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A BEHAVIORAL COMPARISON OF FOUR INBRED STRAINS OF MICE

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Virginia Commonwealth University.

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Abstract

A BEHAVIORAL COMPARISON OF FOUR INBRED STRAINS OF MICE

By Erin Wood, M.S.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2010.

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Isogenic, or inbred, mouse strains are currently the experimental subjects of choice in laboratory studies focused on genetics, pharmacology, and psychological issues.

Understanding phenotypic differences in isogenic strains is important in order to interpret experimental results obtained from inbred mouse strains. Four commonly used inbred strains, C57BL/6NHsd (C57), DBA/2NHsd (DBA), 129S2/SvHsd (129), and Balb/cAnHsd (Balb/c), are investigated in this study using four different behavioral tasks that measure

locomotor activity and cognitive behavior (Morris Water Maze (MWM), T-maze, and operant autoshaping procedures). In the locomotor activity task 129 mice showed significantly less horizontal ambulation than any other strain, while differences in rearing was seen between all strains, with C57 mice producing the most, and 129 showing the least rearing. Thigmotaxia was seen the most in the 129 strain, less so with the Balb/c and DBA strains, and the least in the C57 mice. In the MWM learning across strains was noted but there was no difference between the strains. In the T-maze the Balb/c strain showed the shortest latency to enter an arm, while the 129 strain showed the longest. As expected they also showed the lowest accuracy and the highest percent time-outs compared to all the other strains. In the autoshaping procedure little difference between the strains was observed. Balb/c mice trended graphically towards higher rates however there was no difference with regard to number of contingent responses or number per strain to reach a criterion of 10 or more contingent reinforcers. Finally, locomotor activity was measured again at the end of the study. The activity results were still similar, although the C57 strain showed a decrease in horizontal ambulation as compared to DBA and Balb/c strains; however, the 129 strain still showed the least activity. These results indicate that there are significant differences in locomotor behavior and cognitive processes in these strains that should be considered when interpreting results from studies using these inbred mouse strains.

Behavioral Comparison of Four Inbred Strains of Mice

Overview

Now more than ever the scientific community is looking to the field of genetics to inform our inquiries into the human condition. Predisposition to, treatment of, as well as ultimately the prevention of a variety of human disorders is being investigated through a genetic lens. Cancers, mental disorders, autoimmune diseases, the obesity epidemic and its relatives, diabetes and heart disease, high cholesterol, high blood pressure, and geriatric issues such as Alzheimer's and osteoporosis, are merely a few issues currently considered to have some genetic basis (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gnd>). Even a variety of non-disease conditions are being investigated genetically, such as personality (Benjamin, Ebstein & Belmaker, 2002), taste preference (Bachmanov et al, 2009), and sensory acuity (Vloeberghs et al, 2008). Hence furthering the field from every possible angle is important.

Behavioral studies can offer great information to the field of genetics. Behavioral psychologists, pharmacologists, and geneticists are utilizing isogenic, or inbred, mouse strains to inform transitions between behavior and practical implications of diseases and disorders in humans. Behavioral tasks such as the locomotor activity chamber and the Morris Water Maze (MWM) are standard in such research (Holmes et al, 2002; Crawley et al, 1997). Tasks such as the T-maze and operant models have not been as commonly utilized with genetic objectives, though they also can and do provide valuable information about variations in mice strain behaviors. Researchers in behavioral psychology/neuroscience have long depended upon these tasks and agree that they are useful tools due to their long-standing validity and reliability (Crawley et al, 1997).

Mice in particular are the species of choice for genetic research particularly due to their relatively fast procreation cycles, and they are able to be genetically manipulated in ways that far surpass other commonly utilized laboratory species (Bucan & Abel, 2002; Paigen & Eppig, 2000). Quieting the “extra ‘noise’” injected via genetic background variability allows researchers to focus on their true research inquiries without being concerned that genetic variance is obscuring results and therefore conclusions.

The comprehensive objective for this project is to add resources to the ever-growing database concerning inbred mouse strain differences via behavioral phenotyping. Contributing to community knowledge in a way that strengthens the foundation for future research is of the utmost importance and is a primary goal of this research.

Why the mouse?

Mice have become the medium of choice for biomedical research for a variety of reasons (Knight & Abbott, 2002). They are small, easy to handle, require few resources to house and feed, and their small size requires less drug as compared to species of more substantial size. Mice procreate quickly and are able to be genetically manipulated in ways that far surpass other commonly utilized laboratory species (Bucan & Abel, 2002; Paigen & Eppig, 2000). The ability to create new and differing variations in the mouse is a powerful tool upon which researchers now greatly rely. Isogenic, transgenic, knock-ins, knockouts, knock-ons, or –offs, are only a few of the current buzz words describing how scientists are manipulating the mouse genome in order to provide specific canvases that more accurately represent a disease or treatment model, and sometimes facets of both.

Why inbred strains of mice?

Inbred, or isogenic, organisms are those that have essentially identical genetic compositions. This characteristic is one that is applicable to monozygotic twins in a variety of species, even humans. In species that procreate quickly breeders will often inbreed siblings, or in rodent nomenclature, littermates, continuously for a minimum of 20 generations. At this point approximately 98% of the genome between offspring is identical. Each successive generation that is bred becomes only more genetically alike (Festing & Altman, 2002).

The availability of inbred species for research has major benefits. Festing (2002) has dedicated a large part of his professional life to considering the various ways appropriate research preparation supports valid and responsible science. He remarks that with regard to the “three R’s” (Russell & Burch, 1959), using inbred subjects allows for the reduction in the number of subjects necessary (Festing & Altman, 2002). This is due to the fact that a large amount of statistical variance in organism-based research is derived from lack of uniformity among phenotypes, as compared to isogenic samples. Of course, Festing also takes into account that not all experimental “noise” results from individual genetic differences, and so appropriate, step-wise experimental design must play the primary role in reducing all types of variance. Certainly issues such as environmental effects (e.g. shipping stress, interspecies aggression, lab, and tester conditions) are also noteworthy and should be equally prioritized in terms of minimizing variability. The most direct way of controlling for genetic variability though, is to build mutations upon inbred genetic backgrounds (Crawley et al, 1997).

Over time, as strain breeding has continued and genetic mapping has become commonplace, the identification of specific genes and alleles has grown into publicly

available libraries of data (Festing & Altman, 2002; Paigen & Eppig, 2000). In fact, scientists can access and add onto these databases, while building research designs based on this information. For example, a research group may decide to utilize a particular model with a particular strain of mouse because they know the strain has an excessive amount of a certain receptor that they are interested in studying. Moreover, Waterson et al. (2002) note that having the entire mouse genome in hand will facilitate the genetic manipulation of mice strains by minimizing “unfortunate choices”. Paigen and Eppig (2000) suggest that due to the lack of potentially protective heterozygosity isogenic mice are prone to extreme phenotypic variation, which is also of particular interest to some researchers.

Blake et al. (2001) present the Mouse Genome Database (MGD), though it has actually been publically available since 1994. They describe it as “a community database resource for the laboratory mouse... (providing) standard nomenclature and consensus map position for mouse genes and genetic markers...” Paigen and Eppig summarize the growth of another useful database concerned with the mice strains: “The Mouse Phenome Project” in which one can find physiological, anatomical, and metabolic characterizations, susceptibility to various diseases, behavioral traits, and gene arrays (2000). Originating in 1999, like the MGD, this project is housed at Jackson Laboratory and is connected to the MGD to ease accessibility. Also known as the Mouse Phenome Database (MPD), the website started in 2001 and with contributions from over 200 researchers and data being pulled from public sources for 36 priority strains, data from February 2009 states the site experiences over 58,000 hits per month (MPD, <http://www.jax.org/phenome>). Crawley et al. (1997) were already indicating the need for this in a review article wherein they describe strain differences across a variety of behavioral tasks and drug-induced behaviors. “A

comprehensive database on behavioral phenotypes of inbred strains of mice would provide the information needed by molecular geneticists to make the optimal choice of parental strains and breeding strategies for the expected phenotype of each targeted mutation, and to interpret the results appropriately.”

Rats are known to make higher frequency vocal calls under the influence of rewarding stimuli (drugs of abuse and electrical brain stimulation), and lower frequency calls during aversive stimuli (lithium chloride and footshock; Burgdorf et al, 2007). In this case the higher frequency calls are associated with “positive activation” (Knutson et al, 2002) or a more “positive emotional state” (Burgdorf et al., 2007). In a study undertaken by Burgdorf et al. (2009) rats were bred to selectively exhibit either high or low rates of this particularly high frequency call. These authors note that measure is considered related to the “social-emotionality” of the animals’ states. Animals that expressed more of the higher frequency calls also showed greater center zone activity in an open-field test, lower social aggression, and greater preference for a sucrose solution than those subjects that had a lower expression of these high frequency calls. Interestingly, fecal boli count was higher in low frequency call emitters in several tasks than it was in the higher frequency call emitters. These authors consider this measure to be associated with a higher anxiety-like state, or some refer to this as an “emotional” state, as is similar to the interpretation by authors of many other studies (Bindra & Thompson, 1952; Borelli et al, 2004; Crawley et al, 1997; Fulk et al, 2004; Hall, 1934, 1936; Walsh & Cummins, 1976; Whimbey & Denenberg, 1967). Whimbey and Denenberg (1967) found defecation to be a valid measure of subject emotionality via factor analysis, or “emotional reactivity” as is the phrase used by Denenberg in a 1969 article. Finally, speaking to predictive validity, Borelli et al. (2004) noted a reduction in the rate of

defecation in rats treated with chronic fluoxetine, a serotonin-reuptake inhibitor class of antidepressant, and diazepam, a benzodiazepine derivative that acts to increase GABA activity, as compared to saline-treated subjects. These two compounds are known to successfully treat symptoms in human subjects related to depression, obsessive-compulsive disorder, anxiety-related states, among others (<http://www.nlm.nih.gov/medlineplus/druginformation.html>).

While the following study was published after the MGD but before the MPD, it shows clearly what strides can be made when genetic quantitative trait analyses and behavioral tests are combined. Flint et al. (1995) investigated three behavioral tests (open field locomotion including a fecal boli measure, elevated plus maze, and y-maze) that have often been used as preclinical assays in mice to assess changes in emotionality-like behaviors and reactivity in novel environments and they also conducted a number of genetic tests to locate quantitative trait loci (QTL). They found that the open field activity was heavily influenced by a three loci in the mouse genome, and that these loci also were very active in defecation as well as y-maze activity. Two of these loci were also correlated with entry into the open arms of the elevated plus maze, which is used as an indicator of heightened emotionality-like behavior. The authors concluded, “The nature of these genes is unknown, but the discovery of QTLs determining emotionality in the mouse provides the first step toward their molecular characterization and may lead to the identification of genes responsible for human susceptibility to anxiety.” This hopeful statement clearly indicates that even fifteen years ago researchers understood that it would take a combination of both genomic interest and behavioral research (in addition to many other fields) in order for scientists to realize the “macro-power” of current technologies.

How are inbred strains most often used?

Most often inbred mouse strains are used as a background genetic foundation for various types of genomic mutations, such as transgenics (those strains in which genes are added) or knockouts (those strains that have particular genes or groups of genes inhibited; their activity diminished or stopped completely). Therefore, the behavior observed in the resultant strains is not only from the mutation, but also it is some product of the background inbred genetics with the mutant genomic characteristics (Gerlai, 1996). These strains, inbred and beyond, are often utilized to investigate physical and psychiatric disorders in humans, such as addiction (Jackson et al, 2009). By isolating genes that increase (up-regulate), decrease (down-regulate), or nullify (knock-out) certain receptors or receptor groups in either the CNS and/or PNS, researchers have been able to create very specialized mouse strains that emulate diseases or symptomologies. Not only is the treatment of the resultant phenotype of interest, but it is also intriguing to see how the initial manipulations actually produce the changes in the strains. Clues to how humans develop disorders are discovered this way.

Studying the genetic contributions to nicotine addiction, Jackson et al. (2009) focused on C57BL/6 and DBA/2 mouse strains when investigating the effects of acute and chronic nicotine. They found that C57BL/6 mice tend to be more sensitive to the acute administration of nicotine, while DBA/2 mice are more sensitive to the blockade of nicotinic effects in pain-related models. Dodelis et al. (2002) note that variation in the neuronal $\alpha 4$ -nicotinic receptor subunit cannot merely be the result of normal receptor expression variation, but rather that an amino-acid related polymorphism of the subunit is more likely responsible. In the Jackson et al. (2009) study they found greater reward induced via nicotine administration in C57BL/6 but not DBA/2 mice. They attribute the differences

between these two strains genetically, finding the CHRNA 4 locus modulating the differential sensitivities to nicotine administration seen between C57BL/6 and DBA/2 strain on behavioral assays.

Historically the C57BL/6 inbred strain, specifically the substrain maintained at the Jackson Laboratory since 1948, C57BL/6J, have been shown to have a high alcohol preference (Rodgers & McClearn, 1962) and consume the greatest amounts of alcohol compared to other inbred strains (Belknap et al, 1993b). [This substrain also consumes the most morphine compared to fourteen other strains (Belknap et al, 1993a).] Due to the slight (1-2%) genetic variation between the two substrains, Mulligan et al. (2008) compared the Jackson Laboratory substrain to a comparable substrain, C57BL/6C maintained by Charles River Laboratory. These two substrains show variable phenotypic characteristics, and in this study genetic expression from different parts of the brain were analyzed in alcohol naïve mice. C57BL/6C male and female mice showed lower ethanol consumption as well as preference than C57BL/6J mice, though no difference in sensitivity to alcohol, or taste acuity, was observed. These researchers identified 29 differentially expressed genes associated with increased preference for alcohol and consumption, and 22 of these were associated with enhanced expression in the C57BL/6J substrain as compared to the C57BL/6C substrain. The authors conclude with cautionary advice to those who would use either of these two substrains in future studies not only ethanol-related, but those that may have anything to do with the methods they used herein. If one were not to know the difference in the phenotypic tendencies of these substrains perhaps they may mistakenly assume similar behavior profiles, and genetic expression. Indeed this study illustrates why

and how identifying baseline difference in inbred mouse strains and substrains is of the utmost importance.

In mice the serotonin transporter (SERT) can be nullified via mutation producing anxiety-like behaviors that researchers link to stress-related psychiatric disorders in humans (Holmes et al, 2003b). In a follow-up study it was found that by backcrossing the SERT mutant mice with two different inbred strains there was a large influence of the isogenic strain's genetic background on the exploratory and anxiety-like behavior exhibited on the original mutant strain. The authors concluded that it is important for researchers to choose the appropriate background strain, and that behavioral phenotyping mutant mice on various genetic backgrounds is a strong tool for this type of investigation (Holmes et al., 2003a). Another example is if the final results of a cognitive procedure indicate that a transgenic mouse has learning deficits then it is necessary that the background inbred strain be known to have no such deficit. The opposite is also true: if the mutation seems to increase cognition then it is imperative to know that the original isogenic strain has either moderate or poor learning (Owen et al, 1997).

Not all studies show such impressive differences between conditions, though. For instance, Paylor et al. (1998) attempted to show that because the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) is so heavily expressed in the part of the brain that seems to have much influence on learning and memory, a significant deficiency at these receptors would negatively affect the processes of learning and memory. Interestingly, these mutant mice performed the same as their wild-type littermates when tested for learning on a conditioned fear test, as well as when tested for spatial learning in a water maze. There was conflicting evidence of decreased anxiety in the literature, but overall this is an example of how there

should no expectation that manipulation of genetics will overtly and clearly influence behavior.

Why is it important to note baseline strain differences?

A combination of approaches is necessary to fully describe and therefore appropriately comprehend the impact of the genomic make-up of an organism (Bucan & Abel, 2002). The present work is focused on one such approach, the behavioral consequences of genetic background. Anatomical, molecular, cellular, physiological, and electrophysiological tests are all methods in conjunction with behavior that are necessary to provide the most comprehensive perspective to researchers.

When specialized isogenic strains are utilized in behavioral research it is imperative that there exist a reliable understanding of differences, and just as importantly similarities, in behavioral phenotypes. Interpretation of study results is complicated in its simplest form, and this only grows in complexity when individual differences are exponentially enlarged by genetic strain differences. False-positives and false-negatives could easily be conclusions if there is a lack of understanding about the impact of genetic background phenotypes (Holmes et al, 2002). Another example is that it is now known that the best background strain choice if there is a predicted decrease in activity (or increased reactivity) from a mutation is a highly active background strain (Crawley et al., 1997). The opposite is also true: a low-level locomotor background strain is the best decision when an increase in activity (or decrease in anxiety) is expected from a mutation.

Gerlai (1996) investigated a novel procedure using the T-maze designed to minimize various weaknesses with the traditional procedures. In it he compared tested CD1 strain derived transgenic substrains, S100 β -5 (5 copies of the transgene) and S100 β -8 (70 copies of

the transgene), against their littermate controls. These substrains overexpress a Ca²⁺-binding protein, show hippocampal impairment, and reduced long term potentiation (LTP) which is considered to play a role in relational learning (Gerlai et al., 1995). Results showed the CD1 controls spontaneously alternate significantly more than either of the transgenic strains, and the S100 β -5 mice did so significantly more than the S100 β -8 mice. It is quantitatively clear that the hippocampal dysfunction resulting from Ca²⁺-binding protein overexpression reduces performance in this task. Next C57BL/6, 129/Sv, and DBA/2 inbred strains were compared to a wild type strain, CD1. While the CD1 and C57BL/6 strains performed comparably, they did so significantly better than the 129/Sv and DAB/2 strains. The author specifically noted that the 129 substrain “showed accelerated hypoactivity which manifested as a quick decline of activity in the open field and T-maze.” Due to the fact that a large number of null mutants are derived from 129 strain embryonic stem cell chimeras crossed with a C57BL/6 or CD1 strain these particular phenotypes can create serious situations regarding experimental result interpretation where hippocampal function is being investigated.

Fowler et al. (2001) studied the differences in three strains of mice in part to “begin to lay the foundation for laboratory studies on the genetic influences on vulnerability to drug motor side effects.” They measured disk-pressing in the outbred CD-1 and inbred Balb/c and C57BL/6 strains, as well as duration in the reward hopper as a measure of microcatelepsy, which is considered a measure of extrapyramidal side effects (EPS) or the Parkinsonian liability of certain antipsychotic drugs (APDs). First, at baseline disk-pressing rate was lowest in the C57BL/6 mice as compared to both other strains, and the outbred CD-1 strain had the shortest hopper duration. Next, they conducted a dose curve of 0.08 mg/kg – 1.28

mg/kg haloperidol, which is a typical, first generation APD. The drug treatment significantly affected both dependent measures. Disk-press rate was dose-dependently reduced and hopper duration increased with dose in all strains. C57BL/6 mice showed the greatest sensitization to haloperidol's cataleptic effects. The researchers were able to partially antagonize haloperidol's effects by administering the muscarinic anticholinergic trihexiphenidyl. Here they found an increase in disk-pressing rates as compared to haloperidol alone, and CD-1 mice were particularly affected. For all three strains there was a decrease in hopper duration. A final conclusion was that C57BL/6 mice exhibit a decreased operant rate that is "a genuine, genetically based behavioral trait," but that when the measure was of a grosser movement, such as the hopper duration, these mice performed similarly to the Balb/c strain. The authors note that as compared to previous sensitivity studies with haloperidol in Sprague-Dawley rats (Fowler & Liou, 1998), the three strains of mice used here were relatively resistant to the drug's effects. This particular study illustrates how important it is to identify baseline behavioral differences in isogenic strains of mice. Had the authors not initially identified the pre-drug differential behaviors in the two dependent measures, they could have incorrectly interpreted the data. With that information, they were able to produce a more thorough explanation of the overall study results. Without research on baseline behavioral differences scientists cannot know if the performances they are observing are results of a) the actual conditions they are imposing (e.g. drug treatment, genetic influence, procedural differences), b) genetic background variance, or c) any combination of other uncontrolled experimental variabilities (e.g. observer/handler interference, inaccurate reporting, automated dysfunction).

Currently few if any scientists believe that a trait as complex and specific as behavior will be explained completely through the identification of regulatory genes (Bucan & Abel, 2002). While many continue to address the phenotypic variations across genetic mutants (Morice et al., 2004), possessing a (more) complete map of behavioral differences in the wild type strains is also of value.

What research has been done on baseline behavioral differences in inbred strains of mice?

Many research groups have already begun to address behavioral differences between these mice with inbred genotypes. For instance Holmes et al. (2002) compared three species, C57BL/6J, 129S6, and DBA/2J across a variety of behavioral tasks, including light/dark exploration, elevated plus maze, social transmission of food preference task, trace fear conditioning, open-field activity, Morris water maze, and Barnes maze. In the open-field activity chamber they found that C57s were more horizontally active than DBAs, which were more active than 129s. While C57s and DBAs produced the same levels of rearing, the 129s were less active than both. Finally, C57s spent much more time in the center of the chamber than did either other strain, which did not differ from one another. In the Morris water maze they discovered that for six dependent measures there were very few strain differences. The only exceptions were slight fluctuations between strains across days in the measures of thigmotaxia and latency to find the platform.

In another study, Wright et al. (2008) examined differences in performance in the Morris water maze by four strains of mice: two transgenic, one inbred, and one outbred (wild-type) strain. One of the transgenic strains was the p75 knockout mice which has a disturbed nerve growth factor (NGF)-binding region. Because p75 neurotrophin receptors

cause neuronal death, diminished activity of this receptor could potentially protect against cell death, resulting in improved cognitive processing. The other transgenic strain was the New Zealand Black (NZB) mouse, which has been used as a model of developmental learning disability, compared to developmental dyslexia (Sherman et al, 1985) and dementia in humans (Spencer et al, 1986). This is thought to be due to 40-60% of the animals presenting with neocortical ectopias (Boehm et al, 1996). Various studies have reported differential conclusions concerning any effect on performance in a variety of behavioral tasks (Balogh et al, 2000; Hyde et al, 2000; Wright et al, 2004). The inbred strain used was the C57BL/6 mouse, and the outbred strain was the Swiss Webster mouse. During the acquisition phase, mean latency to find the platform when it was hidden showed that over the course of six days the NZB and C57 strains were the quickest and did not differ from one another. The Swiss Webster strain showed decreased latency over days; however they were slower than the other strains. Interestingly the p75 mice never learned the task; thus, they showed no decrease in latency to platform throughout the task's six days. For distance swam to the platform, again NZB and C57 mice had the shortest distances, and across days these distances decreased slightly. The Swiss Webster mice swam longer distances than the first two strains, with very little decrease in length over time. The p75 transgenic strain swam the longest distance and showed no decrease across acquisition days. It is interesting to note that swim speed for each strain remained constant; there was no effect of trial day on this measure. Also, C57 mice swam significantly slower on each day than all other strains. Wright et al. (2004) determined that these data for the NZB strain were not outside expectations; however, the p75 data were surprising given the original hypothesis.

Apparently the NGF disturbance created some interference with performance on this spatial memory task.

What do behavioral tasks tell us?

Locomotor Activity Chamber

The locomotor activity chamber, sometimes referred to as open-field, exploratory, spontaneous locomotion, etc., is one of the oldest behavioral task, the most commonly used, and has been the simplest way to measure changes in emotional-like behavior in rodents (Crawley et al., 1997). A square field, historically it was open to an observer, but today it is automated and utilizes photocell or infrared beams to create a grid on the floor. Breaks in the beams constitute movement which is generally categorized as either horizontal ambulation or vertical rearing. Occasionally a researcher is interested in stereotyped behavior, which is a repetitive set of movements often seen with the administration of certain types of compounds. The area is often broken into different zones to discern whether a subject spends more time in a certain area than others. The most commonly noted example of this is thigmotaxia, where a subject spends the majority of the trial time nearer to the walls of the chamber versus the center zone. This is thought to express heightened anxiety or emotional reactivity.

Another useful dependent measure is fecal boli, or rate of defecation. In 1934, Calvin S. Hall wrote “Emotional Behavior in the Rat” in which he discussed various arguments supporting the use of defecation and urination as reliable measures of emotionality in rats. Increases in thigmotaxia, decreases in both vertical and horizontal movements, and increases in defecation and urination are signs of heightened anxiety-like in an open-field situation. A decrease in elimination over time is referred to as “emotional elimination” by Hall, as noted

by Bindra & Thompson (1952), and this phenomenon is differentiated from the random, normal processes of defecation and urination in rodents. In fact low activity and high defecation are genetically correlated, and this cannot be linked via physiological tract. While defecation is an autonomic process, activity is modulated via the somatic (voluntary) nervous system (Flint et al, 1995). Increasing light concentration in this task is another mechanism for increasing stress (Holmes et al, 2002).

Locomotor activity chamber tasks are often used in studies measuring anxiety-like behaviors. These constructs may map onto human predisposition for anxiety or neuroses (Flint et al, 1995). This task also has the power to provide information about the actual physical capacity of an organism, such as mobility, olfactory and visual acuity. These data can support disproving false-negatives and false-positives where an underlying locomotive issue is interfering with other tasks (Holmes et al, 1997).

To efficiently investigate strain comparisons across various dependent measures the MPD allows specific study data to be downloaded in various formats. Golani et al. (2003) compared eight strains of mice on 33 different measures from a 30-minute open field locomotor activity test. C57 mice had the highest activity rate, 129s and DBAs were lower but similar to one another, and Balb/c's had the lowest activity rate. When quantifying the proportion of time "spent away from the wall," again C57s spent the most time away from the wall, then 129s and DBAs spent less time away but were similar to one another, and then Balb/c's spent the most time near the wall. This data is in agreement with a review by Crawley et al. (1997) where they note that generally C57s exhibit low levels of anxiety-related measures in conjunction with high ambulation as compared to other strains. DBAs tend to perform moderately, and Balb/c's tend to perform poorly, indicating high levels of

reactivity. Please see Table 1 for a summary of the literature concerning strain differences in performance on this task.

Morris Water Maze (Spatial Learning: Non-Food Motivated)

The Morris water maze (Morris, 1981) is a measure of spatial learning and memory. Because rodents do not care for swimming, this task has the benefit of being a non-food motivated task, which allows the subjects to remain on free feeding as opposed to being kept at a certain percentage of their free feeding body weight. Generally the apparatus used in this model is a large circular tub filled partly with water. The water is often kept a little colder than room temperature in an effort to increase motivation to escape the water. The mice can do this by locating an “invisible” platform that is approximately 1 cm below the water. Current systems often employ video imaging connected to an automated computer system that tracks the subjects’ swim paths, latencies to platform, swim speeds, and thigmotaxia. Visual cues are placed around the tub to facilitate spatial recognition and learning. Often at the end of this task a cued trial will be instituted where the platform will be visually identifiable by some sort of marker or flag. This tests the subjects’ visual acuity to determine whether acquisition performance was hindered by poor eyesight. This particular task is particularly useful when addressing abnormalities in hippocampal or cortical functioning, as performance in this assay is disrupted.

Upchurch and Wehner (1988) were particularly interested in the poor eyesight commonly found in albino species and strains. Therefore they used the Morris water maze task to compare two albino strains, Balb/c and C3H/2, to test optical capabilities. They also tested C57BL/6I and DBA strains which are not known to have visual deficits. Trials with a visual cue as well as trials with a hidden platform were conducted in a between subjects

Table 1.

Locomotor Activity Literature Review Table.

<h1>Locomotor Activity</h1>			
<u>C57BL/6</u>	<u>DBA/2</u>	<u>BALB/2</u>	<u>129S2/Sv</u>
<ul style="list-style-type: none"> •Greatest horizontal ambulation^{3,8} •Least horizontal ambulation⁷ •Greatest rearing^{3,8} •Least thigmotaxia^{3,8} •Habituated to horizontal ambulation but not rearing³ •Longer distances wheel running⁵ 	<ul style="list-style-type: none"> •Moderate horizontal ambulation⁷ •Greatest rearing³ •Most thigmotaxia³ •Habituated to horizontal ambulation but not rearing³ 	<ul style="list-style-type: none"> •Greatest horizontal ambulation^{1,4,6,7} •Less distance wheel running⁵ 	<ul style="list-style-type: none"> •Least horizontal ambulation^{2,3,4,6,8} •Least rearing^{3,8} •Most thigmotaxia^{3,8} •Habituated to horizontal ambulation but not rearing³

¹Baron and Meltzer, 2002; ²Gerlai, 1998; ³Holmes et al., 2002; ⁴Isles et al., 2004; ⁵Johnson, Pesak, and Newland, 2009; ⁶McKerchar et al., 2005 (actometer); ⁷Nikulina, Skrinskaya & Popova, 1991; ⁸Voikar et al., 2001

design. For the cued procedure, neither of the albino strains was able to acquire the task, while both the C57BL/6 and DBA mice showed decreases in latency to find the platform over trials. As expected, the more difficult hidden platform task proved to also be only acquirable by the latter two strains, though the DBA strain did more poorly than they did for they did visible platform task. To further investigate the genetic predisposition to ocular deficits, the authors tested the acquisition of the visible platform task in littermate albino and pigmented mice from segregating generations. Though there was no difference in performance between the groups during the first six trials, the pigmented group did perform better on the last twelve trials than the albino mice. These data support the authors' conclusion that indeed the poor eyesight most likely contributed to the albino groups' poor performance in the task. Also, they found that over the course of all the trials with the invisible platform C57BL/6 mice did better than the DBA strain, but only in the last two blocks of six trials.

In an effort to compare multiple inbred strains to determine suitability for transgenic background strain breeding, Brooks et al. (2005) investigated various behavioral tasks, including the Morris water maze. They implemented a shortened version of the task in which 7-paired trials were run in a single day. They found low to moderate performance in 129, C57 and DBA strains on the latency to platform measure, and interestingly saw a significantly longer latency for the Balb/c's on the last two pairs of trials. With regard to distance traveled, all four strains exhibited decreased path length over the course of the day, though to a lesser degree for the DBAs. For the visible platform task the C57 strain had a much shorter mean time to platform than all other strains. The authors noted that during this last task the Balb/c's had significantly slower swim speeds than the DBAs.

In a study comparing many strains, but particularly C57BL/6J, DBA/2J, 129/SvJ, and Balb/cByJ (the main noted behavioral difference between this strain and the Balb/c used in the proposed research is “better reproductive performance and less aggression” in the Balb/cByJ; Jackson Laboratories, 2009), Owen et al. (1997) address differential performance in the Morris water maze. The procedure lasted for three days, and each subject experienced four blocks of three trials per day. All strains showed significant decreases in latency to escape over the course of the 36 trials, and the Balb/cByJ strain was one of two albino strains (out of six total albino strains) that actually showed significantly decreased latency. All strains also showed significant decreases in latency to platform when a visual cue was placed on it. This cued trial lasted for two days, where each day had twelve trials per day.

Please see Table 2 for a review of the literature from this task.

T-Maze (Spatial Learning: Food Motivated)

The T-maze is a very simple maze that has three arms and resembles a “T” shape. One of the arms, possibly longer than the other two but not always, is the ‘entry’ arm and is the starting point for the subject. The other two arms are the ‘choice’ arms and one is always baited with some palatable food reward, eg. sucrose or grain pellet, or fruit look cereal piece. Generally there is at least one gate at the starting point that is opened when the timing begins. There may also be gates on the choice arms to retain the subject once it has chosen an arm to enter. This model may be automated or be manipulated by an observer. There are many options with regard to procedure. One example is match-to-sample where the first trial has one choice arm baited and the subject must return to that specific arm to achieve subsequent food rewards. Another procedural example is a switch paradigm, or non-match-to-sample (or position). Here, after the first trial where a single arm is baited, the subject must choose the

Table 2.

Morris Water Maze Literature Review Table.

<h1>Morris Water Maze</h1>			
<u>C57BL/6</u>	<u>DBA/2</u>	<u>BALB/2</u>	<u>129S2/Sv</u>
<ul style="list-style-type: none"> • Fastest acquisition of hidden platform^{2,3,4,7} <ul style="list-style-type: none"> • Slowest acquisition of hidden platform^{5,6} 	<ul style="list-style-type: none"> • Fastest acquisition of hidden platform³ <ul style="list-style-type: none"> • Intermediate acquisition of hidden platform⁴ • Slowest acquisition of hidden platform⁶ • Fastest acquisition of visible platform⁴ 	<ul style="list-style-type: none"> • Slowest acquisition of hidden platform³ • Intermediate acquisition of hidden platform⁴ • Slowest acquisition of hidden & visible platform⁶ 	<ul style="list-style-type: none"> • Fastest acquisition of hidden platform^{3,4,5} • Intermediate acquisition of hidden platform²

¹Holmes et al., 2002; ²Nguyen et al., 2000; ³Owen et al., 1997; ⁴Schimanski & Nguyen, 2004; ⁵Voikar et al., 2001; ⁶Upchurch & Wehner, 1988

All studies noted latency reduction to the hidden platform location in all strains. All studies showed all tested strains acquired the location of the visible platform. Holmes et al. (2002) and Owen et al. (1997) observed that all strains showed reduced latency to the visible platform over trials. Holmes et al. (2002) noted no differences in swim speed, latency to hidden or latency to visible platforms between the strains tested (C57, DBA/2, and 129S). Nguyen et al. (2000) found no difference between strains (C57, DBA/2, and 129S) in latency to visible platform.

other arm in order to get the reinforcer. Every time the subject gets the reward the next trial has the opposite arm baited. If the subject fails to choose the correct arm the reinforcer remains in the originally baited arm until it either chooses correctly or fails the trial. At times a researcher will institute a reversal task where the subject must respond to the reward being in the arm opposite to what it had been trained to up to that point. Delays may also be put in place between the initial ‘forced’ trial and the subsequent ‘retention’ trials to study working memory (Baddeley & Hitch, 1974).

This task is generally considered a spatial learning and memory test, however it is used for other constructs. Some scientists utilize the T-maze to deduce tendencies toward routine rigidity or away from flexibility to change, which may inform inquires related to autism and related disorders, or to detect for learning deficiencies which may speak to mental retardation (Crawley, 2007). Common dependent measures are latency to choose an arm, latency to eat the reinforcer, accuracy of arm choice, time to re-learn a reversal task, and latency to acquire procedure.

In this lab the T-maze has been used with a delayed alternation procedure by Pehrson for his dissertation research (2007). He was investigated the effects of early post-natal PCP administration and time delays (3-100 seconds) on reference memory in both male and female C57BL/6 mice. No differences in acquisition between PCP-treated and –untreated mice, regardless of sex were observed. Once the delay response curve was conducted a discrepancy became apparent between drug and saline groups. Male PCP-treated subjects had lower accuracy scores as compared to saline-treated males. Also, there was a significantly lower accuracy at a 100-second delay as compared to the 3-second delay. There was no change in choice latency across treatment groups; however, there were reductions in

choice latency at the 30- and 100-second delay points. There was no effect of PCP treatment or delay on latency to eat the reinforcer.

In the female mice there was no effect of PCP treatment on accuracy throughout the delay response curve. Like the males, though, there was a significant decrease in accuracy from the 3-second delay to the 100-second delay. There was no effect of PCP or saline treatment on the females with regard to choice latencies; however again, as in the males, there was a significant decrease in latency to arm choice at 30- and 100-second delays. Finally, there was no effect of delay or treatment condition on latency to eat the reward.

Pehrson also administered a PCP challenge where subjects that met criteria during acquisition and delay portions of the task were administered 1.0, 3.0, or 10.0 mg/kg PCP fifteen minutes before being tested in the T-maze. In the males there was an effect of treatment group on accuracy in which the saline group performed better than the PCP group. The highest dose of PCP also reduced accuracy, increased arm choice latency, and latency to eat regardless of treatment group. In the female mice both PCP- and saline-treated groups had reduced accuracy at the 10.0 mg/kg PCP challenge dose. There was no effect on arm choice latency in the female mice regardless of PCP dose. Regardless of treatment group, latency to eat in the females at the 10.0 mg/kg PCP dose was increased, though.

Pehrson concluded that the only unexpected difference seen in these results was the sex discrepancy where the accuracy of female mice was not significantly negatively affected by the early postnatal PCP-administration, as it was in the male PCP-treated mice. Pehrson posited this was potentially a result in decreases in processes related to task performance, such as attention.

In a study comparing OF-1 Swiss outbred female mice, Guaryerbas et al. (2002) examined the relationship between the experience of emotional stress and immunosenescence, the aging of the immune system. First the mice were run through a T-maze for fifteen days and then divided into two groups. Those that ran the maze quickly (less than 20 seconds) were referred to as “fast mice,” while those that ran it slowly (greater than 20 seconds) were referred to as “slow mice.” These two groups were tested on a simple tightrope task to measure neuromuscular coordination and vigor. Fast mice performed better on the tightrope task than the slow mice, and they maintained better coordination throughout their lives than the slow mice. Survival analyses showed that fast mice lived significantly longer than slow mice, and the slow mice had less healthy immune systems than the fast mice. Finally, fast mice were significantly heavier than slow mice throughout their lives. The authors suggest that the connection between performance in the T-maze and the immunosenescence is a degradation of neurotransmission processes. What is of particular interest in this study is that the T-maze procedure was utilized as a mechanism for categorizing the subjects before the main dependent measures were recorded. Thus, this assay can serve a variety of purposes, not merely to measure spatial learning and memory, and cognition. Please see Table 3 for a review of the literature for this task.

Patil et al. (2009) note that a combination of mazes for research into spatial and non-spatial constructs is particularly important when using the MWM. The stress induced in this task due to the adverse affects of swimming in mice is such that it could interfere with the exact cognitive properties being targeted for experimentation. Adding in the T-maze allows for another angle, not only in reduction of physical stress, but also a different motivation

Table 3.

T- Maze Literature Review Table.

<h1>T-Maze</h1>		
<u>C57BL/6</u> <ul style="list-style-type: none">• Faster acquisition of task¹	<u>DBA/2</u> <ul style="list-style-type: none">• Slower acquisition of task¹	<u>129S2/Sv</u> <ul style="list-style-type: none">• Slower acquisition of task¹

¹Gerlai, 1998

Gerlai (1998) used a different procedure using the T-maze structure, referred to as T-Cat. This modification allows for minimal subject handling by manipulating movement with the doors versus manual handling. It is said to measure “curiosity.” There was no difference between the three strains in the time to reach 5 trials (locomotion).

(food-motivated versus non-food-motivated task) and different cues (internal, directional cues versus distal visual cues).

Autoshaping Operant Procedure

In behavioral research the interest in the autoshaping component of operant conditioning is most commonly thought to have started with B. F. Skinner and his colleagues during the middle of the 20th century. As operant conditioning became a foundation of behavioralism's interests, along with it came inquiries related to other conditioning arrangements, such as superstitious conditioning (Brown & Jenkins, 1968; Skinner, 1948). Autoshaping is a term used to describe the manner in which a research subject acquires an operant task, or the emergence of said behavior. The origin of autoshaping behavior research began primarily with pigeons (Brown & Jenkins, 1968), and there have also been studies using monkeys (Gamzu & Schwam, 1974; Sidman & Fletcher, 1968), rats (Atnip, 1977; Bankart et al., 1974; Boakes, Halliday, & Poli, 1975; Coveney & Sparber, 1981; Kearns & Weiss, 2007; Mundy & Iwamoto, 1986; Rodriguez et al, 2007), fish (Cole & Adamo, 2005), and mice (Baron & Meltzer, 2001; Goodrick, 1967; McKerchar et al., 2005a; O'Connell, 1980; Vanover & Barrett, 1998). Other studies have focused on the effects of lesions and various compounds on various species' autoshaping behavior (Mitchell, 1983; Ocos, Martinez, & McGaugh, 1988; Reilly, 1988; Steckler, Andrews, Marten, & Turner, 1993).

As noted above the term 'autoshaping' could apply in many experimental situations. Here the apparatus is a standard, two lever operant chamber and the size is appropriate to either rats or mice (or pigeons if that species is being used). Control of the procedure and data collection is completely automated, so this automatization minimizes intrusion by the researcher which makes this a more controlled research environment. Another strength to the

“instrumentation measure(s) relatively discrete behavior in a wholly objective and quantitative manner” (McKerchar et al., 2005).

Brown and Jenkins (1968) categorized various operant tasks by the type of procedure used:

In the usual arrangement for discriminative operant conditioning, reinforcement is conditional on a stimulus and on a response. Food may be delivered to a hungry pigeon only when it pecks a key and only when the key is lighted. By relaxing, in different ways, the conditionality in the rule for delivering food, three other condition arrangements of interest can be generated. The delivery of food may be entirely unconditional, *i.e.*, without regard to the stimulus that is present or to behavior; the delivery of food may be conditional on behavior (e.g. the pigeon must peck a key) but unconditional with respect to stimuli; or the delivery of food may be conditional on the stimulus (*e.g.*, food is delivered only when the key is lighted) but unconditional with respect to responses. (p. 1)

Their particular study found that the “forward pairing” condition, the one focused on both stimulus- and response-reinforcer dependency, and the “fixed duration” [or fixed interval (FI)], focused explicitly on a stimulus-reinforcer dependency [and perhaps implicitly (see Atnip, 1977)], both produced key pecking in the pigeons and were not different in the number of trials to first responses.

Using this paradigm, Atnip (1977) compared five different procedures on how they influenced acquisition of lever pressing in rats. All conditions had a variable interval (VI) of 40 seconds (”) for ten days with 50 trials per day. In the autoshaping condition (stimulus- and response-reinforcer contingencies) if there was no lever press in 10” then a reinforcer was delivered, and if there was a lever press a reinforcer was delivered. In the operant

condition (response-reinforcer) the parameters were the same as above, however if there was no lever press no reinforcer was presented. In the classical conditioning component (explicit stimulus-reinforcer contingency), lever pressing produced no consequence; a reinforcer was delivered every 10" following the extension of the lever. The omission condition was the same as the classical; however, a lever press resulted in lever withdrawal and no reinforcer for that trial. Finally a random control group experienced the "probability of food delivery was equal in the presence or absence of the lever." Lever retraction occurred when the lever was pressed as an additional control. During a second phase of the study the first three conditions were replaced with the omission condition, while the last two remained the same. This occurred for ten days, 50 trials/day, with a VI 40". Atnip (1977) found that autoshaping, operant and classical conditioning procedures resulted in the highest levels of acquisition in Phase 1. Observing individual variability, Atnip noted that autoshaping was the most consistent, and that operant and classical conditioning procedures were less consistent but similar to one another. The omission group had more variability and lower mean acquisition than the first four conditions, and the control group did the worst, producing little responding across the study. Once moved to omission training the autoshaping, operant and classical conditions all had similar drastic reductions in responding. As expected, there was no change in the final two groups, omission and control. The results in the first phase indicated to Atnip that the combination of both response- and stimulus-reinforcer contingencies most greatly supported acquisition of lever pressing as compared to either one alone. Phase 2 results indicated that there was also a particular sensitivity to the response- and reinforcer-contingencies as all the first three groups experienced major

declines in responding, however none dropped to control levels. They all dropped to no less than the omission group level.

Within autoshaping it is also interesting to consider species-specific effects. This is the concept that the specific physiology and anatomy of a certain organism can predispose it to certain types of physical movements. In this case the movement would be the operant behavior, like pecking a key by pigeons or lever pressing or nose poking by rodents. This is why operant behavior is studied in such vastly different environments depending on the subjects' species. The topography of a pigeon's consummatory response to a reinforcer influences the way in which it will respond to a key press (e.g. either as though pecking grain or drinking water) regardless of type of deprivation imposed (Jenkins & Moore, 1973). Gamzu & Schwam (1974) became interested in the transition across species of simple operant behavior when major topographical changes were required, for instance instead of key pecking by a pigeon, how would a squirrel monkey fare? Their conclusion was that due to topographical dissimilarity, key pressing in monkeys is not nearly as salient an association as it is for a pigeon. The stimulus-reinforcer contingency could be much more easily disrupted by variable conditions (omission) than it could in pigeons. This concept was refuted by Atnip (1974) when he found that after changing to an omission condition rats show no relationship between lever contact and actual lever pressing.

The majority of studies investigating autoshaping or operant behavior in mice use the nose poke as the dependent measure (Baron & Meltzer, 2001; Johnson et al., 2009; Vanover & Barrett, 1998), whereas most studies that use rats use the lever press (Atnip, 1977; Bankart et al., 1974; Coveney & Sparber, 1981; Kearns & Weiss, 2007; Mundy & Iwamoto, 1986; Rodriguez et al, 2007). One study focused on the impact of previous behavioral history on

subsequent conditioning procedures was conducted with mice and lever pressing. They found that the serotonin 1B knockout mouse acquired the autoshaping paradigm faster than a wild type strain; however there was no difference in the acquisition of a differential-reinforcement-of-low-rate model (Pattij et al., 2004). Another study by McKerchar et al. (2005a) focused on the correlation between locomotor activity and lever-pressing in a one- and two-lever paradigm. A positive correlation was found between activity and either lever-pressing model in the inbred strains, C57BL/6, DBA/2, 129X1/SvJ, and BALB/c. An interesting study was conducted by Goodrick (1967) in which three inbred strains of mice were trained to bar-press only under a light contingency with no reward. C57BL/6 female mice responded with the highest rates. Continuing to combine mice with a lever press autoshaping procedure allows for the extension and replication of these previous studies, as well as broadens the generality of specifically the autoshaping task to a different operant response.

Please see Table 4 for a review of the literature regarding this task.

Current Research Overview

Taken as a whole, the current body of literature using such tasks indicates that indeed there exist significant behavioral differences between inbred strains of mice. Variability in gross behavior, physical capabilities, as well as cognitive processes such as learning and memory, greatly affect the results of such research. More importantly, ignorance as to the baseline differences in isogenic mice can at the least muddy interpretations of such results. With the exponential advancement of technologies and scientific capabilities there are ever-increasingly intricate and complex experiments taking place the world over. Before they can be properly understood and used to inform inquiries into improving human health outcomes,

Table 4.

Autoshaping Literature Review Table.

<h1>Operant Tasks</h1>			
<u>C57BL/6</u>	<u>DBA/2</u>	<u>BALB/2</u>	<u>129S2/Sv</u>
<ul style="list-style-type: none"> •Increased one-lever pressing from Day 1 → 2⁵ •100% to 50 nose pokes¹ •Highest nose poke rate¹ •Fastest to 50 correct responses⁴ •Fails to extinguish⁶ or extinguishes well³ •Exhibits spontaneous recovery⁶ •Performs intermediately in a leverpress escape/avoidance task² 	<ul style="list-style-type: none"> •Increased one-lever pressing from Day 1 → 2⁵ •75% to 50 nose pokes¹ •Intermediate nose poke rate¹ •Extinguishes well⁶ or poorly³ •Fails to exhibit spontaneous recovery⁶ •Performs best in a leverpress escape/avoidance task² 	<ul style="list-style-type: none"> •Increased one-lever pressing from Day 1 → 2⁵ •91% to 50 nose pokes¹ •Intermediate nose poke rate¹ •Fastest nose poke rate⁴ •Performs intermediately in a leverpress escape/avoidance task² 	<ul style="list-style-type: none"> •Did not increase one-lever pressing from Day 1 → 2⁵ •53% to 50 nose pokes¹ •Lowest nose poke rate¹ •Slowest to 50 correct responses¹ •Performs worst in a leverpress escape/avoidance task²

¹Baron & Meltzer, 2002; ²Brennan 2004; ³Ingram 1982; ⁴Johnson, Pesek, & Newland, 2009; ⁵McKerchar et al., 2005; ⁶Waddell et al., 2004

the research community must first expose the impact of baseline differences caused via genetic manipulation.

Task Summary

Models such as those discussed highlight important behavioral phenotypes that will allow for further elucidation of the genetic basis of behavior. Physical capabilities or deficits therein, are investigated, such as mobility in the locomotor activity chamber and the Morris water maze. Visual acuity is addressed in the Morris water maze and with the signal light portion of the autoshaping procedure. Spatial learning and memory are measured in the

Morris water maze and the T-maze. Motivation from several arenas (e.g. food-based versus non-food based) is addressed with the T-maze, the autoshaping task, and the Morris water maze, respectively. Cognitive processes are studied in the T-maze and autoshaping assays. Clearly the various types of data gathered throughout this study will greatly contribute to the overall body of knowledge about baseline behavioral strain differences in inbred mouse strains.

What is unique about these four specific mouse strains?

C57BL/6

The C57BL/6 strain was developed in 1921 (Harlan, 2010). It is most commonly used for breeding transgenic and knockout mice, and as the background strain for spontaneous mutations (Crawley et al., 1997; Harlan, 2010). The C57BL/6 strain is an “alcohol-preferring” strain, as it readily consumes 10% ethanol solutions (Phillips & Crabbe, 1991). As expected this strain has been used in alcohol preference research, as well as maternal alcohol abuse and other alcohol related diseases studies. Many drugs of abuse (i.e. methamphetamine, LSD, cocaine) and controlled substances (i.e. nicotine, morphine) have been tested in these mice (Harlan, 2010). It is particularly of interest that for the genome of the C57BL/6 strain, the human genome has 99% homologues (Waterson et al., 2002). This is a principle reason why it has been one of the most commonly used strains in translational research.

In open-field locomotor activity and elevated plus maze tests C57BL/6 mice tend to be more active than the 129S2/Sv, DBA/2, and Balb/c strains (Brown et al., 2004a; Golani et al., 2003; Richfield et al., 2003; Wahlston & Crabbe, 2003). Of the four strains, this one has the least fecal boli count (Brown et al., 2003) and it has the lowest thigmotaxia (Wahlston &

Crabbe, 2003) in locomotor activity task. The C57BL/6 shows moderate acquisition and reversal training in Morris water maze, which is better than the 129S2/Sv strain (Brown et al., 2003)

129S2/Sv

The 129S2/Sv strain was developed at Columbia University in 1928. While there are a variety of substrains of the 129, this particular one is derived from a congenic strain made by outcrossing the *steel* mutation (Harlan, 2010). This strain provides embryonic stem cells for creating knockout mice (Crawley et al., 1997). The most common research application outside of transgenesis, is testicular teratomas.

129S2/Sv mice show impairment on many standard behavioral learning tasks, including being categorized as “poor learners” in the Morris water maze (Crawley et al., 1997). Brown et al. (2003b) report this strain as having the slowest acquisition of the Morris water maze task, as well as the poorest reversal training performance (Brown et al., 2003b). These mice are the least active in the open field locomotor activity task (Brown et al., 2004a; Richfield et al., 2003; Wahlston & Crabbe, 2003), and show a moderate fecal boli count as compared to the other three strains (Brown et al., 2003). Finally, 129S2/Sv mice demonstrate moderate thigmotaxia, less than the Balb/c strain (Wahlston & Crabbe, 2003).

DBA/2

In 1909 the parent strain of the DBA/2 was developed, and between 1929 and 1930 crosses of the sublines produced the DBA/1 and DBA/2 strains. Common research applications are coat color, behavior, audiogenic seizures, epilepsy, calcification, metabolism, foetal absorption, immunology and infectious diseases. This strain expresses

low ethanol preference (Harlan, 2010). These mice are also less sensitive to the disruptive effects on operant behavior of cocaine than C57BL/6J and Balb/cByJ (Heyser et al., 1997).

DBA/2 mice performed moderately on the open field locomotor activity task; they were not as active as the C57BL/6 strain, but were more active than either the 129S2/Sv or Balb/c strains (Brown et al., 2003a). They had the highest thigmotaxia of all four strains (Wahlston & Crabbe, 2003), but moderate fecal boli count for the locomotor activity task (Brown et al., 2003). The DBA/2 strain acquired the Morris water maze faster than C57BL/6, 129S2/Sv, and Balb/c strains, as well as showing the best performance during the reversal procedure (Brown et al., 2004b).

Balb/c

The term “BALB” is derived from “Bagg albino.” H. Bagg had the albino stock from whence this strain was developed in 1913. This particular strain was inbred in 1923. While this strain shows low open field activity, they show high levels of spontaneous locomotor activity. High defecation and low alcohol preference are also characteristics of the Balb/c strain (Harlan, 2010). They tend to be more anxious (“spontaneously elevated anxiety”) than several other inbred strains (Belzung & Griebel, 2001).

In the locomotor activity chamber they generally have the highest fecal boli count of all four strains (Brown et al., 2003a). Golani et al. (2003) found the Balb/c strain to be the least active in the locomotor activity task, while Brown et al. (2004) found the 129S2/Sv strain to be the least active in this assay, with the Balb/c being the next least active.

Interestingly, Wahlston & Crabbe (2003) found the Balb/c strain to be nearly as active as the C57BL/6 strain in a five minute locomotor activity trial. Moderate thigmotaxia, second only to DBA strain, was expressed by Balb/c mice (Wahlston & Crabbe, 2003). Finally, they

showed the fastest acquisition of and reversal training in the Morris water maze (Brown et al., 2003b).

Rationale

The focus of the present study is to compile a useful selection of tasks the behavioral community can use to inform certain transitions from behavior to practical implications of disease and disorder in humans. The locomotor activity chamber and Morris water maze are standard tasks in behavioral research. The first offers a great deal of basic motility information, while also providing descriptions of baseline emotional reactivity within and across strains. The second also speaks to physical capabilities, but more so to spatial learning and memory. The T-maze and the autoshaping task have not been used often in mouse strain comparison studies, therefore data drawn from them will be particularly interesting as it is compared with what has been learned in other laboratories. The T-maze is another spatial learning task, however memory performance may also be considered. Finally, the autoshaping procedure can speak to the speed and agility of cognitive processes in these four strains. Researchers in behavioral psychology/neuroscience have long depended on these tasks and agree that they are useful tools due to their long-standing validity and reliability.

The choice of these four particular isogenic strains of mice is a practical one. These strains are currently some of the most commonly used in behavioral, physiological, and molecular studies. These strains have been maintained in several laboratories for around one hundred years now, thus their genomes are particularly well mapped. Additionally many studies have been conducted using them with a resultant large quantity of data which describes particular characteristics.

Overall the greatest impetus for the design of this study is an effort to add resources to the ever-growing database concerning inbred mouse strain differences via behavioral phenotyping. Contributing to community knowledge in a way that strengthens the foundation for future research is of the utmost importance. Perhaps in the future, a reliable, reasonably complete mass of resources on variations in strain behavior will be available for a variety of research inquiries. In that time a scientist will be able to access the data he or she needs in order to properly organize their own study. Because the scientist will not have to worry about these broader and potentially very damaging influences of such ignorance on their results, the scientist will be capable of answering the inevitable advanced yet minute questions that are just now growing into fruition.

As stated in Holmes et al. (2002), “To facilitate the use of novel behavioral paradigms to phenotype transgenic and gene knockout mice, it is important that performance of inbred strains in these tests is carefully characterized”. In particular such a resource will provide invaluable advice used to guide decisions about which genetic backgrounds will be most appropriate for studying various knockout and transgenic mutants (Crawley et al., 1997).

In the present work common behavioral assays (i.e. locomotor activity, Morris Water Maze, and t-maze) are combined with operant models (i.e. autoshaping of level-pressing for food reward) to provide a more comprehensive characterization of the potential differences and/or similarities between these species’ behavioral phenotypes.

Hypotheses

For the first and final task, the Locomotor Activity assay, based on a broad literature review (see Table 1; Baron and Meltzer, 2002; Gerlai, 1998; Holmes et al., 2002; Isles et al., 2004; Johnson, Pesak, and Newland, 2009; McKerchar et al., 2005; Nikulina, Skrinskaya &

Popova, 1991; Voikar et al., 2001), it is expected that the Balb/C and C57 mouse strains will exhibit the highest levels of locomotive activity, with the DBA mice showing slightly less activity and the 129S strain exhibiting the least activity.

The second task is the Morris Water Maze, which also had a high number of previous studies to review (see Table 2; Holmes et al., 2002; Nguyen et al., 2000; Owen et al., 1997; Schimanski & Nguyen, 2004; Voikar et al., 2001; Upchurch & Wehner, 1988). Here it is hypothesized that the C57, DBA, and 129S mouse strains will find the location of the hidden platform and the visible platform faster than the Balb/C strain. This is thought to be due to the decreased visual acuity noted in some albino rodent species (Heiduschka & Schraermeyer, 2008; Upchurch & Wehner, 1988).

In the T-maze, the third task in this study, we expect that the C57 mice will exhibit faster acquisition of the serial reversal procedure than both the DBA and 129S mice, which are not expected to perform significantly differently from one another (see Table 3; Gerlai, 1998). There are no previous data for this procedure with the Balb/C strain so no hypothesis about their performance is being stated.

Finally, the last task will be the Autoshaping and Extinction operant procedure. The umbrella hypothesis is based on a variety of operant procedures (see Table 4; Baron & Meltzer, 2002; Brennan 2004; Ingram 1982; Johnson, Pesek, & Newland, 2009; McKerchar et al., 2005; Waddell et al., 2004) which can be considered to be related to components of this particular assay. It is important to remember though that none of these studies are exact replicates of this study's methodology. Though the data are largely variable across these tasks it is expected that 129S mice will perform the most poorly in leverpress acquisition

behavior and rate of extinction of the behavior as they have typically performed more poorly in a variety of operant tasks as compared to other mouse strains.

Method

Animals

Four strains of mice obtained from Harlan Laboratories, Inc., North America, were used, including C57BL/6NHsd (C57), DBA/2NHsd (DBA), 129S2/SvHsd (129), and Balb/cAnHsd (Balb/c). Thirteen mice of each strain were obtained at the age range of five to six weeks old, totaling fifty-two mice. Weights, as expected, varied with strain, with the C57 and the 129 subjects being the heavier strains, the DBA and the Balb/c strains being lighter. Each subject was individually housed in a temperature and humidity-controlled vivarium on a 12 hr light/dark (0600/1800) cycle. Water was available ad libitum in the home cage and free feeding was allowed for the locomotor activity and the Morris water maze tasks. The last two tasks required food restriction due to the food motivation required by these protocols. For the T-maze body weights were maintained at $85\% \pm 1$ gram of free-feeding weight. The autoshaping task also incorporated food restriction; however, this was managed by providing a certain weight of daily food (2.5g) as per the literature (Barrett & Vanover, 2003; Vanover & Barrett, 1998) unless weights fell below 85% of free feeding weight, at which point more food was provided. All research and procedures were in accordance with the standards set by the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Research Council, 2003), and were approved by the Institutional Care and Use Committee of Virginia Commonwealth University.

General Methods

After the mice were delivered, they were handled and weighed daily for five days in the vivarium to habituate them to laboratory handlers. They were kept entirely in the vivarium at this time due to the first task (locomotor activity) being housed within the vivarium itself. Because of the large number of subjects, the time required to test the mice was quite lengthy and would extend beyond the available time in a single day. Therefore, each subject was randomly assigned to one of two ‘cohorts,’ to be referred henceforth as “Cohort 1” and “Cohort 2,” with an equal number of mice from each strain in each cohort.

Task 1: Initial Locomotor Activity

Apparatus. The apparatus used in the initial locomotor activity assay was four standard open-field rodent locomotor activity chambers (ENV-515, Med Associates, Inc, St. Albans, VT). The activity chambers are made of four clear walls 30.5 cm in height, with a solid white floor, measuring 43.2 X 43.2 cm. While there is no ceiling to the chamber, it is housed inside a larger solid white sound-attenuating chamber (ENV-017M, Med Associates, Inc) which utilizes a noise-masking fan. This apparatus and its software (Activity Monitor 5.0, Med Associates, Inc) monitors locomotion by Infrared (IR) beam breaks. Three arrays of 16 IR beams track horizontal and vertical movements of each mouse, and are placed 2.5 and 5 cm above the floor.

The Med-Associates software allows a real-time onscreen tracking of the subject moving by showing a moving dot and the remaining trail. Screenshots of activity during test sessions were obtained to visually display any strain differences. The software has an option to divide the test chamber into two preset zones. The zones that were used in the present task

were: a center zone measuring 132.25 square inches and the outer zone which measures the remaining 123.75 square inches.

Dependent Measures. The measurements for this task were chosen based off of an extensive literature review for this task (Crawley et al, 1997; Denenberg, 1969; Hall, 1934, 1936; Holmes et al, 2002; Walsh & Cummins, 1976; among others) Those recorded were horizontal ambulation, vertical movements (rearing), thigmotaxia (the tendency of a subject to move more in the outer zone than the open center zone, expressed as a ratio of beam breaks in the outer zone divided by total beam breaks counted,) and fecal boli count, as well as daily horizontal beam breaks “binned” into six 10-minute blocks.

Procedure. Each subject was in the activity chamber for 60-minute sessions each day for three consecutive days. Thus, Cohort 1 ran on days 1-3, and Cohort 2 ran on days 4-6. Each ‘group,’ or the four subjects to be running during the same session, consisted of one mouse from each strain, and their particular test chamber was the same for all three days. Test chambers were randomly assigned across strains. After each test session fecal boli were counted and the inside of the chamber, including the floor and walls, was wiped down with paper towels and a weak alcohol solution. The chamber was allowed to dry thoroughly before the next group session was started.

Task 2: Spatial Learning – Morris Water Maze

Apparatus. The Morris water maze was housed in a separate room with a white curtain separating the pool from the monitoring computer and observer. The pool is a large, circular, galvanized steel tank, measuring 180 cm diameter and 50 cm in height. It was filled to approximately half full with water kept 20-22 degrees centigrade by a large aquarium tank water heater when necessary. The water was colored with white tempera paint to make it

completely opaque. A small circular platform measuring 11cm in diameter and colored white was placed in the water in varying positions in the pool. It was located approximately a centimeter below the top of the water so that it could not be seen by the subjects as they swam. Inside the pool, on the wall above the water line there were placed four distal visual cues: white, laminated papers with unique black and white geometric shapes (e.g. peace sign). A video camera was placed in the ceiling directly over the pool, and its images were recorded by tracking system hardware and software (0121-002M and 0120-252M respectively, Videomex-One, Columbus Instruments, Columbus, OH). This software divides the pool into four quadrants (North, South, East, West,) and each visual cue was placed in the center of each quadrant's wall area. There were also large black and white geometric shapes on the walls of the room above the top of the pool (ex. stripes). These designs are meant to act as spatial cues for the subjects. At the end of each day of running a small amount of bleach was stirred into the water to retain cleanliness. The pool was drained, scrubbed and fresh water and paint added every seven days.

Dependent Measures. The measures recorded were trial endpoint: whether the subject located the platform (hidden or visible) or timed out. This allowed for assessment of learning over time by counting the number of trials per strain per day to reach the platform. Latency to the platform in seconds and total path length measured in centimeters were recorded and also were used in order to calculate swim speed.

Procedure. The subjects remained in their respective cohorts for testing in the water maze. As in the previous task the cohorts were tested consecutively. In the first cohort a total randomization was attempted and the subjects ran in groups of four, one subject per species per group. Originally it was noted that while the sensitivity of the image can be

manipulated manually, historically there have been issues with the back-masking option that causes too much interference for the program to work accurately. It was anticipated that we would have mark the upper back of the neck and back of the Balb/c's (which are white) with a dark grease pencil so that they will show up in the image and be able to be tracked. Indeed this was required. All of the other strains should have had sufficient contrast for the system to track them, though different swimming geometry (more or less of the back above the water) caused issues in tracking as well. These sorts of equipment failure caused a change of protocol for the second cohort wherein the groups were made entirely of a single species. This was implemented due to issues with back-masking and pixilation of the video camera and software. The variation in size and color of the mouse strains required hand adjustment of the hardware between strains, as well as often between subjects.

Due to fatigue and body temperature issues, after each trial each subject was hand dried with a paper towel and returned to their homecage which was warmed with a heat lamp. At the time each subject's turn to swim arrived again, they were warm, rested and dry. Each subject experienced eight total swim days, seven of which were actual trials. The first day was a habituation to the pool, where each mouse had a free swim trial for up to two minutes – less if they appeared to be experiencing fatigue. There was no platform in the pool on this day. In order to keep close watch an observer hid behind the curtain with only their face showing. All other swim trials began with the mouse being released into the pool and the observer completely closing the curtain to avoid distraction.

On days two through seven, each subject had four trials per day, each lasting up to two minutes. The trial ended either at that time or before if the subject founds the platform and climbed onto it. The platform was located in a static, randomly chosen location

everyday per cohort – that is all mice in Cohort 1 experienced the platform in one single location throughout their swims, while Cohort 2 experienced it in another location. While the platform location remained the same for each mouse, the starting location for each trial varied randomly between the other three quadrants from the one the platform is in. Each subject was placed in the water facing the wall of the tank. If the mouse found the platform it was allowed to sit there for 20 seconds, but if it failed to find the platform it was removed from the water by the observer and placed on the platform where it sat for 20 seconds.

On the eighth and final day a visual cue session was run. The platform was moved to a new, random location and a black film canister was placed on the platform to act as the visual cue of the platform's location. This provided information on any strain differences with regard to visual acuity. The assumption being if the subject can see the location of the platform as indicated by the cue then it will immediately go to the platform, therefore having a shorter path length and latency to platform. Instead of four total trials, only three trials were conducted.

Task 3: Spatial Learning – T-maze

Apparatus. The T-maze apparatus used for this task is constructed of acrylic painted black. Each arm of the 'T' is 35.6 cm in length and 7.6 cm wide. The walls are 15.2 cm tall, and each arm has a gate in it that is manipulated by the observer. The entry arm gate sits 10.2 cm from the bottom of the 'T' and the gates that close off the other two arms sit 29.2 cm from the end of each arm, leaving a 7 cm² choice point. At the end of each of the arms there is a small trough into which the sucrose pellet (45mg pellets, BioServ) reward is placed. After each subject was tested the interior of the maze was cleaned with a weak ethanol solution. It was dried before the next subject was placed in it.

Dependent Measures. For this task measures were recorded by the observer. Percentage accuracy for the entire session (correct arm choices divided by total trials where a correct choice could be made), and percent time-out endpoint (number of trials ending in time out divided by total trials where a time out could occur) were calculated.

Procedure. After body weights were stabilized at 85% of free feeding weight, subjects began with two days of habituation to the pellets by having approximately 20 sucrose pellets placed into their homecages. Next, a five day habituation to the maze began. Here each subject was allowed to freely roam the maze for a period of five minutes per day. Food pellets were placed in the feeding troughs of each arm to encourage exploration.

Training then ensued. For fifteen days subjects had eight congruent trials in the maze per day. Each trial began with the mouse being placed into the entry arm with the gate closed. The randomly chosen destination arm (left or right) was baited with a single pellet and the other arm was blocked by a gate. The observer started the stopwatch and simultaneously lifted the gate. The subject exited the start box and proceeded to the open destination arm, hence reference to this first trial as the “forced trial.” Upon entry into the arm (defined as the rear legs crossing the arm threshold,) the observer noted the arm-entry time and slid the destination arm gate closed, keeping the subject from leaving the arm. In this task the first trial is ‘forced’, that is an arm is randomly chosen to be baited. In all subsequent trials for that session (trials two through eight) only the arm opposite to the arm baited in the forced trial was baited. The intertrial interval (ITI) was approximately five seconds, the amount of time it took to remove the subject, place the entry arm gate back in place, remove the destination arm gate, and record dependent measures.

If a subject failed to enter an arm in 60 seconds the trial timed out. Also, if the mouse entered the arm and failed to eat the pellet within 60 seconds the trial time out. If either of these situations occurred during the first trial, the subject was returned to its cage until the other mice in its strain have been tested, and then the first trial was attempted once more. If the mouse again failed to enter an arm or eat the pellet it failed that day's entire session. Likewise, if a subject failed three other trials consecutively, no more trials were conducted and it failed the session.

Task 4: Autoshaping

Apparatus. Five standard, mouse-sized two-lever operant test chambers (ENV-307A, Med Associates, Inc) were be used for this portion of the study. Two retractable levers are 8 cm apart on the front wall of the chamber and a signal light is above each of the levers. The levers extended 0.8 cm into the chamber and are positioned 2.5 cm above a grid floor constructed of parallel stainless steel rods. Centered between them is a recessed food trough into which a liquid dipper delivers 0.02 ml of sweetened-milk (by volume: 150 ml powdered milk, 150 ml sugar, and 500 ml water). The inner test chamber consists of a 15 cm L X 11.5 cm D X 17.5 cm H area surrounded by an aluminum framed box with a single Plexiglas door. On the rear wall of the chamber near the ceiling is the house light. An audio stimulus device (ENV-323HAM, Sonalert Module, Med Associates, Inc) is installed in the box, and provided a 70 db tone. Test chambers are housed in sound attenuating chambers equipped with ventilation fans. Med-PC software (Version 1.17, Med Associates, Inc) was used to control the operant sessions and record data.

Dependent Measures. Measures that were recorded included total lever presses, as well as contingent responses (those responses correctly made during the presentation of the paired cues: the auditory cue and the signal light over the available lever).

Procedure. The cohort breakdown remained in effect for this task. The subjects' food was removed 24 hours before they were scheduled to be tested. The test session was two hours and testing lasted seven days. Upon completion of each test session, when another session was scheduled for the following day, each subject received a 2.5 gram pellet of food, unless their weight had fallen below their 85% of free feeding weight, then it was increased appropriately. When subjects completed the autoshaping task they were returned to free-feeding status.

Specific methodology for this task closely followed that of Vanover & Barrett (1998), Barrett & Vanover (2003), and a study done by Walker & Foley (2010). On day one the session consisted of acquisition of the task, where they experienced a Pavlovian/Instrumental condition. After the subject was placed in the box, (subjects were in the same operant chamber for all test sessions,) the program started with the houselight and the sound-attenuating fan being turned on. A variable interval (VI) schedule of 45 seconds began with a range of 4-132 seconds. A 'trial' was defined as the time between the beginning of a time interval and the beginning of the subsequent time interval. A pairing of the tone and the signal lights above a single lever that had been extended into the box occurred. At this time either the mouse pressed the lever or six seconds passed, either of which produced presentation of the dipper with the reward, as well as a cessation of the signal light and tone. The dipper remained up for four seconds, and then lowered. At this point the next trial began.

After the acquisition session the following four days consisted of ‘retention’ sessions that included only the Instrumental contingency. During these four sessions reinforcer delivery was dependent solely on lever pressing that occurred under the 6-second contingent cued presentation (i.e. an instrumental contingency). The final component was an ‘extinction’ procedure that was identical to the Instrumental sessions except no responses were reinforced – i.e. no rewards were presented regardless of lever pressing behavior. The VI 45” schedule remained in effect for the presentations/cessations of the tone-signal light pairing.

Task 5: Final Locomotor Activity

Procedure. In an effort to identify changes in baseline locomotor activity that may be related to the other behavioral tasks, and/or the potential impact of aging, locomotor activity was also recorded at the conclusion of the study. Subjects were returned to free feeding until their weights stabilized, which took approximately three days. Identical to the initial testing of locomotor activity, the subjects were tested in the activity chamber for 60-minute sessions each day for three consecutive days. Each ‘group’ or the four subjects to be running during the same session, consisted of one subject per strain, and their particular test chamber was the same one all three days. Assignment to activity chamber was again randomized across strains.

The measurements recorded were the same as before: horizontal ambulation, rearing, thigmotaxia, and fecal boli count. Data were also still collected in 10-minute bins.

Data Analysis

As is noted in the figures, the sample sizes for each of the strains dropped from the start of this study to its conclusion. This attrition was due primarily to a variety of unknown

illnesses in the animal colony during the course of the study. There were other cases of data lost due to equipment failure, as is discussed in the results section of the MWM. Finally, some of the data not included for analysis was due to unusable data when the mice failed to learn the task; hence the uselessness of data that was dependent on such learning.

Two-factor, split plot [one between subjects factor (strain) and one within subjects factor (trials or days, as appropriate)] analyses of variance (ANOVA) were used to analyze dependent measures from all the locomotor activity, MWM, T-maze, and autoshaping tasks. The only exception was for the visible trials in the MWM and the Pavlovian/Instrumental component (Day 1) of the autoshaping task. Here a one-way repeated measures ANOVA was used to compare strains. Neuman-Keuls post hoc tests were used where appropriate to analyze significant main or interaction effects on these dependent measures. This particular post-hoc test was chosen due to the moderate level of conservativeness (Bruning & Kintz, 1987). The level of significance was set at $p = 0.05$ for all analyses.

It should be noted that not all of the interaction effects found in all the locomotor activity data were followed with post-hoc Newman-Keuls tests. After statistical consultation (Robert J. Hamm, personal communication, April 2010) the decision was reached to not explore the interactions between strain and days that were orthogonal with post-hoc tests. Thus, only the non-orthogonal interactions between strain and days were explored with Newman-Keuls post-hoc tests.

Power analyses were not conducted for the sample sizes in the present study. While literature review-based hypotheses were considered regarding expectations, this is primarily an exploratory set of studies. Hence sample sizes chosen herein were based on an overview of similar studies found in the MPD (See Tables 1-4; MPD, <http://www.jax.org/phenome>).

The sample sizes used in these four tasks could however be used to calculate non-exploratory sample sizes in future studies.

Results

All statistical results, including presence of main and interaction effects, type of analyses and post hoc tests conducted, observed F-values, degrees of freedom, p-values, effect sizes (Cohen's *d*), and strain *n*'s, are shown in Table 5 and Table 6.

Locomotor Activity

During the first locomotor activity assessment a significant strain difference was seen across the three days where the 129S (1147.00 ± 802.37) mice produced significantly fewer beam breaks than all other strains (DBA= 3390.03 ± 1425.42 , Balb/C= 3796.72 ± 1429.58 , C57= 4423.58 ± 1435.98), who did not differ from one another, see Figure 1, graph A. In the final locomotor activity, Figure 1, Graph B, the same trend is shown, where 129S mice (1828.39 ± 585.49) showed significantly less horizontal ambulation than the three other strains (DBA= 4019.14 ± 1344.32 , Balb/C= 4674.92 ± 1751.20 , C57= 6036.48 ± 1911.36), who did not differ from one another. In the initial task there was also a day effect where more beam breaks were observed on Day 1 (3539.80 ± 1876.19) as compared to Days 2 (3067.10 ± 1752.74) and 3 (2961.10 ± 1743.10), but Days 2 and 3 did not differ from one another, while in the final task here was also a day effect where Day 1 (4688.29 ± 2250.28) had significantly greater beam breaks than both Day 2 (3994.79 ± 1950.36) and Day 3 (3824.33 ± 2225.91), but there was no significant difference between Day 2 and Day 3. No interaction between strain and day was observed in either the first or last activity tasks.

Rearing data in the initial activity task revealed significant differences between all groups where C57 mice (2255.22 ± 378.96) reared more than Balb/c mice (1127.14 ± 455.24),

who reared more than DBA mice (829.14 ± 328.26), while the 129S strain (191.92 ± 245.30) produced the least rearing behavior; refer to Figure 1, graph C. Graph D shows that in the final data slight changes were seen where again, the 129S (515.78 ± 353.69) strain showed significantly less rearing than the other strains (DBA= 1184.17 ± 153.68 , Balb/C= 1538.57 ± 234.61 , C57= 1607.57 ± 543.51), but the other strains failed to differ from one another. In neither task was there an effect of day or an interaction between the strain and day.

Figure 2, graphs A and B, show thigmotaxia data (percent horizontal beam breaks in the outer zone near the walls of the chamber) divided by total horizontal beam breaks. C57 mice (initial: 0.54 ± 0.11 , final: 0.53 ± 0.06) spent significantly less time near the wall than did any other strain in both the first (129S= 0.85 ± 0.15 ; DBA= 0.73 ± 0.11 ; Balb/C= 0.71 ± 0.13) and last task (129S= 0.77 ± 0.18 ; DBA= 0.64 ± 0.06 ; Balb/C= 0.74 ± 0.16), however in the initial activity task the 129S strain showed the greatest thigmotaxia while DBA and Balb/c strains were not significantly different from one another. In the final activity task however, the DBA strain failed to show significant differences from any other strain. Initially thigmotaxia significantly increased every day, with Day 3 (0.74 ± 0.16) showing the most, then Day 2 (0.71 ± 0.18), and finally Day 1 (0.67 ± 0.17) with the least thigmotaxia. In the final task Day 1 (0.62 ± 0.17) showed significantly less thigmotaxia across strains than Day 3 (0.70 ± 0.14), but was not different from Day 2 (0.65 ± 0.15), which also showed significantly less thigmotaxia than Day 3. In neither task was an interaction between strain and day observed.

Table 5.

Statistics Table.

<u>Task</u>	<u>Measure</u>	<u>Factor</u>	<u>F-value</u>	<u>Df</u>	<u>P-value</u>	<u>Effect Size</u>	<u>Inbred Mouse Strain</u>			
							<u>129/Sv</u>	<u>DBA/2</u>	<u>Balb/c</u>	<u>C57BL/6</u>
Activity	Initial Ambulation	Day	6.86	2,88	.002	.135	12	12	12	12
		Strain	19.11	3,44	.000	.566	12	12	12	12
		Interaction	1.46	6,88	.202	***	12	12	12	12
	Final Ambulation	Day	7.32	2,40	.002	.258	6	7	4	7
		Strain	10.58	3,20	.000	.613	6	7	4	7
		Interaction	0.65	6,40	.689	***	6	7	4	7
	Initial Rearing	Day	1.93	2,88	.152	***	12	12	12	12
		Strain	111.13	3,44	.000	.883	12	12	12	12
		Interaction	3.07	6,88	.009	.173	12	12	12	12
	Final Rearing	Day	2.38	2,40	.105	***	6	7	4	7
		Strain	12.96	3,20	.000	.660	6	7	4	7
		Interaction	1.54	6,40	.192	***	6	7	4	7
	Initial Thigmotaxia	Day	7.02	2,88	.001	.138	12	12	12	12
		Strain	18.65	3,44	.000	.560	12	12	12	12
		Interaction	1.57	6,88	.166	***	12	12	12	12
	Final Thigmotaxia	Day	7.25	2,40	.002	.266	6	7	4	7
		Strain	6.85	3,20	.002	.507	6	7	4	7
		Interaction	1.29	6,40	.283	***	6	7	4	7
	Initial Fecal Boli	Day	2.64	2,88	.077	***	12	12	12	12
		Strain	18.36	3,44	.000	.556	12	12	12	12
		Interaction	3.82	6,88	.002	.207	12	12	12	12
	Final Fecal Boli	Day	0.74	2,48	.485	***	6	8	6	8
		Strain	3.43	3,44	.033	.300	6	8	6	8
		Interaction	0.69	6,48	.662	***	6	8	6	8
	Initial Binned Day 1	Bin	34.19	5,220	.000	.437	12	12	12	12
		Strain	22.00	3,44	.000	.600	12	12	12	12
		Interaction	3.87	15,220	.000	.209	12	12	12	12

Task	Measure	Factor	F-value	Df	P-value	Effect Size	Inbred Mouse Strain				
							129/Sv	DBA/2	Balb/c	C57BL/6	
Activity	Final Binned Day 1	Bin	6.38	5,120	.000	.210	6	8	6	8	
		Strain	8.71	3,24	.000	.521	6	8	6	8	
		Interaction	1.48	15,120	.124	***	6	8	6	8	
	Initial Binned Day 2	Bin	51.69	5,240	.000	.519	13	13	13	13	
		Strain	15.64	3,48	.000	.494	13	13	13	13	
		Interaction	2.74	15,240	.001	.146	13	13	13	13	
	Final Binned Day 2	Bin	11.64	5,120	.000	.327	6	8	6	8	
		Strain	8.17	3,24	.001	.505	6	8	6	8	
		Interaction	2.67	15,120	.002	.250	6	8	6	8	
Initial Binned Day 3	Bin	80.36	5,240	.000	.626	13	13	13	13		
	Strain	11.38	3,48	.000	.416	13	13	13	13		
	Interaction	2.13	15,240	.001	.117	13	13	13	13		
Final Binned Day 3	Bin	2.81	5,120	.020	.105	6	8	6	8		
	Strain	4.14	3,24	.017	.341	6	8	6	8		
	Interaction	2.52	15,120	.003	.239	6	8	6	8		
MWM	Endpoint	Day	5.02	5,235	.000	.097	13	13	13	12	
		Strain	1.66	3,47	.189	***	13	13	13	12	
		Interaction	2.41	15,235	.003	.133	13	13	13	12	
	Latency	Day	1.61	5,235	.159	***	13	13	13	12	
		Strain	2.15	3,47	.107	***	13	13	13	12	
		Interaction	1.09	15,235	.367	***	13	13	13	12	
	Swim speed	Day	2.11	5,235	.065	***	13	13	13	12	
		Strain	3.72	3,47	.018	.49*	13	13	13	12	
		Interaction	0.81	15,235	.670	***	13	13	13	12	
	Visible Endpoint*	Strain	1.09	3,47	.363	***	13	13	13	12	
	Visible Latency*	Strain	1.02	3,47	.393	***	13	13	13	12	
	Visible Swim Speed*	Strain	1.43	3,47	.246	***	13	13	13	12	
	T-maze	Accuracy	Day	1.35	14,56	.208	***	13	13	11	12
			Strain	2.47	3,4	.201	***	13	13	11	12
			Interaction	1.18	42,56	.278	***	13	13	11	12
Time-Outs		Day	2.17	14,56	.021	2.82*	13	13	11	12	
		Strain	6.25	3,4	.054	***	13	13	11	12	

<u>Task</u>	<u>Measure</u>	<u>Factor</u>	<u>F-value</u>	<u>Df</u>	<u>P-value</u>	<u>Effect Size</u>	<u>Inbred Mouse Strain</u>			
							<u>129/Sv</u>	<u>DBA/2</u>	<u>Balb/c</u>	<u>C57BL/6</u>
T-maze	Time-Outs	Interaction	1.09	42,56	.373	***	13	13	11	12
Autoshaping	P/I Lever Press*	Strain	2.63	3,25	.072	***	6	8	8	6
	P/I Contingent Responses*	Strain	0.97	3,25	.423	***	6	8	8	6
	I Lever Press Day 2-5	Day	7.31	3,72	.000	1.20*	6	8	8	6
		Strain	0.31	3,24	.821	***	6	8	8	6
		Interaction	0.96	9,72	.460	***	6	8	8	6
	I Contingent Responses Day 2-5	Day	17.57	3,72	.000	1.81*	6	8	8	6
		Strain	2.73	3,24	.066	***	6	8	8	6
		Interaction	1.17	9,72	.328	***	6	8	8	6
	Ext Lever Press Day 1-2	Day	5.89	1,24	.023	.56*	6	8	8	6
		Strain	0.33	3,24	.801	***	6	8	8	6
		Interaction	0.49	3,24	.696	***	6	8	8	6
	Ext Contingent Responses Day 1-2	Day	15.94	1,24	.001	.65*	6	8	8	6
		Strain	0.98	3,24	.417	***	6	8	8	6
		Interaction	1.00	3,24	.411	***	6	8	8	6

This table includes all statistics for all analyses conducted. Unless noted, all analyses were two-way mixed factor ANOVA, with one between subjects factor (strain) and one repeated measures factor (day or bin). *Indicates ES calculated by hand with Cohen's *d*, due to low n. (Thalheimer & Cook, 2002) **Indicates a one-way between subjects ANOVA was calculated. ***ES not calculated for non-significant ANOVAs. ***Indicates the ANOVA was not significant therefore ES values were not calculated.

Table 6.

Summary Effects Table.

<u>Task</u>	<u>Measure</u>	<u>Main Effect: Time</u>	<u>Main Effect: Strain</u>	<u>Interaction Effect</u>
Activity	Initial Ambulation	Y	Y (N-K)	N
	Final Ambulation	Y	Y (N-K)	N
	Initial Rearing	N	Y (N-K)	Y
	Final Rearing	N	Y (N-K)	N
	Initial Thigmotaxia	Y	Y (N-K)	N
	Final Thigmotaxia	Y	Y (N-K)	N
	Initial Fecal Boli	N	Y (N-K)	Y
	Final Fecal Boli	N	Y (N-K)	N
	Initial Binned Day 1	Y	Y (N-K)	Y
	Final Binned Day 1	Y	Y (N-K)	N
	Initial Binned Day 2	Y	Y (N-K)	Y
	Final Binned Day 2	Y	Y (N-K)	Y
	Initial Binned Day 3	Y	Y (N-K)	Y
	Final Binned Day 3	Y	Y (N-K)	Y
MWM	Endpoint	Y	N	Y (N-K)
	Latency [#]	N	N	N
	Swim speed [#]	N	Y (N-K)	N
	Visible Endpoint*	n/a	N	n/a
	Visible Latency*	n/a	N	n/a
	Visible Swim Speed*	n/a	N	n/a
T-maze	Accuracy [#]	N	N	N
	Time-Outs [#]	Y	N	N
Autoshaping	P/I Lever Press*	n/a	N	n/a
	P/I Contingent Responses*	n/a	N	n/a
	I Lever Press Day 2-5	Y	N	N
	I Contingent Responses Day 2-5	Y	N	N
	Ext Lever Press Day 1-2	Y	N	N
Ext Contingent Responses Day 1-2	Y	N	N	

This is a summary table of main or interaction effects found via statistical analyses, as well as any post hoc tests calculated, for the measures taken throughout this study. Unless otherwise noted all analyses are Two Factor Mixed Design with one factor repeated measures design.

*Indicates a One-way Between Subjects ANOVA was conducted. [#]Indicates mean daily data per strain was utilized. (N-K) Indicates a Newman-Kuels post hoc test was calculated.

Figure 1

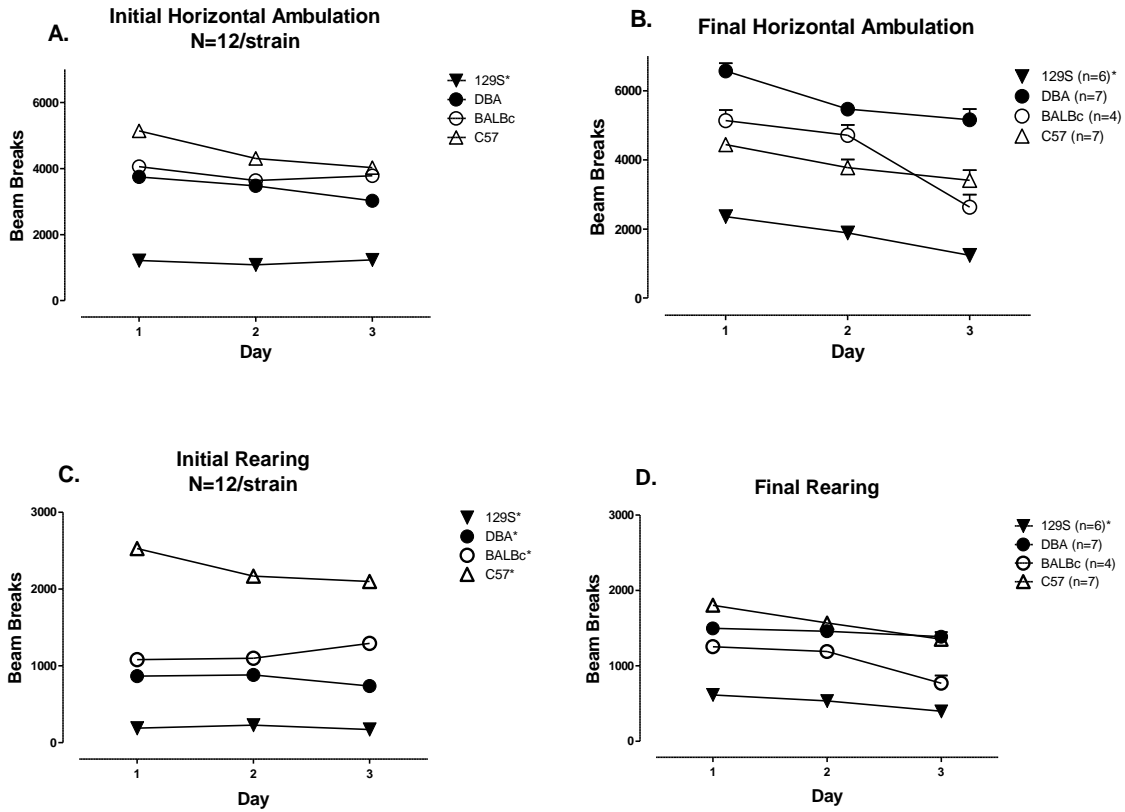


Figure 1. Initial and Final Activity Data. Graphs A and B show horizontal ambulation as measured by beam breaks for both the initial and the final locomotor activity tasks. Graphs C and D show rearing behavior as measured by beam breaks. Strain symbols are defined in the legends, as are any significant difference between strains. Sample size for initial task data is noted under the graph titles, while final task sample sizes are noted in the legend. *Indicates the strain was significantly different than all other strains. Effect of day is noted in the results text, as are any interactions between day and strain. Data presented as mean \pm SEM.

Fecal boli data are shown in Figure 2, graphs C and D. Balb/c mice (initial: 20.94 ± 4.20 , final: 15.89 ± 4.60) produced significantly more fecal boli than all other strains in both initial (129S= 11.72 ± 4.07 ; DBA= 13.83 ± 3.23 ; C57= 10.72 ± 5.95) and final (129S= 8.06 ± 2.80 ; DBA= 12.00 ± 4.22 ; C57= 10.54 ± 6.59) activity tasks, however in the first task the other strains did not differ from one another, while in the final task the 129S mice had significantly fewer boli as compared to the other three strains, but the DBA and C57 strains were not significantly different from each other. There was no effect of day; however an interaction effect was noted (see Data Analysis explanation, page 46).

Figure 3 graphs show binned horizontal beam breaks for the initial and final locomotor activity tasks. In the initial task, during all three days the 129 strain produced the least ambulation of all four strains, and there were no other significant differences with the single exception of Day 1 where C57 mice showed significantly higher beam breaks than all other strains. In the final task however, during Day 1 129S mice showed significantly less horizontal ambulation than the three other strains, C57 mice ambulated on an intermediate level, and DBA mice showed the highest level of ambulation. Balb/c showed significantly greater ambulation than 129S mice, but were not different from either of the DBA or C57 strains.

With regard to effect of bin, for the initial activity task all 3 days showed a significant effect of day where all strains showed a reduction in beam breaks from the initial bin to the final bin. Each day also produced an interaction between day and strain. The final activity task showed a significant effect of bin where all bins were significantly different from one another, except that Bin 2 was not significantly different from Bin 3, which was not significantly different from Bin 4 or Bin 6, which was not significantly different from Bin 5. There was no interaction

Figure 2

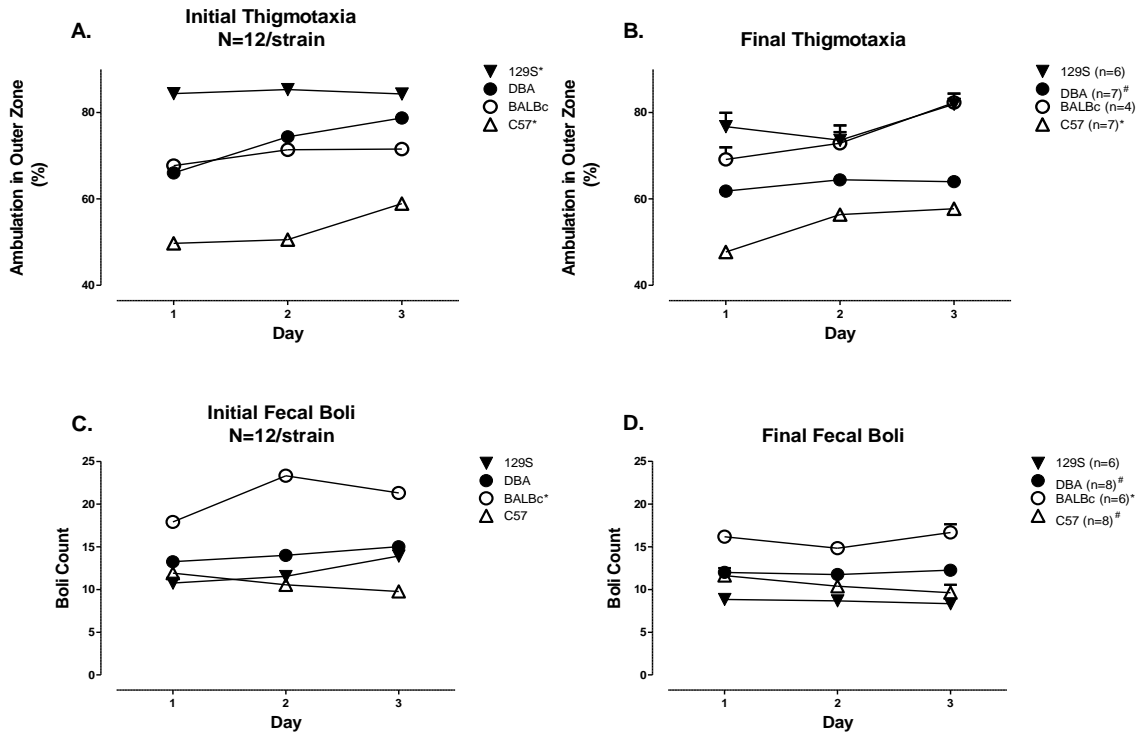


Figure 2. Initial and Final Thigmotaxia and Fecal Boli Data. Graphs A and B show thigmotaxia as measured by horizontal beam breaks in the outer ring of the chamber divided by total horizontal beam breaks, for both the initial and the final locomotor activity tasks. Graphs C and D show fecal boli counts. Strain symbols are defined in the legends, as are any significant difference between strains. Sample size for initial task data is noted under the graph titles, while final task sample sizes are noted in the legend. *Indicates the strain was significantly different than all other strains, except when a [#] is noted. This indicates the strain was not different from the * strain(s) and not different from any other [#]-marked strain(s). Effect of day is noted in the results text, as are any interactions between day and strain. Mean (SEM) is included in all graphs.

between strain and bin. For Day 2, 129S mice ambulated significantly less than all other strains. Bins 2 and 3 were not significantly different from one another, nor were Bins 2 and 6, Bins 3 from Bins 2-6, and Bins 4 from Bins 3-6. All other combinations were significantly different from one another during Day 2. There was an interaction between bin and day. Finally, for Day 3 binned horizontal ambulation data, the 129S showed significantly less ambulation than DBA, but not C57 or Balb/c mice. The C57 and Balb/c strains also were not significantly less than the DBA strain. An effect of bin was seen where all bins were not significantly different except Bin 1 was greater than Bins 2-5, but Bin 1 was not significantly different than Bin 6. Like Day 2 there was an interaction between bin and strain in Day 3 data.

Figure 4 shows representative examples of the activity tracings of horizontal ambulation for each of the four strains. These tracings demonstrate the decreased activity of the 129 mice and show how they tended to remain in the chamber corners for long periods of time.

Morris Water Maze

Figure 5 (panels A, B, and C) shows the data from the MWM, the second task in this study. While there were no significant differences in the average number of trials to locate the hidden platform per strain, there was a significant effect of day where the average number of trials increased as days passed. There was also a significant interaction effect and post-hoc tests revealed that on Day 1 the DBA mice reached the platform significantly less than all other strains, but on Day 2 there were no significant strain differences. On Day 3 the DBA strain reached the platform significantly less than both Balb/c and 129 strains, but not less than C57 mice. On Day 4 the 129 mice found the hidden platform significantly more often than C57 and DBA strains, but not more than Balb/c mice. Finally, on Days 5 and 6 the Balb/c strain located the platform significantly more often than all other strains. On the final day when the platform

Figure 3

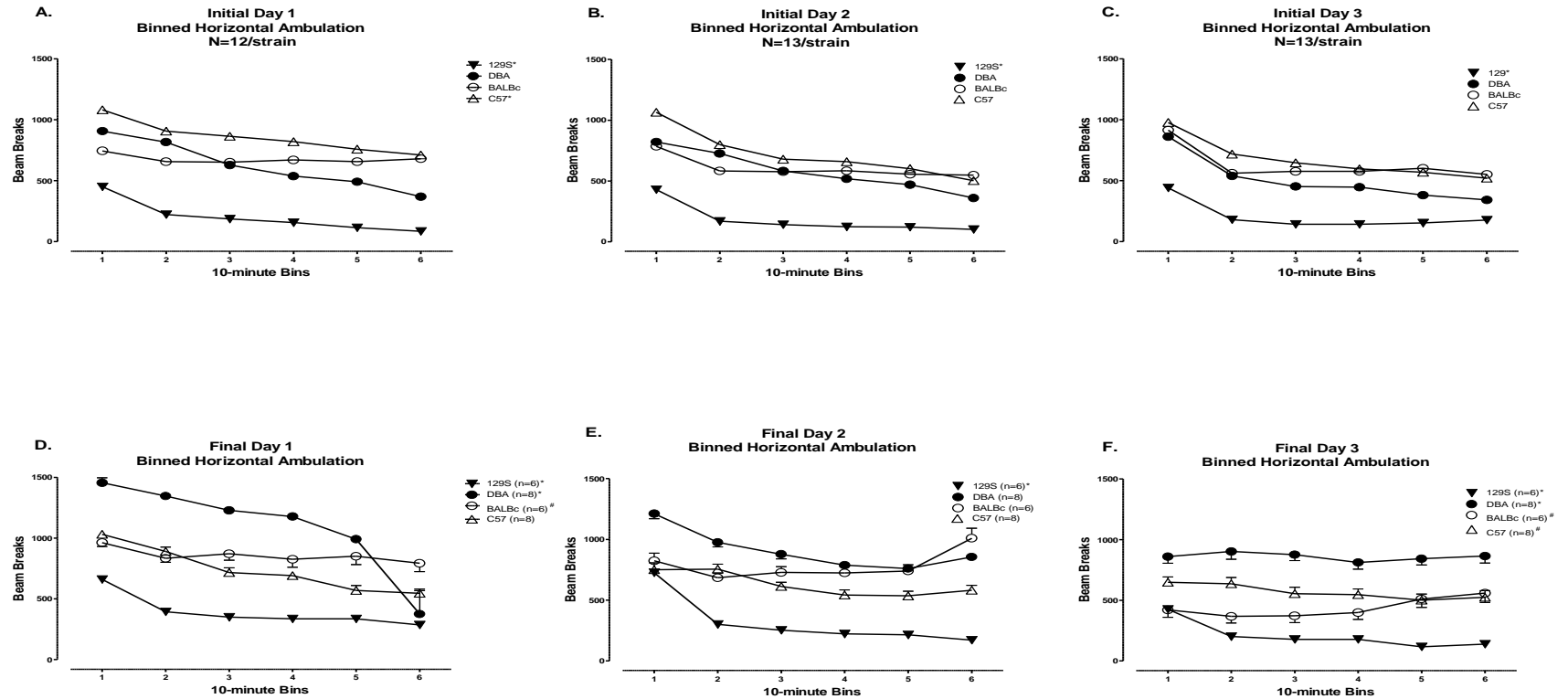


Figure 3. Initial and Final Binned Activity Data. Graphs A, B, and C show the data from the initial locomotor activity task. Graphs D, E, and F show the data from the final locomotor activity task. Strain symbols are defined in the legends, as are any significant difference between strains. Sample size for initial task data is noted under the graph titles, while final task sample sizes are noted in the legend. *Indicates the strain was significantly different than all other strains, except when a # is noted. This indicates the strain was not different from the * strain(s) and not different from any other # -marked strain(s). Effect of day is noted in the results text, as are any interactions between day and strain. Mean (SEM) is included in all graphs.

was visible there was no significant difference between strains with regard to number of trials to locate the platform.

Panel B in Figure 5 shows the average latency to the platform by strain per day. There was no significant difference between strains, nor was there an effect of day, and also there was no interaction between the two factors. During the cued trial there was no significant difference between strains.

Graph C in Figure 5 shows the average swim speed per strain per day. Balb/c mice (12.32 ± 9.22) swam significantly slower than all other strains ($129S=15.76 \pm 3.45$; $DBA=17.04 \pm 5.85$; $C57=17.17 \pm 8.59$). There was no day effect, nor was there an interaction. During the cued trial there were no significant differences in swim speed.

T-maze

Figure 6 shows the T-maze data. In graph A the percent correct arm choices are shown. There were no significant differences in strain performance or across days and the interaction also was not significant.

Graph B, Figure 6, shows the percent trials that ended in a time out. Again, there was no significant difference between strains; however, there was a trend for the 129S strain to have increased numbers of time out trials as compared to the other three strains ($p=.054$). There was a significant effect of day in that the percent of timed out trials was significantly reduced across days (Table 5). There was no interaction between strain and day.

Autoshaping

In Figure 7, graph A the data for total lever presses are presented. No significant difference between strains was noted during Day 1 (the Pavlovian/Instrumental component). During the Instrumental component (days 2-5) there was no effect of strain or an interaction

Figure 4

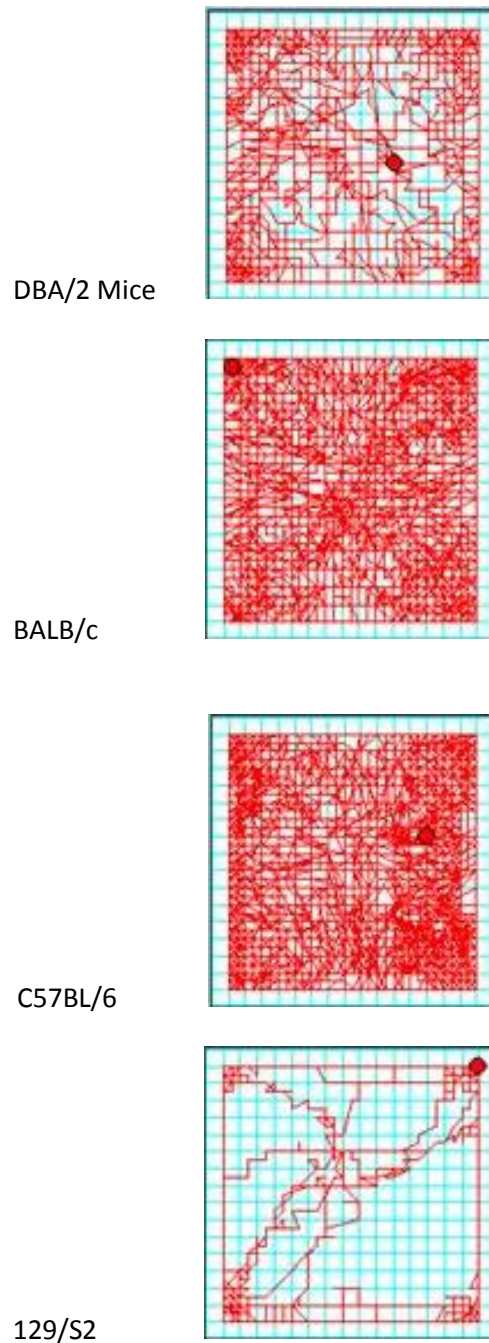


Figure 4. Locomotor Activity Maps. These figures show individual subject paths that represent the overall behavior of the strain. Each mouse strain is noted to the left of the activity maps.

Figure 5

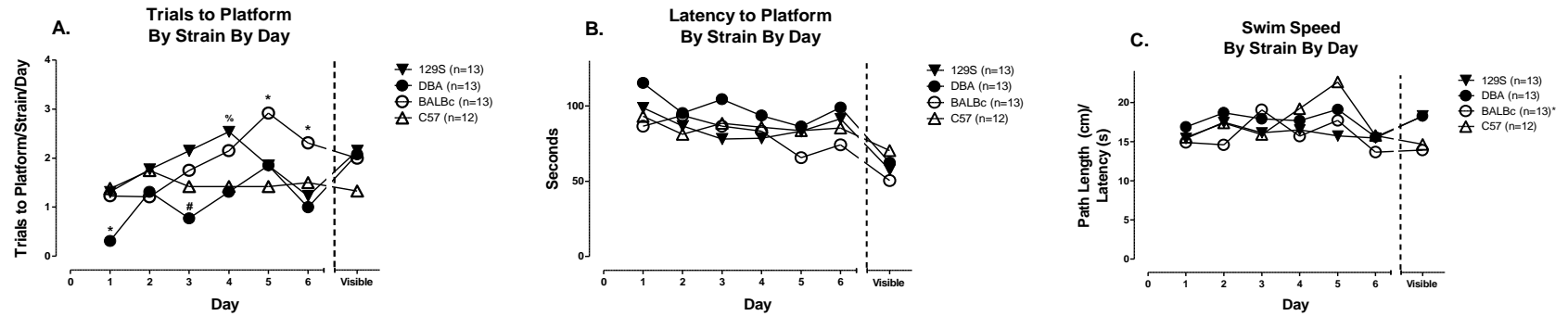


Figure 5. Morris Water Maze Data. Graph A shows the average number of trials per strain per day to reach the hidden platform. Graph B shows the average latency per strain in seconds to reach the hidden platform. Graph C shows the average swim speed per strain per day. In all three graphs the final day is separated as this is the visually cued trial. A separate analysis was done on the visible platform data. Sample size per strain is noted in the legend. *Indicates the strain was significantly different than all other strains, except when a # or % is noted. A # indicates the strain reached the platform significantly less than both Balb/c and 129 strains, but not less than C57 mice. A % indicates the strain found the hidden platform significantly more often than C57 and DBA strains, but not more than Balb/c mice. Effect of day is noted in the results text. Mean (SEM) is included in graphs.

between strain and day; however, there was a significant main effect of day. There were significant increases in the number of lever presses on Days 3-5 (428.68 ± 567.37 , 535.14 ± 636.13 , and 399.46 ± 465.70 , respectively) as compared to Day 2 (64.96 ± 120.41) and on Days 4-5 as compared to Day 3. During the Extinction component (Days 6 and 7) there was no effect of strain or an interaction between strain and day; however, there was a significant effect of day. Lever pressing on Day 7 (160.75 ± 205.79) was significantly less than on Day 6 (314.46 ± 335.63).

In graph B, Figure 7, the number of contingent responses (those made during the presentation of the paired cues; the tone and signal light above the lever) are shown by strain and day. Similar to the results for the total lever pressing data, there was no differential performance in any component dependent on strain. Additionally there was no interaction between strain and day.

Finally, there was no effect of strain during the first component on Day 1, however there was an effect of day during Days 2-5 (3.57 ± 5.87 , 25.07 ± 32.54 , 40.36 ± 41.29 , 50.39 ± 52.81 , respectively). Here significantly different total contingent responses were seen between all days except for Day 4 and Day 5. Also, Day 6 (32.32 ± 31.35) showed significantly more contingent responses than Day 7 (15.36 ± 20.77).

Discussion

Overview

The overall purpose of the present study was to investigate potential baseline phenotypic differences in four isogenic mouse strains. The general motivation for the choices of these tasks and strains is primarily exploratory (T-maze and autoshaping), though portions of the study have literature on which to grow hypotheses (locomotor activity and

Figure 6

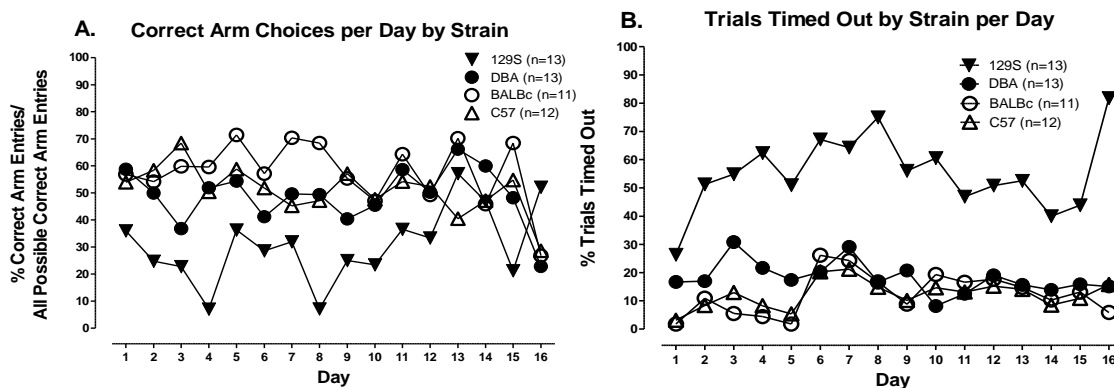


Figure 6. T-maze Data. Graph A shows the percent correct arm choices made per day per strain by day. This is the total number of correct choices possible per daily session divided by the total number of trials where a correct choice was possible. Graph B shows the percent of trials ending in a time out (120 seconds) per strain by day. This is calculated by dividing the number of trials timed out per daily session by the total number of trials where a time out was possible. Sample size per strain is noted in the legend. No significance is noted in these graphs, though effect of day is noted in the results text. No Mean (SEM) data is included in either graph due to observer-based recording of behavior.

MWM). Some results corroborated previous research, while other data was novel and therefore can be used in future hypotheses. Other task results produced data that could not be used for these particular purposes though may be useful in other research.

Locomotor Activity

Based on previous studies (see Table 3; Gerlai, 1998) it was expected that the 129S strain would exhibit the least amount of horizontal activity in the locomotor activity task as compared to the other three strains, (C57, DBA, and Balb/c mice). By the time the final locomotor activity task was undertaken all the strains displayed increases in horizontal activity with the DBA mice displaying the highest level of horizontal ambulation. Nikulina, Skrinkskaya and Popova (1991) reported that DBA mice displayed significantly greater

Figure 7

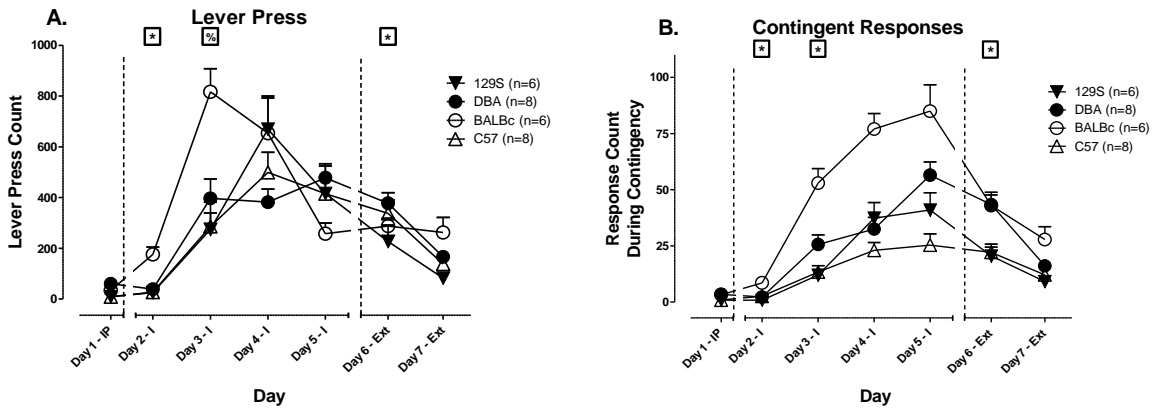


Figure 7. Autosshaping Data. Graph A shows the average number of lever presses per strain by day. Graph B shows the average number of correct responses, or those made during the presentation of the tone-signal light paired, per strain by day. Both graphs are separated into three components: Day 1 is the Pavlovian/Instrumental procedure, Days 2-5 are only the Instrumental condition, and Days 6 and 7 are the Extinction portion. Separate analyses were conducted on each portion. Day 1 data were analyzed with a one-way repeated measures ANOVA. Sample size per strain is noted in the legend. An * in a box at the top of the graph represents that day is significantly different than all other days in its component. A % at the top of a graph indicates Day 3 was less than 4 but not 5. Both graphs include Mean (SEM) data.

ambulation than C57 mice, similar to what was observed in the current study during the second, final locomotor activity task. Also, O'Connell (1980) reported that over the course of a 3-day activity task the Balb/c strain maintained a high activity level, the C57 strain moderate, and the DBA mice increased in activity in the last two days compared to the first day. Overall the Balb/c strain exhibited a relatively moderate activity level in both the initial and final activity tasks as compared to the other three strains of mice. Habituation to the experimental environment seemed to occur in all strains as activity decreased in both the initial and final tasks as the days proceeded, which is unlike the C57, DBA, and Balb/c strain data that were reported in O'Connell (1980) where the DBA and Balb/c strains did not show

decreases in activity over days, but the C67 mice did habituate. Of note is that all of their study's cross-breeding offspring, or hybrids, also showed habituation.

129S mice not only displayed the least horizontal activity of the four strains used in the present study but also showed the least rearing behavior, as well. This significantly decreased activity in the 129S strain was present at the beginning of this study when the mice were approximately 2-3 months old, and again at the completion of the study when they were 13 months old. The strength of this phenotypic difference in locomotor activity also perseverated through the potential impact of the various behavioral tasks that were conducted between the initial and final locomotor activity tasks. It is interesting to note that the two main activity measures, horizontal ambulation and rearing, were highest in the C57 strain at the start of this study, which replicates previous results (Thompson, 1953). In contrast to horizontal ambulation, no habituation in rearing behavior was observed,

The 129S mice displayed the highest levels of thigmotaxia in both the initial and final (only on day 1) assessments of locomotor activity. From a qualitative perspective it was observed that this particular strain, while producing very low levels of horizontal activity, tended to remain close to the walls throughout the 1-hour sessions and sat still in the chamber corners for extended periods of time (see Figure 4). The C57 mice showed the least thigmotaxia during both the initial and final activity tasks; however, this difference was significant only during the initial assessment of locomotor activity as the other three strains showed a decrease in absolute levels of thigmotaxic behavior during the second assessment. These differences in horizontal activity and thigmotaxia between the C57 and 129S strains confirm previous findings (see Holmes et al., 2002; Voikar et al. 2001). The reduced horizontal activity of the 129C mice suggests that this strain would not be a good choice in

memory tasks (like the Barnes maze and 8-arm radial maze) that require a lot of locomotor activity; whereas, the C57 mice should do much better. The increased thigmotaxia seen in the 129S mice supports previous findings that this strain displays increased anxiety-like behaviors (see Voikar et al. 2001) and therefore might be useful in pharmacological studies attempting to identify drugs with anti-anxiety properties.

Balb/c mice had a significantly higher fecal boli count than all other strains in the initial assessment of locomotor activity; however, during the second assessment their fecal boli count had decreased to the point that they were not significantly different from the DBA and C57 strains. Boli count across strains was greater on Day 2 than Day 1 of the initial task, but no differences were seen in boli count across days during the final task. Thompson (1953) also found Balb/c mice defecate significantly more than other inbred strains. Increases in thigmotaxia and fecal boli count have been associated with increased “emotionality” (anxiety-like behaviors) in rodents (Hall, 1934). Based on the present findings, both the Balb/c and 129S mouse strains appear to be appropriate choices for studies interested in measuring anxiety-like behaviors.

Future Directions: Locomotor Activity

A literature review of previous studies (see Table 1) failed to show a differential effect of senescence on locomotor effects in these particular strains, therefore an increase or decrease in activity levels was not expected from the initial to the final assessment of locomotor activity. Even though the rank order of activity changed for the C57, Balb/c and DBA/2 strains, what remained steady was that the 129S strain consistently displayed the least activity. This decreased activity was observed in the other two activity-based tasks, the MWM (floating in the water, often would not even climb onto the platform upon locating it)

and the T-maze (sat still against the walls or in corners regardless of level of food deprivation in place). In a review of inbred behavioral phenotypes, associated assay procedures, and their dependent measures Crawley et al. (1997) notes that generally rodent subjects' activity (horizontal and rearing) levels decrease, fecal boli count increases, and thigmotaxia increases in anxiogenic environments, say when loud noises or bright lights are present. With this in mind it would then be possible to conclude merely based on activity levels, that compared to the other three strains in this study 129S mice are less sensitive to stressful stimuli. It is important to clarify that with regard to relatively low levels of horizontal activity and rearing behavior the 129S behavior may not be so simply explained. While this study's activity data concur with data from studies that have found a positive correlation between horizontal and rearing locomotion (De Fries et al., 1978; Henderson, 1967; Van Abeelen, 1977), it does not agree with the other side of these results, which indicate such increases are negatively correlated with defecation. That is, if lower levels of gross movement indicate increased reactivity to aversive stimuli, then so should these same subjects produce lower fecal boli. Another difference between our results and those noted in the above mentioned studies is that here the 129S had high levels of thigmotaxia which has been considered to indicate heightened stress in aversive environments (Crawley et al., 1997). We did not find these trends with the 129S strain in this study, and via observation the best explanation may be that the generalized, widespread inactivity of these mice affected many if not all of the dependent measures recorded for them.

This strain's decreased levels of activity would undermine its usefulness as a strain in experiments investigating decreases in locomotion due to some applied condition. It may, however, indicate a greater usefulness when considering a potential increase in activity, such

as environmental manipulation or pharmaceutical administration (e.g. a stimulant effect of a drug on activity might be easier to detect in 129S mice). In fact, in Giros et al., (1996) it was hypothesized that a homozygote dopamine transporter knockout mouse strain would exhibit increased activity from normal wild type mice, as well as heterozygote mice. Indeed researchers not only found increased baseline horizontal activity in the homozygote strain, but they also noted decreased sensitivity to administration of both cocaine and amphetamine, which are known to increase locomotion in normal subjects within a certain range of concentrations. Here baseline hyperlocomotion provided a backdrop from which to identify a notable *lack* of change in behavior. Likewise, strains that produce high levels of activity may be more appropriate for studies investigating an expected decrease in activity (Crawley et al., 1997) because they might provide a heightened activity backdrop through which to identify potential decreases in behavior.

DeFries et al., (1978) used an F3 cross between C57 and Balb/c, which they chose due to high and low activity in the locomotor activity task (though the present study didn't find the Balb/c strain to be less active), and found that the same genes are responsible for both activity and defecation. These genetic influences were further studied by Trullus and Skolnick (1993) when they used 16 isogenic strains of mice. They discovered that 75% of the variation in the task under bright illumination is due to genetic factors, but only 44% variation is genetically based when the task is under low illumination. Wehner, Radcliffe and Bowers (2001) describe this result as “a unique set of genes regulate variability in specific measures of anxiety,” referring to the open field locomotor activity task. Further research by Flint et al. (1995) using a variety of behavioral tasks, including Locomotor Activity, Elevated Plus Maze (EPM), and the Y-maze, and many inbred mouse strains has

produced strong evidence that a QTLs on chromosomes 1, 12, and 15 are responsible for the majority of the variance in behavior in the dependent measures taken from these tasks, such as general activity, fecal boli count, Y-maze exploration, and light vs. dark arm entry in the EPM. The authors conclude that these genomic locations “are, at least in part, the genetic basis of emotionality” (for review see Wehner, Radcliffe & Bowers, 2001). There are hopes that these types of interactions between behavioral paradigms and genetic analyses will aid in the realization of the potentially differential impact of environment vs. genetic background on behavior. At that point perhaps a more informed a discussion as to how behavior (inter-) relates to psychiatric disorders in humans can be had, and indeed already is.

Morris Water Maze

Figure 5 shows graphs for the MWM data. Although there was an interaction between day and strain, there were no significant differences between strains on accuracy or across days. While the DBA mice performed significantly worse on Day 1, there was no difference between strains on Day 2, and both 129S and Balb/c strains showed higher accuracy than the DBA mice on Day 3. On Days 5 and 6 the Balb/c mice showed the highest accuracy as compared to the other three strains. This is in direct contrast with the results of Upchurch and Wehner (1988) who found that Balb/c mice could not acquire the position of either visible or hidden platforms. They describe this poor performance as a result of poor eyesight, or inability to see the cued platform location or the distal visual cues around the MWM. However, in the present study there were no strain differences for the visible, cued platform day, which replicates findings reported by Owen et al. (1997). Thus, there do not appear to be deficits in visual capabilities between any of these four strains as measured by the visible platform task in the MWM. Owen et al. (1997) also found that C57 and DBA, but

not 129S and Balb/c strains, showed decreases in latency to reach a hidden platform over trials, however all strains swam significantly more in the trained platform quadrant versus the other quadrants, so all mouse strains displayed some learning.

There were no significant differences between strains or across days with regard to latency to reach the hidden platform. There were no differences for swim speed across days, but the Balb/c mice did display significantly slower swim speeds as compared to the other three strains (although the absolute differences were not that great). Swim speed can be affected by a variety of non-cognitive causes, such as variation in animal size or shape and motivation. Anecdotally, it was noted that the 129S mice tended to spend more time floating than the other three strains, and that a large amount of their backs were above the waterline, while other strains, such as the Balb/C and DBA mice, swam with the majority of their bodies underneath the waterline. The lack of significant decreases in both latency and path length demonstrated that all four strains displayed minimal learning in the MWM task, or at least what learning occurred initially was not maintained. The failure of learning across strains was not anticipated as other studies have shown that these strains are capable of learning across days at some level (see Table 4). It is interesting that on the last two days of the hidden platform the Balb/c strain began to show greater accuracy than the other three strains while other studies have reported poorer performance in the MWM task for Balb/c mice (Holmes et al., 2002; Upchurch & Wehner, 1988); however, one difference in the present study was the relatively poor performance of the other three strains. Holmes et al. (2002) also observed no differences in swim speed, while the present study found that Balb/c mice tended to swim slower than the other three strains. Owen et al. (1997) noted that the poor performance in the MWM and other tasks by 129S mice suggests that they would be a

poor choice of background strain for subjects in learning and memory tasks. This issue has been addressed by creating F¹ hybrids that perform better in such tasks. Wehner, Radcliffe and Bowers (2001) note that QTL analyses have failed to show significant results concerning behavior and performance in the MWM.

T-maze

The T-maze task also failed to reveal any significant differences between strains. It is clear from the graphs (Figure 6) that the 129S strain produced the fewest correct arm choices, as well as the highest number of timed out trials. To qualify this statement it should be noted that similar to the locomotor activity tasks, this strain often displayed less horizontal activity as compared to the three other strains. The high number of timed out sessions was due to the subjects remaining in a single spot in the maze for the entire trial. In a study by Gerlai (1998) that used a different version of the T-maze C57 mice (as compared to 129S and DBA mice) displayed better learning of the task (Balb/c mice were not tested). In another study investigating potential differential spatial and non-spatial learning between C57 and DBA mice in three different radial arm mazes, the C57 strain was again found to be superior (Ammassari-Tuele et al., 1993). Obviously, with the lack of learning in the present study the current T-maze data cannot be compared with that in the literature.

Autoshaping

Though there were no differences between them, all four strains of mice displayed significant increases in both total lever presses and contingent responses during the Instrumental portion of the autoshaping operant task (Figure 7) which shows that they all learned the lever press response during the autoshaping task. During the Instrumental portion of the task (days 2-5), contingent responses (lever-pressing during the contingency) as well

as the previously mentioned lever pressing, increased across days. Towards the end of this period however, the total number of lever presses decreased while at the same time that the contingent responses increased. This also supports the conclusion that all four strains were learning; that is, non-contingent lever presses were minimized while the number of reinforced (contingent) responses were simultaneously increasing. In another autoshaping procedure, though this one used nose-poke as the sought operant behavior, O'Connell (1980) reported that during a baseline condition focused on food consumption there were quantitative differences between DBA, Balb/c and C57 strains, where DBA mice produced the greatest number of responses, then Balb/c, and finally C57 mice produced the least responses in the task. Interestingly, the authors found that this variation was not due significantly to genetic influence, and thus was a product of environmental influence. Likely the most influential environmental stimulus responsible for the variation is the change in contingency schedules from baseline responding to responses during the contingency. In fact, upon the switch from baseline food consumption to the autoshaping procedure a five-fold increase was seen in nose-poke responding across all strains. In the present study we investigated three different contingencies and also noted significant increases in responding as the animals from the Pavlovian/Instrumental condition to the Instrumental condition and then a decrease in responding during the Extinction condition. If the isogenic strains used in the present study represent specific and unique genetic backgrounds, which we believe they do, then the results of the present study agree with those of O'Connell et al. (1980) in that there were no significant differences between the four strains we used in acquiring the operant tasks, and the conditions therein. Thus, in these cases there seems to be little if any

effect of the genome on learning an association between an operant behavior (either nose-poke or lever press) and a food reward.

During the Extinction condition both total lever pressing and contingent responses decreased significantly from Day 6 to Day 7, again indicating that the mice were responding appropriately to the change in the reinforcement schedule. The significant decline in responses during the Extinction portion of this task also demonstrates that all four strains of mice were learning the operant behavior, which was expected based on the reinforcement contingency. One important distinction in the present study as compared to previous autoshaping studies with mice (Baron & Meltzer, 2002; Johnson, Pesek, & Newland, 2009; Papachristos & Gallistel, 2006) is that previous studies have primarily utilized a nose poke response as the desired operant, instead of lever pressing. While no studies have directly examined the differences between the nose poke response and the lever press response, it seems reasonable to assume that the nose poke response requires less physical effort by the mice. It would be interesting to directly compare the rate of learning in different strains of mice with the nose poke response versus the lever press response.

McKerchar et al. (2005a) noted a strong positive correlation between locomotor activity level and rate of lever-pressing in all strains. The present study was not designed to compare locomotor activity data to the lever pressing rate data, as only the autoshaping task was used to look at initial acquisition of the lever press response. McKerchar et al. (2005a) used an intermittent reinforcement schedule to obtain stable (and higher) response rates that could be correlated with activity data.

Thompson (1954) and Crawley et al. (1997) have noted that performance variability within strains should be expected as these types of behaviors (activity, cognitive processes,

etc.) are complex and thus influenced by multiple genetic factors. Interestingly, for the data from the locomotor activity and autoshaping tasks in this study it is quite the opposite. These data had small variability within strain. For example, during the initial locomotor activity task, the SEM for the horizontal ambulation for each of the strains, as a percent of the overall average beam breaks throughout the entire 3-day session was: 129S = 5.30%, DBA = 3.25%, Balb/c = 2.89%, and C57 = 2.53%. For the Autoshaping procedure, the SEM for the contingent responses dependent measure, as a percent of the overall average contingent responses throughout days 2-7 was: 129S = 20.81%, DBA = 12.73%, Balb/c = 13.06% and C57 = 16.96%. This may very well likely be an artifact of automated systems versus observer-based data collection. As Thompson (1954) states, behavior is in itself a complex field thus minimizing variability will be a constant effort, and the major manipulators of behavior are genetics, environment, and interactions between the two. In fact the author even goes so far as to say that the within strain variability observed in his studies must be due to environmental discrepancies that occurred *before* the mice arrived at his location. This particular subject is one that has been a topic of interest in recent years as significant variations in inbred strains of rats and mice have been seen between strains maintained at different laboratories. Wahlston et al. (2003) investigated the “gene-environment interaction” by acquiring eight different isogenic groups of mice (including each of the strains used in the present study) which were then tested in a variety of behavioral assays including: locomotor activity in a small box, the elevated plus maze, accelerating rotarod, visible platform water escape, cocaine activation of locomotor activity, and ethanol preference. Their general findings indicated that there was little to any effect of whether the mice were bred in-house or received by the laboratory via shipment. They also found that sex

differences were negligible, but that the effect of genetics was nearly always large. There was a strong interaction between genes and lab for locomotor activity, cocaine activation, and EPM, but for ethanol preference and the water escape task the results were similar across labs, indicating a large impact of type of task, as well. The authors concluded that while it will never be a perfectly standardized scientific pursuit, even *within* an individual laboratory environment, large effects [genetic, environmental (task, lab, etc.), or an interaction between the two] are likely to be elucidated, while more moderate ones (as could be expected) will be more difficult to uncover across labs. The present study encountered these same issues. The findings from the locomotor activity task clearly replicated previous findings in the literature, while learning and/or performance in the MWM and T-maze did not. Fortunately the Autoshaping task was able to produce useful data, and further investigation of the influences of genotype vs. phenotype on behavior in this task should yield interesting findings.

Future Directions: Autoshaping

In the tasks where data were useable, that is the locomotor activity and operant autoshaping/extinction tasks, we were able to present a comparative *and* a novel data set. In the activity tasks we presented findings that are comparable to the literature, and the within-subjects design allowed for a new perspective on impact of age and experimental conditions on activity, and components within that broad dependent measure (rearing, thigmotaxia, and binning the data within sessions). These two datasets (initial and final) can be used in future studies as comparators when aging and potential impact of various experimental procedures and environments are being investigated.

As for autoshaping and extinction of an operant behavior, the present study used a relatively novel operant, the lever press, which allows these data to be of particular interest in

the field of behavioral genetics. Perhaps due to the relative ease in training complex operant tasks in rats vs. mice, most operant studies have been conducted with rats. That so, demonstrating that four of the most commonly utilized isogenic strains of mice can learn and perform this task (using the lever press response) is important because it demonstrates that behavioral tasks that have primarily been restricted to rats can be used in mice, thus increasing the ability to study the role of genetic factors. Conditions (e.g., brain lesions, pharmacological administration) that have historically been investigated primarily in rats and other non-human populations can now be researched while more easily examining the effects of genetic manipulation. Also, the vast majority of mouse operant studies have used the nose-poke response which has a vastly different topography than the lever press response required in the present study. It has been hypothesized (Gamzu & Schwam, 1974; Hearst & Jenkins, 1974; Jenkins & Moore, 1973) that the nose poke is more closely aligned with the foraging/sniffing behavior that has evolved in mice and other rodents. Therefore the use of the lever press in this task may require the subject to deviate more from their natural behavior topography, indicating this task may be more difficult for mice to perform. The results of the present study demonstrated that lever pressing is not too difficult an operant for mice, even in a complicated, multi-procedure task. Hence, this opens the door for future behavioral researchers who are interested in the various cognitive processes and their related dependent measures to use these and perhaps other inbred mouse strains. Many more such strains should be tested in similar assays to continue to build the comparative data, but pharmacological impact, as well as lesion and trauma studies, can all be used in disease models.

Limitations

This study had some limitations that future studies could eliminate with task adjustment. Primarily in the second and third tasks, the Morris Water Maze and T-maze respectively, learning was not observed in these subjects to the extent to which we could utilize the data to accurately assess the dependent measures to compare strains. Thus, one of the main intentions of this study, to identify behavioral differences between strains, could not be investigated with these tasks. Attempts to eliminate this particular issue (sufficient levels of learning/performance) can be addressed by more closely aligning procedural methodology with experiments that have been successfully conducted in the past. Additionally, various dependent measures should be investigated in order to elucidate other potential areas of learning that could be used to compare strain performance.

Another concern in this study, particularly in the Morris Water Maze, was the use of both observer and automatically based dependent measures. The dependent measures used in the final analysis of this task were the computer-recorded latency to locate the platform and swim speed, along with the observer-recorded total number of trials to find the platform. Widespread equipment failure undermined our original intent to provide the first two measures (along with path length) with assurance of accuracy. The extractable data were used; however, it became apparent that another measure to identify spatial learning and memory was necessary to more completely describe performance in these strains. Evidence suggests that in this particular model, with these types of equipment set-ups, much difficulty is encountered with accurately recording dependent measures across research teams (<http://www.mailtalk.ac.uk/cgi-bin/webadmin?A0=WATERMAZE>; J. Wiebelhaus, personal communication, May, 2009; R. Hamm, personal communication, April, 2009). This seems

to be due primarily to problems of pixilation, interference, and back-masking, which are issues inherent in any video process whereby the movement of an object (or subject) is recorded while a software system “reads” the change of individual pixels from either a light to a dark state, or vice versa. Back-masking is either an automated or manual effort to minimize the light-based “noise” which the software reads as interference. Any one of these problems, but more likely some combination of them, can result in data that are not useable. Future studies using this model should take special precautions to utilize well-tested equipment in viable experimental environments to ensure the utility of any data gathered. Also, when testing strains that have variable coat colors, a between subjects design is advisable as a white water color is necessary when testing mice with dark coat colors, and conversely a dark water color is necessary when testing lighter/albino mice.

A final concern in the present study was the attrition of animals during the study. There were variable reasons for this; however the primary explanation is that this study spanned 13 months. The average lifetime for C57 male mice is 27.2 months, and for DBA male mice it is 23.0 months (http://research.jax.org/faculty/harrison/gerlvi_LifeStudy1.html). Balb/C male mice lifespan median is 9.9-21.6 months (http://www.harlan.com/research_models_and_services/research_models_by_product_type/inbred_mice/balbc.hl). The 129S strain is known in our lab to live approximately the same length of time as the C57 strain. Over the course of this study the n per strain reduced from 13 to 6-8. Some of the animal attrition was due to natural death, although abnormal health issues (undiagnosed illness in the animal colony) began to be observed around study month 9, or when the subjects were all approximately 10.5 months old. We are not aware of any

unusual health concerns specific to these particular isogenic strains. Of course a between subjects design could ameliorate the natural attrition due to death or age-related disease, however our intention with these tasks was to attempt maximum experimental control during every task so a within subjects design was more appropriate for our purposes.

Conclusions

The present study found that for four commonly used inbred strains of mice (C57, 129, DBA, and Balb/c) certain behavioral tasks show similar (autoshaping, MWM, T-maze) and dissimilar (locomotor activity) performance. The constructs addressed include locomotor activity, spatial and non-spatial learning and memory, as well as the acquisition, retention and extinction of an operant autoshaping procedure using a lever-press response instead of a nose-poke response that has been used in most previous studies. Support has been made for the utilization of behavioral genetics as an important tool to elucidate knowledge about human disorders, diseases, as well as non-disease-related points of interest. Information about phenotypic differences provides important information about baseline behavioral differences that can and do affect experimental interpretation in studies using various inbred and outbred mouse strains. Tasks that address wild-type, inbred, hybrid, and mutant strain (Bucan & Abel, 2002) variation in response to different drugs, in tasks such as such as drug discrimination and spatial and non-spatial learning (mazes), are being undertaken in numerous labs. Examples include clozapine drug discrimination in both C57 and DBA strains (Porter et al., 2008), ethanol discrimination in C57 and DBA strains (Shelton & Grant, 2002), differential effects of clozapine and haloperidol on lever-pressing in C57, Balb/c and LP strains (McKerchar & Fowler, (2005), haloperidol catalepsy in CD-1, C57, and Balb/c mice (Fowler, Zarcone & Vorontsova, (2001), and haloperidol and

clozapine's effects on tongue dynamics (Wang & Fowler, 1999). Other measures that can and are being actively investigated by researchers working on identifying and categorizing phenotypes in genetically manipulated mouse strains are blood disorders, hypertension, cancer, and sensory function deficits. The “genotype-phenotype association (which allows for) predictions and facilitates efforts to identify and determine the function of genes participating in normal and disease pathways”

(http://research.jax.org/faculty/molly_bogue.html) is also a useful resource in health and biomedical research, The addition of behavioral phenotyping data from tasks such as the ones used in the present study help provide information that can be used in future studies to better understand the underlying genetic influences on behavior.

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Vita

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