# Identification of Neural Markers Accompanying Memory

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“To my wonderful wife Erika, my daughter Sofia and my two little Angel”

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# 6 Unpacking Memory Processes: Using the Attribute Model to Design Optimal Memory Tests for Rodent Models

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## Introduction

An important consideration in the study of mouse models of any genetic disease is how well the behavior of the mouse serves to actually model the behaviors present in the clinical population being modeled. In order to optimally address this concern is to explicitly develop a behavioral endophenotype of the mouse model in which the mouse is explicitly tested using tasks rigorously designed to explicitly test the cognitive domains affected in the clinical population. To achieve this goal, it is important not only to apply standard behavioral tasks to mouse models of genetic disorders, but also to directly evaluate brain function across all cognitive domains. Furthermore, it is critical for behavioral genetics laboratories to interact with clinical research laboratories to develop comprehensive behavioral endophenotypes for the disorder being modeled. The strength of behavioral genetics and the mouse models is the ability to apply behavioral paradigms known to be subserved by known anatomical loci not only to determine the behavioral phenotype of the model, but also to elucidate candidate brain regions affected by the mutation (Robbins et al., 2012). When research into mouse models of genetic disorders emphasizes patterns of mnemonic strengths and weaknesses across domains (i.e., the behavioral endophenotype), the results will not only directly model the disorder being studied, but also serve both as risk prodrome for disease onset or progression and as outcome measures that can be applied by the clinic in interventional studies.

In this chapter, I will describe a process wherein the behavioral geneticist can identify and select behavioral tests for a mutant mouse with minimal difficulty. In doing so I will (1) describe a comprehensive model of memory processing based on multiple memory systems and parallel processing, (2) discuss the utility of this considering this model prior to designing and selecting behavioral experiments for

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a genetic model, and (3) provide an example from my own work with the CGG KI mouse model of the fragile X premutation to illustrate an example of using the attribute model to guide the development of a behavioral task battery for the mouse model.

## Attribute Model

### *Memory Systems*

At the level of processing, the event-based memory system provides for temporary representations of incoming data concerning the present (i.e., online processing), with an emphasis upon data and events that are usually personal and that occur within specific external and internal contexts. The emphasis is upon the processing of new and current information. During initial learning, emphasis is placed on the event-based memory system, which will continue to be of importance even after initial learning in situations where unique or novel trial information needs to be remembered (Hunsaker, 2012a,b; Hunsaker and Kesner, 2008, 2013; Kesner and Hunsaker, 2010; Kesner, 2013). This system is akin to episodic memory (Tulving, 1983) and some aspects of declarative memory (Squire, 1994) as formulated to describe research using human subjects.

The knowledge-based memory system provides for more permanent representations of previously stored information in long-term memory and can be thought of as one's general knowledge of the world. The knowledge-based memory system would tend to be of greater importance after a task has been learned or given that the situation has become invariant and/or familiar. This system is akin to semantic memory and can be summarized as retrieval and consolidation processes (Tulving, 1983).

The rule-based memory system receives information from the event- and knowledge-based systems and integrates the information by applying rules and strategies for subsequent action (Churchwell and Kesner, 2011; Churchwell et al., 2009, 2010; Cohen and O'Reilly, 1996; Kesner and Churchwell, 2011). In most situations, however, one would expect a contribution of all three systems with a varying proportion of involvement of one relative to the other depending primarily upon the demands of the task being performed.

### *Specific Attributes*

The three memory systems are composed of the same forms, domains, or attributes of memory processes. Even though there could be many attributes, the most important attributes in the attribute model as presently formulated are space, time, response, sensory-perception, and reward value (affect). In humans, a language attribute is also added.

A spatial (space) attribute within this framework involves memory representations of places or relationships between places. It is exemplified by the ability to encode and remember spatial maps and to localize stimuli in external space.

**Table 6.1** Description of the Processes Performed by Different Memory Systems Used in the Attribute Theory as Applicable to Research Using Rodents

| | Event Based | Knowledge Based | Rule Based |
|---|---|---|---|
| Encoding | • Pattern separation<br>• Transient representations<br>• Short-term memory<br>• Intermediate-term memory | • Selective attention<br>• Associated with permanent memory representations<br>• Perceptual memory | • Strategy selection<br>• Rule maintenance |
| Retrieval | • Consolidation<br>• Pattern completion | • Long-term memory<br>• Retrieval based on flexibility and action | • Short-term working memory |

Memory representations of the spatial attribute can be further subdivided into specific spatial features including allocentric spatial distance, egocentric spatial distance, allocentric direction, egocentric direction, and spatial location (Hunsaker, 2013; Kesner, 2013).

A temporal (time) attribute within this framework involves memory representations of the duration of a stimulus and the succession or temporal order of temporally separated events or stimuli, and from a time perspective, the memory representation of the past (Kesner and Hunsaker, 2010).

A response attribute within this framework involves memory representations based on feedback from motor responses (often based on proprioceptive and vestibular cues) that occur in specific situations as well as memory representations of stimulus−response associations.

A reward value (affect) attribute within this framework involves memory representations of reward value, positive or negative emotional experiences, and the associations between stimuli and rewards.

A sensory/perceptual attribute within this framework involves memory representations of a set of sensory stimuli that are organized in the form of cues as part of a specific experience. Each sensory modality (olfaction, auditory, vision, somatosensory, and taste) can be considered part of the sensory/perceptual attribute component of memory.

## *Processes Associated with Each Attribute*

Within each system attribute, information is processed in different ways based on different operational characteristics (Table 6.1).

For the event-based memory system, specific processes involve (1) selective filtering or attenuation of interference of temporary memory representations of new information and is labeled pattern separation, (2) encoding of new information, (3) short- and intermediate-term memory for new information, (4) the establishment of

**Table 6.2** Primary Neuroanatomical Correlates Underlying Each Attribute in Rodents

| Attribute | Event Based | Knowledge Based | Rule Based |
|---|---|---|---|
| Spatial | Hippocampus | Parietal cortex | Infralimbic/prelimbic[a]<br>Retrosplenial cortex |
| Temporal | Hippocampus<br>Basal ganglia | Anterior cingulate<br>Infralimbic/prelimbic[a] | Anterior cingulate<br>Infralimbic/prelimbic[a] |
| Sensory/perceptual | Sensory cortices | TE2 cortex[b]<br>Perirhinal cortex<br>Piriform cortex | Infralimbic/prelimbic[a] |
| Response | Caudoputamen | Precentral cortex<br>Cerebellum | Precentral cortex<br>Cerebellum |
| Affect | Amygdala | Agranular insula[c] | Agranular insula[c]<br>Infralimbic/prelimbic[a] |
| Executive function | Basal ganglia | Infralimbic/prelimbic[a]<br>Parietal cortex | Infralimbic/prelimbic[a]<br>Parietal cortex |
| Social<br>Proto-language | | Unknown networks | |

Murine homologs of:
[a]medial prefrontal cortex.
[b]inferior temporal cortex.
[c]orbitofrontal cortex.

arbitrary associations, (5) consolidation or elaborative rehearsal of new information, and (6) retrieval of new information based on flexibility, action, and pattern completion.

For the knowledge-based memory system, specific processes include (1) encoding of repeated information, (2) selective attention and selective filtering associated with permanent memory representations of familiar information, (3) perceptual memory, (4) consolidation and long-term memory storage partly based on arbitrary and/or pattern associations, and (5) retrieval of familiar information based on flexibility and action.

For the rule-based memory system, it is assumed that information is processed through the integration of information from the event- and knowledge-based memory systems for the use of major processes that include (1) the selection of strategies and rules for maintaining or manipulating information for subsequent decision making and action, (2) short-term or working memory for new and familiar information, (3) development of goals, (4) prospective coding, (5) affecting decision processes, and (6) comparing actions with expected outcomes.

## *Attributes Map onto Neural Substrates*

On a neurobiological level each attribute maps onto a set of neural regions and their interconnected neural circuits (Table 6.2). For example, within the event-based memory system, it has been demonstrated that in animals and humans the

hippocampus supports memory for spatial, temporal, and language attribute information; the caudate mediates memory for response attribute information; the amygdala subserves memory for reward value (affect) attribute information; and the perirhinal and extrastriate visual cortex support memory for visual object sensory/perceptual attribute.

Within the knowledge-based memory system, it has been demonstrated that in animals and humans the posterior parietal cortex supports memory for spatial attributes; the dorsal and dorsolateral prefrontal cortex and/or anterior cingulate support memory for temporal attributes; the premotor, supplementary motor, and cerebellum in monkeys and humans and precentral cortex and cerebellum in rats support memory for response attributes; the orbital prefrontal cortex supports memory for reward value (affect) attributes; the inferotemporal cortex in monkeys and humans and TE2 cortex in rats subserves memory for sensory/perceptual attributes (e.g., visual objects); and the parietal cortex, Broca and Wernicke's areas subserve memory for the language attribute.

Within the rule-based memory system, it can be shown that different subdivisions of the prefrontal cortex (and rodent homologs; Preuss, 1995; Rose and Woolsey, 1948a,b; Uylings et al., 2003) support different attributes. For example, the dorsolateral and ventrolateral prefrontal cortex in humans support spatial, object, and language attributes and the infralimbic and prelimbic cortex in rats supports spatial and visual object attributes; the premotor and supplementary motor cortex in monkeys and humans and precentral cortex in rats support response attributes; the dorsal, dorsolateral, and mid-dorsolateral prefrontal cortex in monkeys and humans and anterior cingulate in rats mediate primarily temporal attributes; and the orbital prefrontal cortex in monkeys and humans and agranular insular cortex in rats support affect attributes (Kesner, 2000).

### *Interactions Among Attributes*

Despite the relative independence and parallel processing of the different attributes, it bears to mention that the neural systems that underlie memory of all forms interact and contain similar nodes (i.e., hippocampus processes space and time and in special cases can process sensory/perceptual information and affect—but all these attributes are processed in larger networks made up of disparate elements). To provide a concrete example for interactions among attributes, the interaction among temporal, spatial, and sensory/perceptual attributes will be discussed based on different interactions on task demands.

The nature of the interactions between memory systems can be evaluated to dissect out the processes involved in both episodic and nonepisodic behavioral experiments. For illustration, two hippocampus-dependent tasks involving specific and easily identifiable sensory/perceptual stimuli (what), spatial information (where—computed from a combination of sensory/perceptual and temporal attributes), and temporal relationships between the stimuli (when) will be compared and contrasted. One task will require event-based memory processes and the other task can be solved via knowledge-based memory processes. The nature of the interactions

between these three attributes corresponding to what, when, and where will be analyzed to differentiate between the two tasks.

The knowledge-based memory task requires that a pair of associations be acquired over multiple training trials. It is an object−trace−place paired-associate task involving sensory/perceptual stimuli (what), a temporal stimulus in the form of a temporal discontinuity (trace interval; when), and spatial information (where). This task is designed as follows: when a particular spatial location (a) and a Garfield toy (1) are paired across a 10-s trace interval (an underscore), the animal is rewarded (a_1+). Also if a different spatial location (b) and a truck toy are matched (2), the animal is rewarded (b_2+). If spatial location (a) and the toy truck are paired, there is no reward (a_2-), similarly for spatial location (b) and (a) Garfield toy (b_1-). The trace interval separates the sensory/perceptual stimulus and the presentation of the spatial location (Hunsaker et al., 2006). If the association presented during a trial were rewarded, the rat would receive a reward upon displacing a block in the correct spatial location, which is then represented by the affect attribute, signaling a correct choice. This should bind the sensory/perceptual stimulus and spatial location association across the temporal discontinuity (an association involving what, when, and where). Then, the animal is presented with a new sensory/perceptual stimulus and spatial location association. If rewarded, then the process continues as before; if not rewarded, the animal does not receive any reward, and the affect attribute signals an error. Learning this task within only the event-based memory system would be difficult because the event-based memory system is susceptible to trial-by-trial interference. Both temporally adjacent (e.g., subsequent) and spatially adjacent (e.g., occurring in the same or very similar spatial locations irrespective of temporal contiguity) episodes would interfere and degrade each other during acquisition.

Learning these associations involves comparing accumulated behavioral episodes or events within the knowledge- and rule-based memory systems to develop appropriate rules, goals, and schemas to perform the task efficiently. Also, these two latter systems generate and apply abstract rules and generalize temporal, spatial, and internal contexts. In other words, the knowledge- and rule-based systems read the accumulated behavioral episodes, clarify the relevant contextual information, and apply this information to guide future actions. Once the knowledge- and rule-based memory systems have processed the data and generated the schemas necessary to perform the task, the event-based memory system does not significantly contribute to performance of this task since the four discriminations or associations (a_1+, b_2+, a_2-, b_1-) have been efficiently encoded and only need to be discriminated from each other (O'Reilly and Frank, 2006; O'Reilly and Rudy, 2001).

In contrast to the above biconditional discrimination, a task developed by Day et al. (2003) and modified by Kesner and colleagues (2008) allows rats to perform a very similar sensory/perceptual stimulus and spatial location association in an event-based manner. During the study phase, the animal receives two rewarded object−place pairings (i.e., single sensory/perceptual stimulus in a spatial location defined by the sum total of sensory/perceptual stimuli in the environment)

separated by a short temporal interval. Since there are two distinct behavioral episodes in close temporal proximity to each other, information pertaining to temporal relationships between stimuli (e.g., temporal contiguity) discriminates the two episodes and facilitates retrieval (Hunsaker and Kesner, 2008). During the test phase, the animal is provided with a retrieval cue. The animal has to learn that the sensory/perceptual stimulus provided as a retrieval cue is a signal to displace a neutral block in the corresponding spatial location previously paired with the cue (or to the sensory/perceptual stimulus cued by a spatial location). Since none of the 50 sensory/perceptual stimuli and 48 spatial locations are frequently paired (there are nearly 2500 possible combinations), each pairing is trial (or behavioral episode) unique. Since the animal receives two distinct behavioral episodes followed by a retrieval cue to signal which of the two episodes needs to be recalled, the animal has not only to remember the relevant episode to receive reward, but also to discriminate between the relevant episode and the episodes presented either immediately before or after the relevant episode, as well as all previous episodes that occurred in the same or a similar spatial context.

The critical difference between the two tasks is not the cued-recall nature of the latter task *per se* but that the associations to be remembered are trial unique. This allows each behavioral episode to be coded as unique but increases potential interference from previous or subsequent behavioral episodes. To overcome this interference and to guide efficient recall of the correct behavioral episode, this task is performed with the contribution of the knowledge- and rule-based systems such as traditional biconditional discrimination tasks, but the trial specific episodes make it necessary to depend on the event-based memory system to compare the retrieval cue to the stored episodes to efficiently recall the correct, and only the correct, behavioral episode to guide behavioral decisions and actions.

## Applying the Attribute Model

### *General Advice*

Despite the need to move beyond limiting behavioral research to the standard behavioral paradigms (i.e., water maze, contextual fear conditioning), it is by no means necessary to avoid these tasks all together. Rather, it is important to integrate these tasks more through behavioral analysis necessary for elucidating behavioral endophenotypes (Table 6.3 for lists of tasks used for general phenotyping as well as behavioral endophenotyping; Hunsaker, 2012a,b for references to the specific tasks).

To begin, it is important to evaluate the basic sensory function in all mice, because any deficits in basic sensation or perception confound interpretations of behavioral results (Crawley, 2007). Sensory deficits do not preclude the behavioral analysis of a mouse model. When a mouse shows sensory deficits, either the model can be bred onto a different background strain over numerous generations—commonly $>10$ generations backcrossed onto the C57BL/6J strain—or else behavioral tasks can be

**Table 6.3** Summary of Behavioral Tasks Commonly Used in Behavioral Phenotyping Strategies Organized by General Domain

| Attribute Tested | Behavioral Phenotyping | Behavioral Endophenotyping |
|---|---|---|
| Memory (spatial, temporal) | Water maze<br>Radial arm maze<br>Barnes maze<br>Active/passive Avoidance<br>Contextual fear conditioning | |
| Spatial memory | | Categorical (Metric) Processing<br>Coordinate (Topological) Processing<br>Touchscreen pattern Separation<br>Delay match to place with variable interference<br>Delay match to place with variable cues |
| Temporal memory | | Trace fear conditioning<br>Temporal ordering of stimuli<br>Sequence learning tasks<br>Sequence completion tasks<br>Duration discrimination |
| Associative | | Biconditional discrimination<br>Cued-recall task for trial unique associations |
| Affect | Classical fear conditioning<br>Open field<br>Elevated plus maze<br>Porsolt test | |
| Valence | | Reward contrast with variable Reward value |
| Anhedonia | | Anticipatory contrast task<br>Species relevant sexual behaviors |
| Approach-avoidance | | Hyponeophagia<br>Defensive burial |
| Fear processing | | Defensive test battery<br>Classical, contextual, trace fear conditioning |
| Motor (response) | Rotarod | |
| Visuomotor | | Skilled forelimb reaching<br>Capellini handling task<br>Seed shelling tasks<br>Parallel beam or ladder walking tasks |

(*Continued*)

**Table 6.3** (Continued)

| Attribute Tested | Behavioral Phenotyping | Behavioral Endophenotyping |
|---|---|---|
| Motor learning | | Acquisition of Skilled Reaching<br>Acquisition of Rotarod (initial training)<br>Working Memory for Motor Movements |
| Sensory/perceptual | Prepulse inhibition<br>Acoustic startle<br>Hot plate analgesia | Prepulse inhibition<br>Acoustic startle<br>Psychonomic threshold |
| Social | Three chamber social novelty | Social dyadic behavior<br>Resident intruder tests<br>Social transmission of food preference<br>Social dominance |
| Executive function | Operant conditioning<br>Holeboard exploration<br>Reversal learning | |
| Cognitive control | | Contextually cued biconditional discrimination<br>Serial reversal learning<br>Stop signal task<br>Probabalistic (80/20) reversal learning |
| Attention | | 5 Choice serial reaction time task<br>Covert attention tasks |

Also summarized are behavioral tasks proposed to be useful for behavioral endophenotyping organized by component attributes (Hunsaker, 2012a,b for references for each task that emphasize the methods for each paradigm).

chosen that minimize the contribution of the particular sensory modality that is not being processed, as Farley and coworkers (2011) demonstrated in an experiment emphasizing behaviors to test space and temporal processing while concurrently not requiring vision in mice that are blind from an early age.

After evaluating basic sensory function in the mouse model, it is critical to determine the pattern of behavioral strengths and weaknesses in the population being modeled by the mouse. With this information from the clinical population, it is important to either create or adopt behavioral tasks to evaluate the same cognitive attributes or domains as tested in the clinical population. For example, if a disorder being modeled shows global memory deficits (measured by intelligence (IQ) and neuropsychological tests) without concomitant impairments for executive function, then the mouse model needs to be tested for memory across a number of domains or attributes evaluated by the neuropsychological tests in order to better dissect cognitive function in the model. In this example, executive function should also be evaluated, but in this case to verify intact executive function in the model.

More concretely, a general memory deficit may be mediated by an inability to encode new information, consolidate/retrieve encoded information, or understand the rules required to perform correctly on a given test. All of these factors can be tested in mice and can further be evaluated across domains: spatial, temporal, and

response memory can be specifically evaluated in the mouse, as can the contribution of affect to memory, anxiety, and depressive behaviors. With these data, research into the mouse model may actually serve to inform the clinic as to more specific domains that can be tested in the clinic—emphasizing a direct interaction across the research in the clinic and the behavioral genetics laboratory (Hunsaker, 2012a,b; Table 6.3).

There are a number of difficult questions that must be answered in the development of a comprehensive behavioral endophenotyping approach, including: How many of these behavioral tests could be conducted on a single cohort of mice? Would multiple tests on the tasks listed lead to potential confounds? What are the potential contributions/confounds of environmental effects, such as housing conditions, on mouse behavior? These are important factors, because small changes in experimental design can make the difference between a successful analysis of a behavioral phenotype and a collection of uninterpretable data.

The question of testing the same group or mice across multiple experiments is sometimes more complicated as it seems, but a solution can be designed if viewed through the attribute model. The most critical aspects that need to be taken into account when performing multiple experiments on the same mice are twofold: any role for negative effect on task performance, and the rule-based memory system succumbing to interference across tasks. It is important to keep in mind the role for negative effect for task performance not only on the present task, but also on the future tasks performed with the same group of mice. If a mouse is to receive fear conditioning in the middle of an experimental design, it will take a week or so of handling for the mouse to unlearn any associations between the fear conditioning and the experimenter (Rudy and O'Reilly, 2001).

For the rule-based memory system, it is important to remember that mice and rats take a significantly longer amount of time to learn and apply rules to guide behavior than humans. As such, if a researcher wants to perform any experiments that require the mouse to learn a rule or set of rules to guide behavior, and are not just exploiting the natural tendency of mice to explore their environment and behaviorally respond to novelty across domains (i.e., novelty detection), then intervening tasks not requiring rule learning/implementation need to be presented prior to the usage of another task taxing the rule-based memory system. Also important in this case is the use of very different apparatus for each rule-based learning task, or else previously learned rules will have to be explicitly extinguished prior to beginning training on a new task (Cohen and O'Reilly, 1996).

As a rule of thumb, it is best to perform at most a set of experiments evaluating basic sensory function in mice (Crawley, 2007), followed by tasks evaluating each attribute in turn, followed potentially by the water maze/avoidance tasks and fear conditioning as the final task to prevent carryover from experiments interfering with subsequent experiments. In this, most likely, more than one experiment tasking executive function/complex rule learning can be performed. More than one of these tasks will result in interference that will confound interpretation of subsequent rule-based tasks. Additionally, aside from the water maze and fear conditioning experiments, it is recommended that series of experiments

be counterbalanced across animals and groups, preferably using a Latin square design to reduce the contribution of task order to any observed effects. Additionally, it is often worth testing the ability of mice to perform the behavioral task battery by evaluating a few wild-type mice of the background strain being used to verify that they can perform all the tasks without being overwhelmed by excessive testing.

In addition to genetic background, the treatment of mice prior to and during experimentation is critical. It has been shown a number of times that alterations to the cage environment is an important factor for later behavioral improvement—such that an enriched environment results in better performance on behavioral tasks and increased gray matter and dendritic complexity. As such, mice should preferably be housed in a standard fashion, either with a set number of mice per cage or else singly housed, but it is important to note that mice do not do as well singly housed as rats; they tend to show increased anxiety levels, which may affect task performance (Van de Weerd et al., 2002). As such, multiple housed is recommended unless precise drug dosing or food deprivation is required that precludes group housing. Furthermore, the amount of stimuli available to the mice within each cage should be uniform across cages. As such, a standard environment needs to be maintained among all mice during experimentation.

## *Specific Application of the Attribute Model*

To properly apply the attribute model to optimally select behavioral paradigms for the study of genetic models, a researcher must ask themselves a number of questions (in no particular order). (1) What are the pathological effects of the mutation on anatomy? In other words, in what way is the structure of brain regions, neuroendocrine regions, receptors, etc. altered by the mutation. The answer to this question suggests a starting point for task selection.

For considering the pathological effects of mutations on anatomy, the most efficient way to specifically quantify this effect is in collaboration with a veterinarian or (neuro)anatomist that studies the species. A full histological profile can be performed by core facilities at most universities and the necessary sectioning and staining techniques are easily trained to skillful technicians. Depending upon the hypotheses being evaluated, it may be helpful to evaluate the organ systems of the mutant rat or mouse. A key example from my own work has demonstrated that key neuropathologic features of a mouse model for the fragile X premutation (the CGG KI mouse) were also present in the peripheral nervous system as well as somatic organs and cardiac muscle—similar to that observed in parallel in postmortem tissue from human premutation carriers (Hunsaker et al., 2011a,b). Unfortunately, it never occurred to any of the researchers involved to look at these features in mice years before we finally did so. Such information would have been able to guide human research into the pathology of the fragile X premutation years before this report. Using the information provided in Table 6.3 along with a more thorough literature search would inform a first wave of behavioral tasks to consider for evaluating the behavioral consequences of the genetic mutation.

Additionally, we were able to identify pathology associated with the premutation throughout the brain, but specific patterns of development were identified that led us to evaluating hippocampus and parietal cortex function in the CGG KI mice (Hunsaker et al., 2009; Schluter et al., 2012; Wenzel et al., 2010).

(2) Is there anything known from human research concerning the effects of mutations within the genetic locus being evaluated? If so, then this provides a starting point, that of generating a behavioral model in a mouse or rat for the research that has been undertaken in the clinic. Upon replicating (or more to the point recapitulating) these effects, logical extensions to better characterize the deficits can be undertaken.

This is a generally self-explanatory process, but nonetheless it bears extrapolation. Often, there are subclinical effects for a number of populations with genetic mutations that are important for animal researchers to know as a mouse model may be able to tease out these subclinical effects in the nonhuman models in ways not possible to do in patients. What this means is that, in addition to reading the literature available on a given mutation or disease, it is often much more efficient to initiate a collaboration, or even just initiate contact with a clinician and probe their experiences and ask their impressions on what is worth studying in the population.

For a specific example from my own work in the CGG KI mouse model of the fragile X premutation, the process by which experimental hypotheses arose will be discussed. The full clinical manifestation of the fragile X premutation is a late onset neurodegenerative disorder called fragile X-associated tremor/ataxia syndrome, or FXTAS. FXTAS is primarily a movement disorder characterized by Parkinsonism, intention/kinetic tremor, and cerebellar gait ataxia, along with a dysexecutive syndrome and cognitive decline leading to dementia. In the initial screen of the mouse, no convincing phenotypes were found to model FXTAS (van Dam et al., 2005). Upon discussion with collaborators studying the clinical population, it was brought to our attention that the population constantly report "muddy thinking," and what can best be described by general clumsiness and subclinical apraxia (reports of tripping over their own feet, not being athletic, or spilling their milk at lunch).

Based upon these verbal reports and models being developed to account for these reports, it was determined that evaluating spatial and temporal processing was an appropriate starting point and evaluating motor tasks was worth a try in the mouse model, so long as the tasks were sufficiently difficult. Though not reviewed here, these experiments were able to identify early onset, progressive deficits for spatiotemporal memory as well as motor difficulty on difficult, but not relatively easy, motor tests (Borthwell et al., 2012; Diep et al., 2012; Hunsaker et al., 2010, 2012, 2011, 2009). In other words, the clinical manifestation of FXTAS was not recapitulated in the mouse model, but the subclinical manifestation of the fragile X premutation observed by our clinical collaborators were recapitulated.

(3) What molecular cascades are affected by the mutation? The answer to this question will set up research questions into whether the molecular cascade disrupts more short-term learning, or consolidation, or long-term retrieval processed preferentially.

One way of thinking about this is to consider that there are mutations that affect induction of long-term potentiation or else affect early-phase Long Term Potentiation (LTP) separately from mutations that preferentially affect late-phase Long Term Potentiation (LTP). As memory processes can be similarly parceled, the timescale of the mutations effect on behavior be considered. Again with an example from my own research, it is important to assess what precisely is the molecular pathology associated with the fragile X premutation, since the same parametric mutation underlies both premutation ([50,200] CGG repeats) and full mutation (230 + CGG repeats) underlying fragile X syndrome. It has been demonstrated in the Fmr1 KO mouse model of fragile X syndrome that the lack of Fmrp results in an exaggerated long-term depression dependent upon Group 1 mGluR receptors (mGlur1/5; both paired pulse low-frequency stimulation in the presence of D-(-)-2-Amino-5-phosphonopentanoic acid (APV) APV as well as bath application of (*S*)-3,5-Dihydroxyphenylglycine (DHPG) DHPG; Huber et al., 2002). Since the fragile X premutation and the CGG KI mice show reduced, but nonzero, Fmrp, it was unknown whether the characteristic findings of enhanced mGluR1/5-dependent Long Term Depression (LTD) would be present in the CGG KI mice. What was found was that, in fact, the CGG KI mouse showed a CGG repeat length-dependent impairment in plasticity induction for both NMDA LTP, LTD, and mGluR1/5 LTD, but no enduring effects after plasticity was induced. We interpreted these findings as suggestive of more general cellular dysfunction not particularly dependent upon one receptor system over another (Hunsaker et al., 2012).

Intriguingly, an independent group demonstrated in a different mouse model of the fragile X premutation (CGG-CCG mouse) that shows much more profound reductions in Fmrp levels phenocopied the Fmr1 KO mouse for mGluR1/5 LTD. They did not look at NMDA-receptor dependent plasticity (Iliff et al., 2013). These findings, although they appear discrepant, actually make perfect sense based upon the hypothesis that reduced Fmrp levels results in exuberant mGluR1/5 activity; and as such both support theories of premutation-dependent molecular pathologies.

As can be seen from the specific examples provided earlier, there are a number of steps to choose a behavioral paradigm to test a rodent model. Importantly, although presented earlier as a holistic approach, it is possible to ask the questions serially based on the laboratory expertise. If a laboratory has a particular focus on molecular mechanisms, then obviously the elucidation of biochemical pathways affected by the mutation are the logical starting point. Similar logic applies to cognitive neuroscientists and anatomists in approaching research questions.

## Conclusions

An important consideration in the study of mouse models of any genetic disease is how well the behavior of the mouse serves to actually model the behaviors present in the clinical population being modeled. In order to optimally address this concern is to

explicitly develop a behavioral endophenotype of the mouse model in which the mouse is explicitly tested using tasks rigorously designed to explicitly test the cognitive domains affected in the clinical population. To achieve this goal, it is important not only to apply standard behavioral tasks to mouse models of genetic disorders, but also to directly evaluate brain function across all cognitive domains. Furthermore, it is critical for behavioral genetics laboratories to interact with clinical research laboratories to develop comprehensive behavioral endophenotypes for the disorder being modeled. The strength of behavioral genetics and the mouse models is the ability to apply behavioral paradigms known to be subserved by known anatomical loci not only to determine the behavioral phenotype of the model, but also to elucidate candidate brain regions affected by the mutation (Robbins et al., 2012). When research into mouse models of genetic disorders emphasizes patterns of mnemonic strengths and weaknesses across domains (i.e., the behavioral endophenotype), the results will not only directly model the disorder being studied, but also serve both as risk prodrome for disease onset or progression and as outcome measures that can be applied by the clinic in interventional studies.

In addition to this interaction, it is rather difficult to elucidate a behavioral endophenotype using only a limited toolkit. As such, the attribute model is unique in that the theory itself was designed to not only explain and describe processes underlying memory, but also provide hypotheses that can specifically be tested. As mentioned earlier, Table 6.3 contains a list of the behavioral tasks available to the researcher today that have been validated in mice and rats to test specific memory processes (Hunsaker, 2012a,b for references corresponding to the tasks). Using subsets of these tasks in turn with collaborations with molecular and clinical neuroscientists we were able to design a battery of test to evaluate a behavioral endophenotype in the CGG KI mouse. Importantly, research into the CGG KI mouse has critically informed the clinic and provided rationale to formally identify subclinical apraxia and postural issues early in life as well as emphasized the need to evaluate spatiotemporal processing in the fragile X premutation (Goodrich-Hunsaker et al., 2011a,b,c; Narcisa et al., 2011; Wong et al., 2012).

In broader application, it is quite possible that applying models such as the attribute processing model concurrent with translational interactions among molecular, behavioral, and clinical researchers may result in a paradigm shift in behavioral genetics. At present the field is working toward developing standardized sets of behavioral cores that can process large numbers of genetic models in relatively short times. This is commendable, but if the research culture opens up to free exchange of theories and hypotheses between molecular genetics and clinical neuroscience laboratories as well as general psychiatry and medical research into clinical populations, then the mouse model will be a tool to identify neurocognitive as well as molecular endpoints of mutations states. Such a tool is begging to be developed to facilitate the testing of candidate compounds prior to phase I clinical trials to reduce the tendency of compounds to succeed in the mouse and fail in the human. Additionally, such tools also would facilitate the elucidation of what the behavioral consequences of specific alterations to biochemical processes actually are at a much higher resolution than has been possible to date.

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