampus generally has a high level of corticosteroid hormone receptors, and it is known that glucocorticoids can have a neurodegenerative effect in this area of the brain which may ultimately decrease the hippocampus’ regulatory ability on the HPA axis.  Studies in NHPs found that the neurobiology of the hippocampus was altered in infants that experience maternal separation, and these changes included a decrease in corticosteroid receptors.  In macaque models, hippocampal volume was decreased in adult animals whose mothers had been exposed to variable foraging stress when the subjects were infants.  Some adult wild-born macaques spontaneously exhibit depressive-like symptoms despite not being exposed to early life stress.  The behavioral symptoms and neuropathology changes (particularly in the hippocampus) closely resemble that seen in human depression.  While it is still unclear if hippocampal atrophy is the result of depression or simply a trait of the disease, but the NHP model offers the ability to directly assess the impact of early experience on hippocampal development.

Serotonin is a ubiquitous neurotransmitter in the brain that influences a wide variety of CNS functions, including many systems that can be dysregulated in depression.  In humans, lower levels of serotonin in the CNS are implicated in the symptomology of depression, as well as the dysregulation of the HPA axis and hypercortisolemia seen in the disease.  There are correlations between behavior and serotonergic profile in NHPs, with animals with lower levels of serotonin metabolites engaging in more impulsive, aggressive behaviors, lower levels of social interaction, attaining lower social rank, and elevated mortality and alcohol consumption rates (in experimental setting).  Experimental administrations of therapies known to increase CNS levels of serotonin result in increased social behaviors and decreased anxiety and aggressive behaviors in NHPs.  NHP models have shown that there is a relationship between early stress and decreased levels of serotonin and its metabolites in the CNS, and not just in experimental manipulations such as peer-rearing but also in maternal abuse of group living infant macaques.  Complicating the study of stress, serotonin and depression is there is a serotonin transporter gene that regulates levels of serotonin transporter protein in the CNS, and both humans and rhesus macaques show a repeat-length polymorphism in the promoter region of the serotonin transporter gene.  This yields a long (l) and short (s) allele; humans and rhesus macaques that possess an s allele show decreased transcriptional efficiency and lower levels of central serotonin when compared to animals with two l alleles.  In humans, the s allele has been associated with increased incidence of depression, suicide, anxiety, and impulsivity; a greater HPA axis response to stress; and a greater propensity for negative physiologic and behavioral responses to a variety of experimentally administered environmental and social challenges.  NHP females with the l/s genotype were also significantly more likely to be abusive mothers, and abused infants with the l/s genotype had higher cortisol levels and more negative behavioral responses to stressful situations than those with the l/l genotype.  Further studies in NHPs and humans have shown that infants with an s allele rate higher in negative emotionality, have reduced gray matter volumes, and have altered metabolic activity in brain regions involved in emotional reactivity.  NHPs have proven to be excellent models in which to explore the relationship between the serotonin transporter gene and early life experience.  The results of these studies has shown that effects associated with the short allele are differentially expressed depending on early experiences and are consistent with human data showing a relationship between possession of an s allele, early adverse experience, and depression.

NHP models of early adverse experience have provided significant support for the Diathesis-Stress, or Two-Hit, model of depression.  These models are particularly useful because the behavioral, neuroendocrine, and neurobiologic profiles are consistent with that seen in human depression, and they allow direct manipulation risk factors not possible in human studies.  The ability to manipulate variables and follow infants over time has allowed scientists to determine the effects of stress on behavioral and physiological development.  NHP studies have also demonstrated the interaction between early adverse life experiences and genetic or physiologic profiles in the development of depression.

QUESTIONS

1.  The model of depression that suggests stress interacts with underlying predispositions to produce a CNS that is primed to express psychopathology when confronted with stressful experiences later in life is

a.  Two Hit model

b.  Dialysis-Stress

c.  Diathesis-Stress

d.  Two Stress

e.  a and c

2.  Peer-reared macaque infants show which of the following behavioral changes?

a.  Increased fearfulness

b.  Increased stereotypy

c.  Decreased play

d.  All of the above

e.  None of the above

3.  Which of the following has NOT been suggested to have a causal link to depression in this article?

a.  Serotonin CNS levels

b.  Human growth hormone levels

c.  Hippocampal neurodegeneration

d.  HPA axis dysregulation

4.  Possession of an s allele in the serotonin transporter gene has been associated with which of the following in macaques?

a.  Maternal abuse

b.  Lower cortisol levels in response to stress

c.  Easily consolable infants

d.  Increased gray matter volume

ANSWERS

1.  e

2.  d

3.  b

4.  a

**Novak et al. Use of the Cross-Translational Model to Study Self-Injurious Behavior in Human and Nonhuman Primates, pp. 274-283**

Domain 3

Primary Species: Macaques (Macaca spp.)

SUMMARY: In this article, the authors focus on the idiopathic development of SIB (self-injurious behavior) in rhesus macaques and assess the utility of the rhesus macaque model for the development of nonsuicidal self-injury (NSSI) in the general human population. A combination of retrospective, statistical, and empirical procedures are used to understand SIB disorder. The authors use a systems approach to examine SIB in monkeys from five different perspectives that reflect increasingly complex yet interdependent levels of analysis. These are (1) description, (2) etiology, (3) contextual factors (i.e., triggering events), (4) function/maintenance, and (5) therapeutic intervention. This articles shows the value of the cross-translational model (macaques to humans and humans to macaques) in understanding SIB.

Description (Form, Sex Differences and Prevalence Rates): As is the case with rhesus macaques, the physical damage in humans, ranges from skin abrasions and superficial cuts to more serious burns and deep lacerations. Compared with college students, in which NSSI prevalence rates vary 7–34%, the prevalence rate in rhesus macaques falls within the range but appears to be slightly lower than that reported for humans.

Etiology: Similar vulnerabilities to those described in monkeys are often associated with the development of NSSI in humans. Self-injuring behavior is frequently associated with early life stress, often in the form of child abuse or neglect. Although peer rearing and surrogate peer rearing monkeys exhibit species-typical behavior and appropriate parental behavior, they show altered emotional regulation, which includes anxious behavior and higher levels of fear and aggression, as compared with maternally reared infants. A strong relationship also exists between NSSI and poor emotional regulation, with individuals reporting more frequent and escalating negative affect.

In the monkey model, we now have considerable evidence regarding a role for the HPA axis and the stress response system in this disorder. Existing evidence in humans with NSSI similarly suggests a dysregulation in HPA function.

There is growing evidence for a contribution of genetic differences to the development of SIB in rhesus macaques and a deeper understanding of the more immediate triggers of self-directed biting than is currently available for humans with NSSI.

Contextual Factors/Triggering Events: there is a deeper understanding of the more immediate triggers of self-directed biting in macaques than is currently available for humans with NSSI. In humans, most of the research on NSSI has focused on identifying risk factors such as altered emotional regulation and self-dissatisfaction.

Function/Maintenance: SIB has been described in humans by 4 models (the pain model; the social interaction model; the emotional cascade model and the anxiety/tension-reduction model). Behavioral and physiological findings in macaques support a role for anxiety in the expression of SIB. Administration of the benzodiazepine (BDZ) diazepam, an anxiolytic compound, resulted in significant reductions in self–directed biting and the incidence of wounding in monkeys. Surprisingly, however, the treatment worked in only half of monkeys; These findings suggest the existence of at least two subpopulations of macaques with SIB, one of which has a form of reactive SIB in which the disorder arises as a consequence of certain lifetime experiences and is responsive to compounds that alter anxiety. The other population is currently uncharacterized.

Therapeutic Intervention: Despite all that is known about NSSI in humans and SIB in macaques, effective treatment of this disorder in both human and nonhuman primates remains elusive. Treatments generally fall into two categories: psychotherapy and/or pharmacotherapy, the latter of which is most relevant to nonhuman primates. A number of pharmacological agents have been used to treat NSSI, most of which target either the opioid system (e.g., the opioid antagonist naltrexone) or the serotonergic system (e.g., selective serotonin reuptake inhibitors [SSRIs]). Both naltrexone and buprenorphine (an opioid partial agonist), led to marked reductions in severe NSSI in humans, an effect which continued into a post-treatment period.

More recent studies suggest a promising role for opioid antagonists in treating SIB in macaques. Indeed, treatment with extended-release naltrexone markedly reduced the time monkeys engaged in self-directed biting by 54%, an effect that was maintained for at least 4 weeks after treatment

A substantial effort has been directed to identifying possible environmental changes as a form of treatment for SIB in macaques. At best, the results are mixed.

Conclusion: Going forward, the authors anticipate that the rhesus macaque models of SIB will continue to have important cross-translational value in bridging the gap between preclinical and clinical research and therapy.

QUESTIONS

1. Why is the rhesus macaque an ideal model for understanding causes and circumstances surrounding the development of abnormal behavior such as SIB?

2. What are strong predictors of SIB development in rhesus macaques?

a. Length of individual cage housing

b. Number of veterinary procedures

c. Surrogate peer rearing condition (infants reared with an inanimate surrogate and given 2 hrs of daily peer exposure

d. All of the above

3. True or false: housing monkeys in outdoor pen environments has been shown to reduce the rates of biting behavior in monkeys with SIB regardless of whether they are housed individually or in pairs

4. True or false: pairing SIB male monkeys with female monkeys actually led to increases in SIB

ANSWERS

1. Because of their highly complex social nature and their ability to adapt to diverse environments (traits characteristic of humans).
2. d
3. True
4. False: pairing SIB male monkeys with female monkeys actually led to decreases in SIB, but this strategy of opposite sex pairing may require pregnancy prevention (e.g., vasectomy)

**Lutz. Stereotypic Behavior in Nonhuman Primates as a Model for the Human Condition, pp. 284-296**

Domain 3 – Research; Task 3 – Design and conduct research

Primary Species: Macaques (Macaca spp.)

SUMMARY:

* Stereotypies – repetitive, unvarying, and seemingly functionless behavior patterns
* Reported in a wide range of species, including non-human primates (NHPs) and humans
* NHP examples – self-clasping, rocking, eye poking, pacing
* Human examples – rocking, eye poking, hair twirling
* To be effective, an animal model should have the following criteria:
* Face validity – similarity in behavioral output
* Construct validity – similarity in cause or origin
* Predictive validity – similar treatment responses
* Behavioral assessment methods in NHPs and humans – self report (human only), behavioral assessment, and physiological measures (e.g. heart rate, cortisol)
* Types of stereotypy (humans and NHPs)
* Whole-body (e.g. pacing, bouncing, rocking)
* Self-directed (e.g. eye poking, digit sucking, hair pulling, self-grasping)
* Humans may show more complex stereotypies (e.g. rocking while flicking the hand in front of the face)
* Abnormal repetitive behaviors may occur in individuals with autism, intellectual disabilities and obsessive-compulsive disorder (OCD)
* Lower-order motor movements – e.g. stereotypies, repetitive manipulation of objects
* Higher-order behaviors – e.g. rituals, preoccupations with aspects of the environment, or compulsions (irresistible impulse to perform a seemingly irrational act in order to prevent or reduce anxiety or distress)
* Autism – neurodevelopmental disorder in humans that results in impaired social interaction and communication as well as stereotyped patterns of behavior (e.g. head banging, body rocking)
* Intellectual disability – diagnostic criteria for humans include:
* Below-average intellectual functioning
* Deficits in adaptive functioning
* Ability to be independent or handle common demands in life when compared to others of a similar age and background
* Stereotypic behaviors are commonly noted but are not a diagnostic criteria (e.g. hand motions, body rocking)
* OCD – a human disorder in which the individual has obsessions or persistent thoughts, images, or urges that cause anxiety and compulsions or repetitive acts that aim to mitigate this anxiety (e.g. cleaning/washing, checking)
* Causes/origin of stereotypies
* Inadequate rearing (e.g. isolation rearing, nursery rearing)
* Environmental restriction later in life (i.e. non-stimulating environments)
* Environmental overstimulation
* Stereotypies are an arousal-reducing response to situations of frustration or aversive stimuli
* Treatment for stereotypies
* Environmental enrichment
* Behavioral treatment or training
* Positive reinforcement training – NHPs
* Functional analysis approach – humans (identifies environmental variables that reinforce or maintain stereotypies so that effective behavioral treatment methods can be developed)
* Drug therapy – altered brain functioning has been associated with stereotypies in humans and NHPs
* Dopamine antagonists (e.g. chlorpromazine, haloperidol, thioridazine)
* Serotonin uptake inhibitors (e.g. fluoxetine, fluvoxamine)
* Opioid antagonists (e.g. naltrexone)
* Conclusion: NHPs with stereotyped behaviors may serve as excellent models for humans with neurodevelopmental or obsessive-compulsive disorders as there are important similarities between both (form of the behavior, cause or origin of the behavior, and the types of interventions that are effective).

QUESTIONS

1. These are relatively benign repetitive behaviors that occur normally in NHPs and that are often associated with uncertainty and anxiety (e.g. scratching, self-grooming, yawning, and body-shaking).
2. T/F: Abnormal stereotypic behavior can be considered pathological in NHPs if it occupies a substantial portion of the animal’s time budget, interferes with biological functions, or induces tissue damage.
3. This is considered the best form of environmental enrichment for NHPs as it is more like to vary and is less likely to produce habituation than other forms of enrichment.
4. This is a behavioral method that involves eliminating the relationship between a behavior and its sensory outcome.

ANSWERS:

1. Displacement behaviors
2. True
3. Social housing
4. Sensory extinction

**Slattery and Cryan. The Ups and Downs of Modelling Mood Disorders in Rodents, pp. 297-309**

Domain 3: Research, Task: 1 and 2; K2 animal models

SUMMARY: Major depressive disorder (MDD) and bipolar disorder (BD) are two complex disorders that are modeled in the laboratory.  MDD may affect more than 340 million people worldwide, and BD may cause the greatest loss in work days per year.  Although treatment options exist, 30-40% of patients do not respond to any therapy, underlying the urgent need for more efficacious and novel-acting treatments.  Potential reasons for failure rate of new drugs include high placebo response, lack of objective diagnostic criteria and the translatability of animal models.  While traditional models have their uses, the authors suggest that better tests of antidepressant activity are needed.

Definition of a test vs a model:  a model has both an independent variable (inducing manipulation) and a dependent variable (behavioral/neurochemical readout).  A test is only a dependent variable.  Recent research focuses on endophenotype based approach, which attempts to assess only one symptom or marker of the disease.  There are three strategies used to develop animal models:  1) genetic manipulation, 2) selective breeding for behavioral extremes, 3) environmental, physical or pharmacological manipulations or a combination of above.

TRADITIONAL ANIMAL MODELS AND TESTS OF DEPRESSION

Forced Swim Test (FST) and Tail Suspension Test (TST):  When mice or rats are subjected to an inescapable situation, after initial escape-directed behavior, the animal quickly adopt an immobile posture.  Clinically effective antidepressants will increase the time spent in escape directed behavior.  There are some differences in the neurobiology of the two tests.  For example, GABAB  receptor antagonists and knockout mice have been shown to have antidepressant-like properties in the FST but not the TST.   Studies identifying the circuitry are also being done. A human study showed that inactivation of Brodmann Area 25 using deep brain stimulation could alleviate depressive symptoms in treatment resistant patients. A study looked at the inactivation of the infralimbic cortex resulted in an antidepressant-like phenotype in normal rats.

Learned Helplessness: Rodents are subjected to inescapable shocks and then reintroduced to the same environment, with escape possible.  The controls are subjected to escapable shocks each time.  Some animals will exhibit a reduced number of escape attempts, which is indicative of a depressive like phenotype.  Pharmacological reversal of helpless behavior requires at least 3 to 5 days of anti depressant administration.

Olfactory Bulbectomy: Removal results in long term alterations of behavior, immune and neurochemical functions.  The most consistent behavioral alterations are hyperactivity in a novel, brightly lit open field arena and deficits in passive avoidance learning.  Both are responsive to chronic, but not acute, anti-depressant administration.  Other neurochemical adaptations can also be reversed by chronic antidepressant treatment.

Chronic Stress Exposure (Non Social):Chronic stress is known to be a risk factor for MDD.  Chronic mild stress (CMS) paradigms are also used.  Examples of CMS are social instability, 24-hour light exposure, restraint, food and water deprivation.  Making these stresses prolonged in duration and unpredictability makes the CMS paradigm. These are reversed by chronic anti-depressant treatment.

Anhedonia:  Anhedonia is the inability to feel pleasure.  It is the traditional endpoint for CMS and maternal separation studies. Sucrose preference is often measured.  The female urine sniffing test is a recent approach, where the amount of time spent sniffing by males exposed to bedding with female urine is measured.  Recently, anhedonia has been studied after animals have genetic or environmental manipulations, using paradigms that were first used in the field of drug addiction, such as progressive ratio responding or intracranial self-stimulation (ICSS).

Cognitive Processes:   Patients can have difficulty in concentrating and display more attention and processing of negative stimuli than controls (negative bias).  Affective bias- acute antidepressant treatment can lead to a positive shift in emotion processing in humans.  Rats are given two separate positive experiences of equal value on different days under neutral conditions or during pharmacological or affective state manipulation.  Affective bias is assessed using a preference test where both positively rewarded substrates are presented together. A study could show that acute antidepressant treatment (of a new drug) leads to a positive bias towards the substrate paired with the admin of drug.

Social Stress Models:  Social defeat, chronic subordinate colony housing in mice, social defeat overcrowding, or chronic subordination (in tree shrews) have been shown to result in numerous behavioral and physiological alterations closely resembling those resulting from chronic stress in humans.  Alterations may also persist a long time after termination of the stressor.  Models respond to chronic anti-depressive treatment.

Genetic Predisposition: There is still a lack of knowledge regarding the cause and pathophysiology in humans.  Some rodent genetic lines have been created by selective breeding:  Flinders Sensitive Line rats, or High vs Low-anxiety-related behavior rodents.

Sex Differences In Modeling MDD:Note that forms of stress that are effective in males may not be effective in females.  Female rodents tend to exhibit greater hypothalamic-pituitary axis responses to stress and display less habituation to repeated stress compared to males.  Studies of FST, TST and sucrose preferences have been done.  More studies are needed.

Modeling Treatment Resistant Depression: Antidepressant responder vs. non responders could be assessed to look for molecular differences between the group or strains.  Studies have been reverse translated back to animals.

Modeling Depression In Old Age: More studies are needed.  Hippocampal neurogenesis has been shown to decrease with age, and become less sensitive to antidepressant treatment in aged rats.  Studies are needed to demonstrate a causal link.  Immune activation has been showed to be elevated in both aged humans and rodents.

Animal Approaches To Study Bipolar Disorder: Mania can be modeled by looking at drug-induced hyperactivity. Sleep deprivation and social stress, as well as strain differences can also be studied.  Mood stabilizers can be administered to study effect.

QUESTIONS

1. Define a model and a test in the context of this review.

2.  What brain areas are recruited in the forced swim test?

3.  True or false.  The reversal of learned helplessness takes 3 to 5 days of anti-depressant administration.

4.  True or false.  Anhedonia is the inability to feel pleasure.

5.  Which of the following are factors for choosing the drug-induced hyperactivity model in rodents?

a. High throughput possible

b. Ease of use

c. Inter-laboratory reliability

d. Responsive to mood stabilizers

e. All of the above

6. True or false.  The time required for therapeutic efficacy in chronic social stress paradigms is close to that required for clinical benefit of current antidepressants.

ANSWERS

1. A model has both an independent and dependent variable, also known as the inducing manipulation and behavioral/neurochemical readout.  A test is only the dependent variable.

2.  The hippocampus, infra limbic cortex and ventral tegmental area to nucleus accumbens pathway and central dopamine signaling pathways.

3. True

4. True

5. e. All of the above

6. True

**Vorhees and Williams. Assessing Spatial Learning and Memory in Rodents, pp. 310-332**

Domain 3: Research; Task T1: Facilitate or provide research support

Primary Species: Mouse (Mus musculus)

SUMMARY: Navigation is the ability of organism to learn to find their way through the environment without getting lost, which require memory for locations and routes. No organism can survive without the ability to leave their nest, forage for food, explore,... and return safely where they started.

Types of Navigation: There are at least 2 distinct types of navigation: Allocentric way finding which is referred to as spatial navigation (cues/landmarks located outside and at some distance from the organism; the other one is Egocentric wayfinding which is characterized by the ability to navigate using internal cues (they means limb movement, speed, sequence of turns,...), optokinetic flow as the organism moves past surrounding objects and signpost. Allocentric navigation requires visual cues while the egocentric navigational accuracy is reduced but it is still operating, even in darkness. Some researcher go further in the study of egocentric navigation and they differentiate between Route-base navigation (which relies on internal cues of rate of movements) and path integration (which has a vector addition). When a memorized operation is overlearned, it can become habits, shifting its localization in the brain and reclassified as implicit or procedural memory. Path integration is seen as the ability of the organism to leave its home-base and move to different locations and then return by a other, more direct, path. Both kind of navigation has different neural networks but they overlap extensively and it is quite complicate disrupt the memory control of one navigational type without affecting the other in a greater or lesser extent.

Brain Regions Mediating Navigation: For the case of allocentric navigation, primary regions crucial for this ability are hippocampus and entorhinal cortex. The place cells located in the hippocampus form a neural map of the organism's environment. The medial entorhinal cortex have place cell connected to hippocampus' place cells. Dorsal and ventral regions of entorhinal cortex form tiling patterns with response from smaller to larger fields of environment. Finally, entorhinal cortex has the control of head direction and border cells which limit the known environment of the organism. This network provides spatial information in rodents but it is implicated in semantic and episodic memory in humans. So, research groups focus in testing the allocentric learning of mice for their memory models, just to translate the results in the semantic memory of human.

In respect of non-spatial navigation, the brain regions are less studied in comparison with spatial navigation. Data at the moment show an overlap between both systems. Egocentric memory are strong linked to head direction cells. Despite being discovered in entorhinal cortex and presubiculum (allocentric network), these cells are also located in thalamus, mammillary nucleus, retrosplenial cortex and dorsal striatum. Depending the brain region of study, both system could be differentiated (lesions in the hippocampus show deficits in allocentric learning but not nonspatial memory in mouse); but, in humans, it has been proved that egocentric path integration recruits neural activity in the hippocampus and parietal cortex.

Principles of Navigational Assessment: The assessment of memory studies based in the navigational function of animals has to point out the role of spatial navigation, the non-spatial navigation and the learning-performance distinction. For the spatial navigation, the key is to differentiate between the distal cues from the proximal and internal cues required to find the way through the environment. In the allocentric learning, tests should eliminate any proximal cue, a challenge that offer to open pool the principal device. On the other hand, navigation in darkness is designed to avoid distal cues and evaluate the internal and proximal cues in egocentric navigational studies. Besides, to evaluate the role of egocentric learning in non-spatial navigation test, sometimes it required to create an environment where a few distal cues are included. In this way, the subject decides which cues to use during the training phase and then test which cues are dominant when the arrangement of cue position is changed. Finally, learning-performance distinction are critical for all tests of learning and memory. As with any learning and memory assessment, methods which use multiple measures of behavior are best to provide and interpret results reflecting navigational learning-memory; instead of those that performs factors such as differences in motivation, the tendency of thigmotaxis, or motor deficits.

Assessing Navigation in Rodents: The tests most widely used are Morris Water Maze (MWM) and Radial-arm Maze (RAM).

MWM is a circular pool of water that was featureless on the inside  but had many cues on the outside. Reinforcement was provide by having a small platform either invisible, by submerging it, or visible, by protruding it above the surface of the water. To evaluate how subjects use distal versus proximal cues, four test conditions could be performed to learn how to find the scape platform:

Place - keeping a white platform in a fixed place (near the centre of the pool), animals cannot see it due to be submerged in white-colored water. In each trial, animal start form a different randomized place 8cardinal points).

Place + cue - keeping the "place trial conditions' except that the platform is black and arise above the water level

Cue only - in this kind of trial, both platform location and starting points are randomized

Place-Random - it is used the white submerged platform but randomizing both platform location and starting point

Obviously, animals taking cues as reference learn more quickly than those in absence of cues. However, in absence of distal cues, animals firstly show a disorganized patterns of swimming to hunt the platform and later, they learn to perform a specific swimming pattern  around the centre of the pool, which allow them to get the platform in a efficient way.

RAM has a central hub and arms radiating out from the centre, like spokes of a wheel. There are two main procedures: the working memory and the working/reference memory versions. Arms numbers range from 4 to 12 or 17, being this last focus to increase the difficulty and improve the method for working/reference memory version. This test maze require training and a motivation, normally a food reward; so animals should be restricted for food consumption before the trials. Once animals have learned the system, they are left in the centre and allowing them to explore with a single reward at the end of each arm. Data recorded are which arms are visited once versus those visited more than once (scored as error).

The test present some problems, animals may solve the maze in ways other than relying on spatial working memory. Once of these is use of chaining or a serial strategy (ex. always turn right or turn left). Another issue is that the food restriction should be equal between groups and the point that after getting the first reward, it is necessary to keep the motivation for keeping the mouse farrowing. To resolve the chaining problem, when a subject enter in an arm, the others were closed. Once the animal re-center in the maze, all the armed would be blocked for a period of time after the reopening and allow it to get a choice again. To avoid some of the problems with appetitive motivation, swimming version of RAM have been developed (submerged platforms are located at the end of the arms, so animal learn which arms has platforms and which ones not.

In the second version of RAM, some arms are baited on each trial and some not. The animal should visit only the always-baited arms and never the always unbaited-arms. Revisiting a baited arm after the bait is taken as an error. In RAM the learning process should be slower than in MWM, being an advantage or disadvantage form different points of view. MWM is recommended for assessing spatial navigation and RAM for assessing spatial working and associative learning memory.

The author support clearly the MWM in memory study, they analyze the characteristics of this maze.

Features of MWM for Rodents

1. Rodents are natural swimmers but prefer to be out of the water
2. A pool is easy to construct: uniform shape and featureless inside and rich cues outsides
3. The task is amenable to assessing proximal cues by using visible platforms
4. Assessing swim speed in training period, it is possible to determine the motivation to scape and ability to swim

Advantages

1. Water is an equal-opportunity motivator
2. Nearly of 100% of rodents complete the task
3. Nearly of 100% of controls animals master the task (with strain independence)
4. Minimal training is required
5. Short experimental period is required to obtain validated data
6. Water motivation is so efficient in the first trail as the last one.
7. Flexibility, MWM is adaptable to many experimental conditions

Disadvantages

1. Some strains exhibit floating behavior or persistent thigmotaxis rather than active searching for the goal. Inbred strains as C57BL learns more easily than other mouse strains as BALB/c or 129/Sv (being this one the strain which require more training in MWM)
2. Low sensitivity for working memory.
3. The main criticism is the test is unduly stressful.

Nevertheless, there is some controversy in the stress impact being a helplessness or a motivation in a depending manner. Inescapable stressors, such as inescapable shock, lead to learned helplessness which is used to model depression. Similarly, Porsolt forced swim test of swimming despair, also induce a form of immobility resembling  helplessness; however, the test environment is designed to maximize frustration by making the swimming chamber small, the walls high, and the confinement long to induce defeat, very much in contrast with the MWM, in which escape is reinforced. MWM trials lengths are shorter than Porsolt stress but in addition, if the animals fails to find the platform, it is not allowed to remain in the water and become fatigue or discouraged. In other studies, stress is not always negative. Stress in relation to performance is an inverted U-shaped function Too little as well as too much is counter-productive but in a midrange has been observed to improve the test performance.

Procedures for the MWM

Pool And Platform Size: Depending of rodent specie and strain, animals learn more easily in smaller pool than in greater. The standard pool is 132 cm in diameter, but for rats 210-cm works better. In mouse 150-cm offers more advantages but if the pool increase to 210-cm they show no learning.

Search Area To Target Ratio: Clearly, the larger the pool and smaller the platform, the more difficult the task becomes; therefore, considering the ratio of pool surface area to platform surface area permits a systematic examination of this factor. Mice learn well in a ratio 149:1 (122-cm pool diameter with a 10cm of platform), but there is no learning above ratio of 441:1 (210-cm to 10cm of platform). Rats are different, they learn in all ratio tested form 149:1  to 2386:1 (244-cm to 5 cm of platform), even when they present deficit in allocentric memory. The difference between groups (control rats vs affected animals) are more evidence when the challenging is high at the beginning, but  it quite smaller when the challenging is gradually increasing.

Cue: At minimum, rats needed two distinct distal cues by which to navigate. Moreover, the two cues had to have a minimum angular separation (distance between prominent cues located around the pool) to provide adequate navigational information, but in addition to this, rats always used distance to the edge of the pool as a cue. The advantage of providing the cued version of the MWM first is, as previously noted, it has the effect of reducing or eliminating problems that animals have with sensorimotor function and, for learning subordinate skills such as staying on the platform and searching away from the wall, effects similar to those learning by using nonspatial pretraining methods. Providing cues firstly is desired in mice because they are more prone to thigmotaxis and to floating and other behaviors that reduce the rate of learning. Cued trials eliminate most of these nonspatial behaviors and serve as a control procedure to determine that proximal cue learning is intact.

Hidden Platform Reversal And Other Phases: One of the most useful ways of getting more information from MWM testing is to add a reversal phase. This is done by moving the platform to the opposite quadrant.  There are many examples of treatments that produce small or even no differences during acquisition but significant deficits during reversal.

Memory/Probe Trials: The probe or memory trial is a standard feature of MWM in which the platform is removed. Where the animal spends its time is a measure of spatial bias. If the animal has learned the platform's location, it should show a preference for the quadrant in which it was located and even for the site where the platform was within the target quadrant. Another import issue concerning the probe trials is how long they should be. It recommended to perform trials of 30-45 seconds. If it required more time, this should be fractionated and analyze the trial in intervals.

Water Temperature:  Under typical conditions, water temperature of 20–22ºC works well for both rats and mice.

Intertrial Interval: It has long been known that massed practice reduces learning and memory performance compared with distributed or spaced practice in almost all settings. In general, spaced trials enhance learning and memory. However, although the acquisition data suggest that spaced trials improve performance in rats, the effect is much less  pronounced than in mice and insignificant on reversal or shift.

Sex Difference And Ontogeny For Allocentric Learning: In most tests of allocentric navigation in rodents, male animals show an advantage compared with female animals. Not surprisingly, age has an influence on MWM performance. In a rat study, the P21 and P28 (days of age) groups were slower to learn the hidden version and never became proficient, and the P21 group never became proficient in the cued condition, but overall the cued groups performed better than the hidden version groups, a finding that shows that the cued version is easier to learn than the hidden version. The study concluded, that before P23, spatial learning in rats is reduced.

Experimental Design: There has been much discussion recently that many animal studies have not proven to be replicable. A variety of sources of this problem have been identified and include inadequate sample sizes, lack of randomization of animals to groups, nonblinding of experimenters, and inadequate control of litter effects. In the performance of MWM studies, authors suggest the following recommendation:

Sample Size:  group sizes less than 10 can be unreliable and it is recommended the use of 15 to 20 animals per group, especially for mice, whose date are more variable than rats.

Litter Effects: The basic issue in multiparous species such as rats and mice is that litter-mates have overlapping genetic, epigenetic and experimental influences that make litter-mates more similar to one another than offspring from another litter (similar than fraternal twins).  One of the basic assumptions of inferential statistics that subjects be independent and treatment groups inside of a offspring imply a statistic complication. The problem of oversampling multiple offspring per litter and treating them as if independent has been well described elsewhere, as have solution. Solutions include sampling only one pup per litter (or only one male and one female pup) or, if breeding heterozygotic mice for a genetic mutation, sampling only one pup of any given genotype per litter or per sex per litter.

Randomization:  Although most experimenters are taught that animals should be randomly assigned to groups, it is not always done and even less often reported. Many experimenters have come to accept the practice of arbitrary selection as an approximation to random assignment. but the lack of reproducibility of animal experiments suggests that this assumption is flawed. Authors approach is to take a group of animals before an experiment is begun and assign each animal an arbitrary temporary number, then use a random number chart or computerized random number generator to determine which temporary number is assigned to which treatment group. Whatever the method, it should be described in the methods in the paper.

Blinding: This is an important safeguard no matter what the experiment, including tests of learning and memory. Inadvertent bias can affect handling and outcome of tests without the person even recognizing it. Routinely blinding (and stating as much in the methods) is essential.

As conclusion, MWM has a remarkable ability to motivate learning and memory with little training to high levels of proficiency. The other characteristic that makes the MWM stand out is that by testing allocentric navigation it assesses a conserved form of learning and memory essential for survival and is convergent with semantic and episodic memory systems in humans, functions often adversely affected by disease and injury and which are the targets of research to develop treatments. As such, the assessment of MWM spatial navigation is likely to remain a central methodology in the study of mechanisms underlying how the brain captures, consolidates, and retrieves allocentric information to navigate through the world.

QUESTIONS

1. Define landmark vs. signpost.

2. Re-classified this egocentric navigational behaviors:

a. Flying movement sequence: straight-left-right-left-left

b. Riding a bicycle

c. Taking a shortcut

3. Which is semantic memory?

a. Knowledge of the movement to drive a car

b. Memory of facts as well as places

c. Memory of meaning of words

d. All of them

4. List the stage of learning of a rat during MWM studies

5. T/F. N-methyl-D-aspartate (NMDA) antagonist impaired sensorial function and degrade MWM performance

6. List other Mazes

ANSWERS

1. A landmark is a farther away cue which provides relational information as to where the organism is compared with other landmarks. On the other hand, a signpost is an animal-closed marker of where to change the direction along a path but does not give a relational information.

2. a. Route-based; b- Route-based; c- path integration

3. b.

4. First thigmotaxis (involuntary response for water feeling), swimming around the perimeter. This is followed by a swimming at a distance from the edge of the pool accompanied by weaving and circling, finding at the end the platform. Later, animal learn the strategy to get the scape platform. And finally, operator test what rat has learned removing the platform form its fixed location and giving a fixed length of time to search for the platform's former location and records the animal's bias for the goal area.

5. T

6. T-maze; Hole-board maze and Star maze.

**Coleman and Pierre. Assessing Anxiety in Nonhuman Primates, pp. 333-346**

Domain 3 – Research, K2 and 3 – Research Methods and Animal Models

SUMMARY

Anxiety = apprehension over anticipation of a potential threat (not inherently harmful)

* Anxious state = a heightened emotional response to a potentially threatening event

Anxiety disorders = dysregulated biobehavioral responses to novel or potentially threatening stimuli

* Trait anxiety = heightened behavioral response across a larger spectrum of experiences and/or maintenance of responses for a lengthened time

Displacement behaviors = inappropriate behaviors to the stimulus that provoked them

* Believed to reflect anxiety in primates
* Include: scratching, auto-grooming, shaking, yawning, bared-teeth grins, and piloerection

o   Marmoset specific: slit-stare, flat-tufted ears, and anogenital presentation

* May include vocalizations (“coo” in young macaques, “tsk-tsk” and “geckering” in marmosets)
* Many have been validated as indicators of anxiety by response to pharmacologic challenge (decreased w/ anxiolytics, increased w/ anxiogenic compounds)
* Important note: ALL are appropriate in certain contexts, ONLY indicative of an anxious phenotype when performed out of context or for prolonged periods

NHPs are used as animal models of anxiety d/t several similarities to humans including: extended lifespan with comparable developmental stages, comparable brain complexity, and similar physiological systems. NHP anxiety models rely on inferences about behavioral or physiologic outcomes that parallel human conditions. No single test captures all elements of anxiety, and how animals respond to one stimulus does not predict how they will respond to other stimuli.

Home Environment Assessments: Highlights natural variation in anxious behavior without an overt threat (analogous to human anxiety).

Home Environment Observation: Utilize focal or scan observations to quantify behavior and determine animal’s activity budget; can be performed on group-housed or caged, singly housed animals

* Used to assess social anxiety, maternal separation anxiety, and generalized anxiety
* Cynomologus macaques in single housing identified with 5 distinct behavioral profiles

o   Displacement and aggression to observer interpreted as analogous to anxious behavior

Observer Rating Method: 2 or more observers score subjects on various predefined traits (“active” and “apprehensive”); with analysis, factors can be correlated with behavioral responses

* Rhesus macaque infants with a highly nervous temperament (similar to human neuroticism) were more likely to develop stereotypical behavior later in life

PROVOKED RESPONSE TESTS

Home Intruder Test (HIT): Measure an individual’s response to potentially threatening social stimulus of an unfamiliar human intruder (mostly performed with infant rhesus macaques)

Design: 10 minute stages of novel stimuli including: a novel cage/room, no eye contact human intruder (NEC), and direct eye contact human intruder (Stare, ST).

* Normal response in infants (rhesus m):

o   Novel cage/room (Alone, without dam) – coos/locomotion (interpreted as distress)

o   NEC stage – freeze (interpreted as adaptive response)

o   ST stage – threats or aggressive vocalizations (defensive behavior)

* Infants w/ excessive responses during NEC stage (excessive freezing) had higher basal cortisol levels compared w/ normal infants; suggestive of anxious temperament

o   Similar cortisol levels to human infants w/ immotile fear response in novel environment

* Infants w/ no response to human intruder (NEC or ST stages) showed a blunted growth hormone response to pharmacologic challenge w/ clonidine and growth hormone releasing hormone

o   Associated w/ development of depressive/anxious behavior in humans

Pharmacologic Manipulations (Behaviors Were Regulated By Different Neurotransmitter Systems):

* Morphine, opiate agonist 🡪 reduced cooing; Naloxone, opiate antagonist 🡪 increased cooing

o   Suggests vocalizations are related to the distress response, not anxiety

* Diazepam, anxiolytic 🡪 reduced freezing and defensive behaviors

o   Indicative of contribution to an anxiety-like response

Variation used w/ marmosets: Human intruder stands in close proximity to home cage

* Normal response: retreating , slit stare, scent marking, flattened ear tufts, and piloerection
* Pharmacologic manipulations: reduced behaviors with anxiolytics

Predator Confrontation Test: Assesses response to a predatory threat (used in marmosets)

Design: Subjects acclimate to a testing apparatus/maze; a taxidermized Ocilla cat (natural predator) is placed in a far corner so that subjects “happen across” it during exploration

* Normal response: threat/fear-related response (increase in alarm calls, vigilance, displacement behaviors, and decreased exploratory behavior); habituation occurs after prolonged exposure
* Lack of habituation and heightened fear response interpreted as anxiety

Pharmacologic Manipulation: Anxiolytics (diazepam and buspirone) 🡪 dose-dependent reduction in displacement behavior and increased exploratory behavior and proximity to predator

Similar Tests In Other Species (Rhesus macaque): Utilizes snakes (real and model)

* Normal: fear response similar to marmosets w/ diminished response after repeated exposure
* Large amount of individual variation in habituation w/ some subjects never habituating

Associative Conditioning and Startle Response Tests: Uses subject’s innate harm avoidance/reflexive escape response; heightened response indicates anxiety

Design: Subject presented with a neutral or conditional stimulus (CS) followed by a noxious unconditioned stimulus (US) known to cause a reflexive unconditioned or startle response (UR)

* Magnitude and duration of the motor response are measured
* Repeated pairings of the CS with the US results in association; so the animal responds with the UR even in the absence of the US
* Exaggerated emotional responses to seemingly unthreatening stimuli are a hallmark of anxiety disorders, expressed as either hyper- or hyporesponsiveness
* Variations (i.e. Potentiated Startle Test, Prepulse Inhibition Test, or Unsignaled Acoustic Startle) indicate that a lack of reduction/inhibition in response after prepulse stimulus or repeated exposure to an unsignaled stimulus is indicative of an “anxiety sensitive” individual (similar to humans with psychopathologies or anxiety disorders)

Cognitive Bias Testing:Evaluates emotional regulation; based on the idea that emotional state can affect cognitive functions (i.e. judgement, attention, and memory) in humans and animals. Individuals in an anxious or negative emotional state are more likely to interpret ambiguous stimuli as threatening.

Judgment Bias Test Design: Subjects are trained to pair specific cues with specific behaviors (red, touch a square; blue, touch a triangle) to achieve various outcomes (positive and negative, or positive and less positive). Subjects are then presented with a neutral cue (purple) and asked to choose a behavior.

* Response to ambiguous cue is thought to indicate emotional state: positive emotional state – likely to respond optimistically, negative state – more likely to respond pessimistically
* Capuchins w/ high levels of head twirls (stereotypy) were more likely to respond pessimistically

Attention Bias Test Design: Monkeys exposed to 2 monitors displaying either threatening or neutral conspecific face after undergoing either a negative event (health exam) or a positive event (enrichment)

* Subjects looked at aggressive faces more quickly, regardless of the positive or negative condition
* After the negative event, animals disengaged from aggressive faces more quickly
* Interpreted as emotion-mediated avoidance of threatening faces (similar to humans w/ high levels of social anxiety spending less time attending to emotional compared to neutral faces)

Anxiety Assessments and Care of Captive Primates:Behavioral characteristics in NHPs can be used to: identify animals predisposed to clinical/behavioral problems and predict responses to various stresses; alter husbandry practices for anxious individuals to reduce anxiety; inform subject selection for research studies.

* Inhibited/anxious NHP infants may have attenuated behavioral responses to stressful events, and may be more likely to develop stereotypic or self-injurious behavior
* Inhibited rhesus m infants are more likely to exhibit airway hyperresponsiveness (a characteristic of asthma, similar to human children)
* Rhesus m with low sociability (similar to social anxiety) exhibit altered regulation of the HPA axis and altered immune function when in an unstable social environment
* Rhesus m are more likely to affiliate with conspecifics having a similar, rather than dissimilar, temperament measured by HIT or home environment assessments
* Inhibited rhesus m were less likely to be successfully trained using positive reinforcement
* Anxious cynomologus m are more likely to develop amenorrhea in response to room changes

QUESTIONS

1. Piloerection in nonhuman primates is an indication of:

a. Pain only

b.  Anxiety only

c. Affiliation only

d.  a and b

e.  b and c

2. Based on pharmacologic manipulations of the Human Intruder Test on infant rhesus macaques, which of the following characteristics is likely associate with a distress response, and not a good indicator of anxiety in NHP anxiety models:

a.  Excessive freezing

b.  Lack or response to a human intruder

c.  Increased vocalizations when placed in a novel cage

ANSWERS

1. d. Both a. and b. (piloerection is considered as a sign of pain and a displacement behavior in NHP, an indicator of anxiety in inappropriate environments)

2. c. Increased vocalizations when placed in a novel cage (vocalizations decreased with administration of morphine, and not with diazepam, indicating that they are more likely associated with a distress response instead of an anxiety response)

**Meyer and Hamel. Models of Stress in Nonhuman Primates and Their Relevance for Human Psychopathology and Endocrine Dysfunction, pp. 347-360**

Domain 4: Animal care

SUMMARY: This review details models of stress in adult non-human primates of varying species, followed by stress models occurring at earlier stages of development in similar species.

Key Points

* + Homeostasis is defined as mechanisms that hold constant a controlled variable by sensing its deviation from a ‘set point’ and feeding back to correct error.
  + Allostasis is defined as mechanisms that change the controlled variable by predicting what level will be needed and overriding local feedback to meet anticipated demand.
  + Slow incorporation of cortisol into hair over time provides an index of long-term adrenocortical activity, and in some cases, a measure of the Hypothalamic-pituitary-adrenocortical (HPA) response to major life or environmental stressors.

Stress in Adulthood

* + Adult primates living in a social setting can experience significant stress when removed from the social group or when the social hierarchy of the group is unstable.
  + Relocation of long-tailed macaques (*Macaca fascicularis*) to a novel colony room or to new cages within the same colony room is a stressful experience that initially results in activation of the HPA axis and suppressed patterns of activity.
  + Pigtail macaques (*Macaca nemestrina*) in similar studies were less reactive than long-tailed macaques; while male Rhesus macaques (*Macaca mulatta*) show elevated plasma cortisol levels up to 1 year after relocation.
  + A combination of relocation plus social separation activates a strong initial stress response.   The response has been shown to disrupt the female baboons’ (*Papio sp*) menstrual cycle.  It can also alter the reactivity of the HPA axis.  However the stress response may be attenuated by the presence of a social partner.
  + Relocation can be especially stressful when it involves the introduction of unfamiliar conspecifics.
  + Psychosocial stress of forming a social group with unfamiliar conspecifics can be exacerbated by realignment of a previous dominance hierarchy and the aggressive interactions that may accompany social organization.
  + The stress response of a new social group can be shortened or attenuated when dominance hierarchies are formed without direct physical aggression.
  + Social rank is another important factor that can contribute to individual stress responses.  Subordinate monkeys are more highly stressed than the dominant animals.
  + The relationship between social rank and stress is complex however, depending not only on rank but also on structure of the animals’ society and individual’s characteristic behavior within that society.
  + When dominance hierarchies are stable, physiologic stress responses may be greatest for either low- or high-ranking animals.

Stress in Development

* + Prenatal stress during early (day 50-92) or late (day 105 to 147) gestation exerts long-lasting effects of increased emotionality and hyperactivity of the HPA axis.
  + Infant rhesus macaques physically separated from their mother exhibited severe disturbance behaviors.  Totally isolated infants showed high cortisol levels but few vocalizations, whereas the opposite was seen when the mother was present nearby but in a separate cage.
  + Behavioral and endocrine responses to separation stress in infant rhesus macaques depend on a complex interaction between the rearing experiences of the animals and genetically mediated serotonergic function.
  + When Variable Foraging Demand (VFD) animals were compared to Low Foraging Demand (LFD) monkeys (food easily obtained), they were found to be socially subordinate.
  + Maternal abuse occurred in a small percentage of rhesus macaque mothers and included dragging, crushing, hitting, dropping and stepping on infants.  It was shown to be transgenerational.
  + “Stress Inoculation” – a primate model where infants of socially housed squirrel monkeys were subjected to weekly 1-hour separations from the mother and the rest of the social group beginning at 17 weeks of age and continuing for a period of 10 weeks. A variety of behavioral, endocrine and neurobiological outcome measures are then down.
  + This model showed that certain kinds of stress relatively early in development may program both the nervous and endocrine systems to cope better with late stressors by enhancing inhibitory control over behavioral and HPA axis responses.

QUESTIONS

1. What are the current two ideas that are used to possibly explain the concept of “Stress”?

2. Name the model that incorporates elements of Homeostasis and Allostasis.

3. Discuss the use of Corticotrophin-releasing factor (CRF) in stress studies.

4. What does a “grunt” mean in baboon colonies?

5. When baboons approach sexual maturity what do the males and female do with respect to the natal troop?

ANSWERS

1.  The two ideas are:

a.  Stress involves a threat to homeostasis

b.   Stressors are stimuli that are unpredictable and/or uncomfortable

2. The Relative Scope Model

3.   It is used as a test to assess overall system reactivity at the levels of the pituitary and adrenal glands

4.   It is a benign vocalization that signals lack of aggressive intent

5.  The males emigrate; while females remain within their natal troop

**Dettmer and Suomi. Nonhuman Primate Models of Neuropsychiatric Disorders: Influences of Early Rearing, Genetics, and Epigenetics, pp. 361-370**

Domain 3; TT3.6

SUMMARY: Nonhuman primates are commonly used for studies examining neuropsychiatric-like disorders such as anxiety, impulsive aggression, and alcoholism.  Studies on anxious/depressive behavior in NHPs generally studies social separation such as separation of mother from young.  Early studies did not mimic human studies since such extreme social contact deprivation are not replicated in humans.  This lead to studies looking at surrogate-peer-rearing, where monkeys are exposed to age conspecifics.  These NHPs are considered good models for anxious/depressive behaviors in children due to similar social behavior, endocrine function, brain structure, and mother-infant nurturance as well as fulfilling Ainsworth's criteria of attachment such as unequivocal distress upon complete separation.

The majority of NHP studies on alcoholism use free-choice drinking paradigm.  This paradigm is considered a good model because they show similar blood alcohol levels, impairment, and withdrawal.  NHP models of alcoholism demonstrate individuals exposed to adversity are more prone to alcohol abuse.  Also, NHPs exposed to alcohol is correlated to excessive aggression.  One downfall of these models is the difficulty to monitor blood ethanol concentration.  Peer-reared monkeys exhibit more anxious behaviors and behavioral inhibition which make them more at risk for excessive aggression and alcohol consumption.

Candidate gene studies aim to identify allelic variations of genes that underlie specific disorders.  Candidate genes are selected for the trait or pathology of interest, such as serotonin-transporter-linked polymorphic region, mu-opioid receptor, monoamine oxidase A gene promoter and corticotropin releasing hormone promoter. It has been found that NHPs which exhibit differential expression of these neurohormones is exacerbated by early life adversity. Genome-wide association studies scan the entire genome for common genetic variation.  These studies compare DNA of individuals with a disorder and individuals without the disorder.  These studies are still rare in NHPs but are gaining more popularity.  Epigenetics studies changes in gene activities that are not caused by changes in DNA sequence.  These studies have disadvantages including the fact that epigenetic changes are diverse and analysis may be technically difficult.  These types of studies have shown early life adversity affects the brain and immune system from a very early age and imparts genome-wide effects.

Neuropsychiatric disorders NHP models have yielded critical information and the future holds potential for studies to address the gaps of knowledge in this fields.

QUESTIONS

1. Why are free-choice alcohol drinking paradigm in NHPs considered good models?
2. What are genome-wide association studies?
3. What behavioral paradigm do anxious/depressive behavior studies study?

ANSWERS

1. Free-choice alcohol drinking paradigms in NHP model are good because they show similar blood alcohol levels, impairment, and withdrawal.
2. Genome-wide association studies scan the entire genome for common genetic variation.  These studies compare DNA of individuals with a disorder and individuals without the disorder.
3. Studies on anxious/depressive behavior in NHPs generally studies social separation such as separation of mother from young.