THE IMPACT OF SOCIAL SUBORDINATION ON STRESS REACTIVITY AND COGNITIVE ABILITIES IN CD-1 OUTBRED MICE

by

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ABSTRACT OF THE DISSERTATION

The Impact of Social Subordination on Stress Reactivity and Cognitive Abilities in CD-1 Outbred Mice

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Imposed social subordination, such as that acquired through defeat or alienation, has been shown to negatively impact cognitive performance in both human and animal populations. In the present study we examined whether domain-specific and/or domain-general learning abilities (c.f. general intelligence) are differentially influenced by the imposition of social subordination. Further, we examined whether any observable differences in learning abilities were the direct result of subordination or if they represented deficits which are intrinsically expressed in individuals that are innately disposed to subordination.

Subordinate and dominant behaviors were assessed in two groups of CD-1 male mice. In one group (IMP), social stratification occurred prior to the assessment of learning

abilities, while in a second group (INN), this assessment occurred after learning abilities were measured. Domain-specific learning abilities were measured as performance on individual learning tasks while domain-general learning abilities were measured as the aggregate performance across a battery of learning tasks.

We found that the imposition of subordination decreases exploratory tendencies and to some degree affects domain-specific learning performance. However, the most staggering results came in our analysis of general learning abilities whereby we observed that subjects who assumed a submissive role prior to the assessment of cognitive function were severely impaired. Similar decrements were not seen in subjects that were determined to be subordinate after learning had been assessed. The latter finding indicates that absent the imposition of subordination, individuals with subordinate tendencies do not demonstrate learning impairments. This observation could have important ramifications for those who suffer bullying in school or workplace settings.

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General Introduction

Stress has been shown to be a potent modulator of learning and memory processes. However, the direction, degree, and duration of stress effects on cognition depends greatly on the characteristics of the stressor, the type of learning being assessed and the organism being studied (for review see: Shors, 2006; Roozendaal, 2002, 2003; Joëls, Pu, Wiegert, Oitzl & Kruger, 2006; de Kloet, Oitzl, & Joels, 1999; Lupien, McEwen, Gunnar & Heim, 2009; McEwen & Sapolsky, 1995). The consequent variability in reported stress effects on learning highlights the need to focus on stressors that are both highly relevant and conserved across both human and non-human animal species. Numerous mammalian species, including humans, live in complex social groups and are subject to intense and often unpredictable stress as a result of social interactions. As such, a relatively new area of study has emerged with the goal of investigating the learning effects induced by stressors which are primarily social in nature. One line of inquiry in particular focuses on biobehavioral and learning changes that occur as result of social subordination.

In humans, subordination resulting from alienation or social defeat (e.g. bullying) has been shown to exert a negative influence on cognitive performance. For example, Baumeister, Nuss and Twenge (2002) examined the effects of social exclusion on IQ and performance on the Graduate Record Examination (GRE) test. They had individuals complete a personality inventory which predicted their "future aloneness". Subjects who were told they were likely to end up alone in life showed a significant decline in performance of complex cognitive tasks that involved logic and reasoning (compared with controls). A similar study by Johnson (2005) showed that undergraduate college

students who self report strong feelings of alienation have impaired academic performance, evidenced by lower levels of mastery of course content than their non-alienated counterparts. It has been widely reported that threat of stigmatization (largely due to one's gender or race) also impacts cognitive performance across a wide range of tasks (for review see Schmader, Johns & Forbes, 2008). Likewise, students who are bullied (i.e. made subordinate through non-physical threat or physical force) often experience deficits in school performance evidenced by a drop in class grades (Hoover; Oliver; Hazler, 1992; Olweus, 1978). A significant negative correlation has also been witnessed between language abilities (Knox & Conti-Ramsden, 2007), intelligence (Perry, Kusel, and Perry, 1988; Horwood, Salvi, Thomas, Duff, Gunnell, Hollis, Lewis, Menezes, Thompson, Wolke, Zammit, Harrison, 2008) and level of victimization in males.

While the results are intriguing, human studies of social stress effects are limited. In the laboratory, researchers employ stressors which may be mild in comparison to the stress of real life events. As such, the results from these studies may not fully demonstrate the impact of subordination on learning performance. Studies which examine natural instances of subordination stress in humans are flawed as well. For example, these studies often rely on self reports or personal perceptions of subordination which may not accurately reflect actual events. Further, environmental or individual background differences may interfere with assessments of cognitive abilities and emotionality. Lastly, due to ethical and methodological constraints it is difficult to study the potential biological mechanisms which underlie changes in cognition in human participants.

Animal models provide researchers with a greater degree of controllability over the stressor and thus allow for more detailed examination of the physiological substrates which may be responsible for alterations in cognition. Laboratory models can be used to study the acute or chronic effects of social stress. The preponderance of studies of social subordination in animals look at effects in rodents (e.g. rats, mice, voles) or small primates (e.g. tree shrews). Most studies utilize male subjects since females only express overt aggressive/dominance-like behaviors under certain conditions (e.g. in the defense of pups post-parturition).

There are several laboratory models of social defeat which are used routinely, although in many instances, researchers modify a particular model to custom fit the needs of their study. The Resident/Intruder model involves one or more dyadic agonistic encounters between a resident and an intruder animal. Residents are singly housed and as such become highly territorial and defensive of their home environment. Occasionally, researchers utilize resident subjects that are bred for high aggression or are larger or older to ensure defeat. When an intruder is removed from its own (or a shared) cage and introduced into the cage of a resident, it is typically attacked and defeated. Some experimenters allow subjects to remain in sensory contact for the period(s) in between physical encounters. Sensory contact is achieved by separating subjects with a wire mesh or plexiglass partition with holes so that the subjects can receive visual and olfactory cues from the other subject while remaining physically separated. Sensory contact has been reported to prolong the stress effects of defeat in the subordinate intruder animals (Kurdysteva 1991a, b; Martinez Calvo-Torrent, Pico-Alfonso; 1998).

Researchers may also study social subordination by pairing subjects that were previously from different cages (i.e. singly housed) or social groups (i.e. group housed) in a neutral arena. Unlike the resident/intruder paradigm, in this model neither animal has had the opportunity to establish territorial dominance in the arena prior to the pairing. Through observation of the agonistic encounter(s) one animal is deemed the loser/subordinate and one the winner/dominant **(**Blanchard, McKittrick, Hardy & Blanchard, 2002; Martinez Calvo-Torrent, Pico-Alfonso; 1998).

The colony model, which is primarily used to study rodents, calls for the group housing of subjects in male same-sex groups of three or more either with or without female subjects. These conditions (i.e. group size and co-housing with females) are designed to mimic the natural living conditions of wild rodents living in demes. During colonization, researchers observe and score behavioral interactions between the subjects. Species-specific behaviors associated with subordination and dominance have previously been identified and are used to determine the social status of the individuals in the colony (Grant & Mackintosh, 1963; Miczek, 2001; Martinez Calvo-Torrent, Pico-Alfonso; 1998).

For the better half of a century, researchers have utilized the models described here to examine the behavioral and endocrine correlates of social subordination. It has been widely reported that subordinate animals express increased anxiety-like behavior when they are tested outside the agonistic context. In the elevated plus maze (EPM), a common test for anxiety in rodents, animals are allowed to explore both the dark and exposed arms of the maze. Animals with high anxiety tend to avoid entering the exposed arms of the maze, remaining in the closed arms instead (Rodgers, 1997). Avoidance of

open arms has been documented in both subordinate rats (Ruis et al, 1999; Menzaghi et al, 1994; 1996; Heinrichs et al. 1992; 1994; Berton et al. 1998; 1999) and mice (Avgustinovich, Gorbach & Kudryavtseva, 1997; Rodgers & Cole, 1993; Haller & Halasz, 1999) in the EPM. In the open field test, subjects are placed in an open arena and given the opportunity to explore. Entry into the internal areas of the field is considered to be indicative of increased exploratory or decreased anxious tendencies **(**Rodgers, 1997). After defeat, subordinate subjects make fewer entries in the center areas of the field (rats: Peres & Leite, 2002; mice: Kudryavtseva et al 1991 a,b). While it is possible that the differences in exploration in the EPM and open field reflect differences in exploration independent of anxiety, other tests confirm the notion that subordinate animals are more anxious. For example, in a light discrimination task, subordinate mice displayed anxiety-like behavior indicated by an increased aversion of the light section of the black/white test box (Keeney & Hogg, 1999). Likewise, subordinates demonstrate increased immobility to silence and forced swim (Keeney & Hogg, 1999; Ruis et al, 1999) as well as an increase in the number of ultrasonic vocalizations in response to startle stimuli (Vivian & Miczek, 1998, 1999; Tornatsky & Miczek, 1994). Reports on the duration of these behavioral stress effects are variable. Korte and de Boer (2003) witnessed alterations in these behaviors immediately after social stress but not one, two or three weeks after defeat. However, Ruis et al. (1999) claimed that these behaviors persist up to fourteen days after social stress. Single housing subjects after subordination or defeat can prolong the behavioral (and endocrinological) effects of subordination (Buwalda et al., 2005).

One of the most widely used biological markers of endocrine stress activity is function of the hypothalmo-pituatry-adrenal (HPA) axis. In response to stress, the hypothalamus secretes corticotrophin-releasing factor (CRF), which in turn binds to specific receptors on pituitary cells, which produce adrenocorticotropic hormone (ACTH). ACTH then travels to the adrenal glands where it stimulates the production of hormones known as glucocorticoids (e.g. corticosterone or cortisol in humans in primates). Glucocorticoids can bind to receptors in the hypothalamus and pituitary to provide negative feedback control of the stress response (deKloet, Schmidt, Meijer, 2005). They can also bind to receptors and exert their effects in other brain regions, some of which have been widely implicated in learning processes (Shors, 2006; Buwalda et al, 2005). Both field and laboratory studies have provided converging evidence that that basal levels of glucocorticoids are increased in subordinate rodents (rats: Hardy et al, 2002; Monder, Sakai, Miroff, Blanchard & Blanchard, 1994; mice: Dong et al 2004; Blanchard, Sakai, McEwen, Weiss & Blanchard, 1993, 1995; Francia et al, 2006; Fitchett et al, 2005; Veenema et al, 2003; guinea pigs: von Holst, 1977) and non-human primates (Monogue et al, 1975; Coe et al, 1979; Sapolsky,1983). These differences are most often observed early in the subordination experience with CORT levels in subordinate animals adapting across time to levels comparable to those seen in dominants (Bronson, 1973, Ely & Henry, 1978; Krugers et al, 1997). During periods of social instability, especially when their status is challenged, dominant animals may produce elevated levels of glucocorticoids. In humans, losing a contest competition results in a greater in salivary cortisol levels in individuals with high implicit power motivation which is the non-conscious need to dominate or have impact on others (Wirth, Welsh & Schultheiss,

2006). In situations where both dominants and subordinates have elevated levels of glucocorticoids, hormone levels return to baseline more quickly in dominant individuals (Blanchard, McKittrick, Hardy &, Blanchard, 2002).

Exposure to non-social stressors has also been shown to alter HPA activity in subordinate organisms. For example, an elevated HPA response to a novel stressor has been witnessed in subordinate rodents (Ely & Henry, 1978; Dijkstra et al, 1992; Huhman et al, 1992; Haemisch, 1990) and primates (Coe et al, 1979; Sapolsky, 1983). Conversely, Blanchard et al. (1993, 1995) found a blunted HPA response in a subset of severely stressed subordinates after exposure to restraint stress. It is has been suggested that upregulation of HPA activty, such as that seen in highly stressed animals, may lead to HPA dysregulation and a dysfunctional response to subsequent stress exposure.

Increases in the production of glucocorticoids due to social stress (with or without additional stressors) can lead to compensatory changes in the expression and binding efficacy of glucocorticoid receptors. There are two types of glucocorticoid receptors; Type 1 mineralcorticoid receptors (MR) have a high affinity for glucocorticoids and as such are saturated under basal conditions. Type II glucocorticoid receptors (GR) have a much lower affinity for glucocorticoids and are therefore predominantly occupied when levels of these hormones are high, such as during periods of stress (Shors, 2006; Zoladz & Diamond, 2009). Glucocorticoid receptors are located in high densities in brain areas implicated in both stress and learning, specifically in the hippocampus, amygdala and prefrontal cortex. In response to social subordination stress GR mRNA in hippocampus, specifically the CA1 and CA3 subfields, is decreased (tree shrews: Johren et al, 1994a, b; rats: Blanchard, McKittrick, Hardy & Blanchard, 2002; Chao et al, 1993). Buwalda et al.

(1999) found decreased GR binding in the hippocampus and hypothalamus one week after a social defeat. Interestingly, by three weeks after defeat, GR binding had normalized to control levels while a decrease in MR binding became evident. Decreased MR binding can be indicative of impaired feedback control of the HPA axis. Dysregulation of the HPA axis can lead to further increases or decreases in production of stress hormones which may further impact learning and memory processes in the key brain regions where these receptors are located. Indeed, morphological and neurophysiological changes in the hippocampus, prefrontal cortex and amygdala have been reported after exposure to social stress (Shors, 2006; Buwalda et al, 2005; Gould, Tanapat, McEwen, Flügge, Fuchs, 1998; Bangasser & Shors, 2010; Magariños, McEwen, Flügge, Fuchs, 1996; McKitrick et al., 2000; Von Frijtag et al., 2000).

Given the potentially deleterious effects of glucocorticoids on the brain, one might expect to see widespread cognitive impairments in socially subordinate animals. Indeed there is a great deal of evidence that subordination leads to learning deficits although there is also evidence of no (Buwalda et al., 2005; Barnard & Luo, 2002; Francia et al., 2006) or even positive (Buwalda et al., 2005; Bartolomucci, de Biurrun, Czéh, van Kampen & Fuchs, 2002) effects on cognition due to social stress.

Nearly all of the animal studies of social stress effects have focused on only one domain of learning; i.e. spatial learning. Another key difference among these studies is the type of social subordination they examine (imposed versus innate). Here, the effect on cognitive abilities of imposed subordination refers to subordination that occurs prior to assessment of cognitive function, as a result of agnostic encounters with conspecifics. In

contrast, assessment of the effects of innate subordination on cognitive abilities requires that cognitive function be determined prior to the assessment of subordination.

A number of animal studies have revealed that the imposition of subordination negatively impacts upon learning abilities. For example, rat subjects tested in a radial arm water maze (RAWM) show deficits in both acquisition and memory for the task (Alzoubi, et al., 2009). The RAWM is a structure that consists of a central hub from which a set number of equidistant arms extend. In this maze the arms are water filled and animals must swim through the maze to find a hidden escape platform. The location of the platform remains the same but the start location changes between trials. The benefits of using the RAWM have been detailed extensively. One distinct advantage is that odor cues are eliminated which ensures that the animals performance is not scent-guided (Shukitt-Hale, McEwen, Szprengiel, Joseph, 2004). In their experiment, Alzoubi et al. (2009) intermittently placed intruder rats in resident rats' homecages over a 12-week period. Stressed subjects showed impaired learning as indicated by committing significantly more errors than untreated controls. Additionally, the stressed subjects showed impairments in short and long-term memory evidenced by deficits in recall of the platform location when tested five and 24 hours after training, respectively.

Fitchett et al. (2005, 2009) used a spatial version of a T-maze alternation task to examine the relationship between imposed subordination and spatial learning abilities. In their first study they report learning impairments in subordinate mice from highly aggressive pairs which persist even when subjects are housed alone. In the later study, they report no impairments due to social rank. However, in the latter study, they

combined the data of subordinates from high and low aggression dyads which may have negated the effects witnessed in the original study.

In a study by Krugers et al. (1997), rats exposed to a single social defeat followed by eight days of sensory contact with their attacker were tested in a holeboard task to assess spatial learning abilities. The holeboard typically consists of four rows of four holes which can be baited with food. Subjects are trained to locate food in baited holes which are arranged in a set geometric pattern. Revisits to baited holes and visits to non-baited holes are counted as errors. By examining the relationship between visits and revisits to baited and non-baited arms researchers can determine a subject's reference and working memory abilities. In their study, Krugers et al. (1997) found that defeated subjects showed deficits in the acquisition of both reference memory and working memory which persisted throughout the 10 day training period. Interestingly, giving naïve subjects corticosterone (CORT) at levels comparable to those seen during social stress only mildly affected spatial memory performance, suggesting that CORT is sufficient to produce the impairments in holeboard performance although other mechanisms may be involved in producing the stress-related impairments witnessed in this study. Findings from another study suggest that these effects on spatial performance may be short-lived (Buwalda et al., 2005). Using a similar stress procedure, Buwalda et al., 2005 found that defeated rats showed no differences in performance in the holeboard task three weeks after defeat. Since the latter study (Buwalda et al., 2005) did not examine performance at both time points (i.e. immediately and three weeks after stress) one cannot conclusively state that learning is initially impaired but deficits diminish across time. It is possible that the lack of effects seen three weeks after defeat may be due to methodological differences

between the two studies and that Buwalda et al. (2005) may have found no effect if they tested their animals immediately after defeat.

Imposed social stress has also been show to alter spatial holeboard performance in tree shrews although the direction of these results appears to depend on the pattern of stress exposure and/or the performance criterion in the individual studies. In a series of studies, the Fuchs laboratory examined hippocampus (reference/declarative) and non-hippocampus (working) mediated memory performance in the spatial holeboard task. They found that exposure to intense, chronic (10 or 23 weeks, respectively), alternating non-stressful and stressful conditions produced decrements in hippocampus-mediated memory that persisted for up to ten weeks after the cessation of stress exposure (Ohl & Fuchs, 1998; 1999). No effects on hippocampus-independent memory processes were witnessed. In a separate study the same group found that exposure to five weeks of constant chronic psychosocial stress enhanced learning in animals performing the hippocampal-dependent task, whereas no stress-induced effect was found in the hippocampal-independent versions of the task (Bartolomucci, de Biurrun, Czéh, van Kampen and Fuchs, 2002). There are at least two possible reasons for these discrepant findings. First, it is possible that the chronicity and duration of stress exposure determines the stress effects on learning such that shorter periods of constant stress may facilitate performance while longer periods of chronic alternating stress may cause performance deficits. In the greater stress and learning literature there is evidence that acute stress can enhance while chronic stress can impair learning (Bangasser & Shors, 2010; Sandi & Pinelo-Nava, 2007**;** Joels, 2006), although, the demarcation between what constitutes an acute or a chronic stressor remains unclear.

The second possible reason for differences in the findings between these studies is that in the experiment where they saw enhanced spatial performance, subjects were excluded if they did not engage in or complete the task within a certain time window. The authors claimed this would control for motivational differences and allow for more rigorous examination of learning abilities. However, previous studies have shown that socially stressed animals showed decreased exploratory tendencies (Peres & Leite, 2002; mice: Kudryavtseva et al 1991 a,b). By excluding animals with high latency to approach the apparatus, they may have inadvertently excluded the more severely stressed animals from their study. It is possible then that their findings reflect performance of a less-stressed subpopulation of tree shrews. In all of the studies by the Fuchs laboratory, the stress procedure increased urinary free cortisol concentrations (Ohl & Fuchs, 1998; 1999; Bartolomucci, de Biurrun, Czéh, van Kampen and Fuchs, 2002). In the studies in which spatial deficits were reported, cortisol levels returned to baseline although learning impairments persisted, suggesting that cortisol levels do not need to be consistently elevated to affect learning. It may be that glucocorticoids exert their deleterious effects early on and that these effects are long-lasting. In the experiment where a learning enhancement was observed, cortisol levels were elevated in stressed subjects that showed enhanced hippocampal-dependent learning. While at first this may seem counterintuitive, one must remember that the relationship between glucocorticoids and learning is complex. It has been reported that an inverted U-shaped relationship exists between glucocorticoids and learning whereby physiological levels do not affect learning, slightly elevated levels promote learning and higher levels negatively affect learning processes.

It is possible then that the elevation in cortisol seen in the subjects in this experiment was capable of facilitating spatial performance.

Like the radial arm water maze (RAWM), subjects tested in the spatial Morris water maze (MWM) must locate a hidden platform however; in the MWM, the platform is hidden in a fixed location within a circular pool of opaque water. During training trials, latency to find the platform and or path length to the platform from a random start location is recorded. During probe trials, the platform is removed, and the time spent in the quadrant that normally contains the platform is compared to the time spent in other quadrants. Francia et al. (2006) examined water maze performance in young adult and middle age mice exposed to five days of agonistic encounters. While they found no performance differences in the young adult mice, middle-aged dominant mice outperformed same aged subordinates in a probe trial. The authors suggest that the effect may be attributable to the observed increase in overall exploratory behavior displayed by the dominant mice in this study and not differences in cognitive abilities due to social status. While this may be the case, it also possible that the length of stress exposure was not sufficient to affect learning acquisition or that only retention for the task was impaired. Another group did report significant deficits in spatial water maze acquisition in middle-aged rats exposed to six months of exposure to high social stress when they were tested two weeks after stress ended (Bodnoff et al., 1995). In the latter study, these deficits were not seen in subjects that had been adrenalectomized (with low-level corticosterone replacement), thereby implicating glucocorticoids in the effects of social stress on spatial memory in these animals. In a final experiment, mid-aged rats were treated with corticosterone at levels that mimicked peak physiological levels or levels

seen in response to social stress. Only rats exposed to stress levels of corticosterone displayed impaired performance coupled by a decrease in cellular long-term potentiation (LTP). Long-term potentiation (LTP) has been proposed as a putative cellular mechanism for storage of certain forms of learning and memory. Numerous studies have linked a lack of LTP to poor spatial performance, as such the deficits in LTP may underlie the spatial impairments seen in this study (for review see: Bliss, Collingridge &Morris, 2004).

In another study, Touyarout, Venero and Sandi (2004) observed deficits in MWM acquisition in subordinate rats, however these effects were only observed in subjects that were measured a s being highly behaviorally reactive (e.g. showed increased locomotor activity) when exposed to a novel environment. Meaning, only the most stress reactive animal showed learning effects. In another study, Moragrega et al. (2003) attempted to correlate aggression with spatial learning performance in the spatial Morris water maze (MWM). They found no differences in acquisition or retention of the MWM task between subjects with high (short attack latency/SAL) or low (long attack latency LAL) aggression. Conversely, Buwalda et al. (2005) found that subordinate rats showed increased acquisition of learning in the water maze three weeks after social defeat.

Kvist et al. (1989) examined learning in mice that were selectively bred for dominance like behavior (i.e. high or low aggression). They found that the mice selectively bred for aggression showed facilitated acquisition of spatial maze learning but not a passive avoidance response. Passive avoidance is a one trial task in an animal learns to remain behaviorally passive to avoid an aversive stimulus. Passive avoidance was more rapidly learned in the low aggression mice. However, in this study learning was quantified by

measuring the time spent on an electrified grid upon stepping down from a platform. It is possible that the differences documented here reflect decreased exploration in the low aggression mice and not a learning effect. As reported elsewhere, subordinate mice tend to have lower exploratory tendencies. It is plausible then that low aggression mice may also be less exploratory (i.e. behaviorally passive) which could have resulted in an increased amount of time spent on the platform and less time on the grid.

While the studies detailed thus far indicate that subjects exposed to subordination prior to learning possess cognitive deficits, it is unclear if these decrements exist in subjects that have not been made subordinate. To determine this, one we need to examine learning prior to the imposition of subordination. Unfortunately, only a small number of studies have examined the relationship between innate social subordination and cognition and the results of these experiments are mixed. Spritzer, Meikle, and Solomon (2004) looked at innate differences in spatial performance in the water maze in meadow voles and found that dominant males had better acquisition but not memory for the water maze task. These findings indicate that absent the stress of subordination, innately subordinate individuals display impairments in learning but not memory function.

Barnard & Luo (2002) tested spatial learning performance in the radial arm maze in subjects both before and after paired housing. This allowed them to assess the relationship between both innate and imposed subordination and cognitive abilities. The radial arm maze (RAM) consists of equally spaced arms (the number of arms can range from 3-48) that extend out from a small circular platform. For testing, subjects are placed in the center hub of the radial maze. A fixed number of randomly chosen arms are baited

with food pellets. The subject is given the opportunity to visit all the arms and collect all the available food pellets. After a retention delay, the subject is returned to the maze. In pre-pairing test trials, mice that were later revealed to be subordinate showed no differences in learning performance in a 7-arm RAM when they were compared to future dominants. However, after paired housing, subordinates had impaired performance, evidenced by significantly more incorrect arm choices. This deficit was evident in the early trials across all three days however, by the end of each test day subordinates and dominants performed at comparable levels (Barnard & Luo, 2002). Greater deficits were seen in pairs with high levels of aggression. It is plausible that subordinates from these groups were more severely stressed and therefore more susceptible to the negative effects of social stress on spatial learning performance.

The mixed findings from the studies of animals with tendencies toward subordination underscore the need for a more thorough examination of whether differences in learning abilities due to social subordination represent innate deficits or whether those deficits arise in response to imposed subordination.

Both the studies of innate and imposed subordination have one major shortcoming; they focus exclusively on domain-specific learning abilities. Yet, it has been established that both domain-specific (e.g., spatial abilities) as well as domain-general (general intelligence) factors influence cognition (Kolata, Light, Matzel, 2008). In humans, general intelligence or "g" has been called the "single most dominant cognitive trait ever discovered (Plomin, 1999). General intelligence is a single dominant factor that is purported to influence all domain-specific learning abilities. Like humans, CD-1 outbred mice express individual differences in their "general" cognitive abilities such that

performance in each task in a battery of diverse learning tasks is positively correlated. Through the application of principal components analysis a general learning factor can be identified that accounts for 25-48% of the variance in the performance of individuals. This mouse general learning factor is believed to be analogous in many respects to general intelligence in humans (Kolata et al, 2005, 2007; Light et al, 2010).

To date, no animal studies have attempted to examine the relationship between social subordination and general learning abilities. There is evidence from the human literature however which suggests that social subordination may negatively impact cognitive function. Recall that Baumeister, Nuss and Twenge (2002) found that subjects who were told they would end up alone in life (i.e. socially excluded) had lower IQ scores when compared to controls that were not given a prediction of "future aloneness". It is known that performance on a number of standard IQ tests is influenced by a domain-general intelligence factor (Gottfredson, 1998). If social subordination negatively affects performance on the IQ test then it seems plausible that it may exert similar effects on general learning abilities in animals. Additional evidence suggests that general learning abilities may be impaired as a function of social stress. In humans, threat of stigmatization has been shown to impair working memory; an individual's ability to store task relevant information under conditions of competing demands. Working memory has been shown to be highly related to IQ test performance and general intelligence (Light et al, 2010; Conway, Kane & Engle, 2003). Therefore, it is possible that in animals the imposition of subordination may impair working memory and general learning in a similar manner.

The goal of the current study was to determine if domain-specific (spatial and non-spatial) and domain-general learning abilities in mice are altered as a function of social subordination. Based on previous studies, we believed that animals that were made subordinate would show at least some degree of learning impairment. Our second goal was to see if any observed learning deficits were the result the imposition of subordination or whether they were intrinsically expressed in individuals with a subordinate phenotype. Lastly, we wanted to determine if an animal's stress reactivity was related to either its position in a social hierarchy (i.e. submissive or dominant) and/or its learning abilities.

Experiment I

The goal of the current experiment was to determine whether an individual's domain-specific and/or domain-general learning abilities are altered by the imposition of social subordination (in a colony setting) in a manner similar to that seen in previous studies. Additionally, we wanted to determine if cognitive differences do exist in animals that undergo subordination; whether they occur as a result of the imposition of subordination or if they represent a disposition for poor learning which is intrinsic to animals that are innately subordinate. The latter question can only be answered by utilizing an animal model since social isolation is required prior to cognitive assessments, a condition which is not feasible in humans. It should be noted that this is the first systematic investigation of the relationship between social subordination and general learning abilities.

Domain-specific learning abilities were measured as performance on individual learning tasks while domain-general learning abilities were measured as the aggregate performance across a battery of learning tasks. Stress-induced levels of the adrenal hormone, corticosterone were also measured since prior work has shown a differential activation of the HPA axis in subordinate and dominant subjects in response to stress. Further, corticosterone has been implicated as a possible modulator of cognitive function thereby making any discovery of differences in its expression of particular interest.

This experiment was a four-group between subjects design which compared behavioral and hormonal measures in imposed dominant (IMP DOM), imposed subordinate (IMP SUB), innate dominant (INN DOM) and innate subordinate (INN SUB) mice. To characterize the general cognitive performance of individual animals, the performance on the learning tasks was subjected to a principal component analysis,

which groups variables into "factors" which best account for the overall pattern of correlations between them. Based only on data in the learning tasks, a single factor was extracted (which accounted for 29% of the variance across all tasks), and this factor yielded a factor score for each animal which served to represent its general cognitive ability. Statistical comparisons of groups were conducted using either analysis of variance (ANOVA) or independent samples t-tests to examine between group differences in general learning ability and performance on individual learning tasks. Correlations between factor scores, measures of behavior (exploratory, social, and sensory/motor function) and stress reactivity (stress induced CORT) were also assessed. Timelines of the experimental procedures for the imposed (IMP) (Figure 1A) and the innate (INN) (Figure 1B) groups are depicted below.

Methods

Subjects: 48 outbred CD-1 mice (*Harlan Sprague Dawley Inc., Indianapolis, IN*) weighing 25-30 grams upon arrival (approx 40-45 days of age) which is typical of previous experiments in our laboratory. Since animals were obtained pre-pubescence, it was generally accepted that they had not yet stratified into social hierarchies. Subjects were non-littermates since previous work has revealed that aggressive behaviors are more readily quantifiable in rodents that are unrelated (Zook & Adams, 1975). Upon arrival and prior to the start of the testing all subjects were housed individually and maintained on ad libitum food and water (unless noted otherwise) in a temperature-controlled vivarium on a 12-hour light/dark cycle. They were allowed to acclimate to the vivarium and were handled (removed from the home cage and held by the experimenter for

60s/day) for two weeks prior to behavioral testing (which began at approximately 60-65 days of age).

Colonization Procedure: Subjects were randomly assigned to one of two colonization conditions [imposed (IMP): n=24, innate (INN): n=24]. Subjects in the imposed (IMP) group were housed in groups of three (i.e. triads) from 68-82 days of age. Imposed group colonization took place prior to performance in the learning battery so that the effects of social stratification on learning performance could be assessed. Subjects in the innate (INN) group were colonized in triads after the learning battery (at 133 days of age) so that the relationship between innate tendencies toward subordination/dominance and learning performance could be examined and compared with any relationships between these factors that exists as a result of the imposition of subordinance or dominance (i.e. imposed group performance).

At the start of the colonization procedure, subjects were transported in their homecages to an isolated testing room (300 lux). To examine social interactions, three subjects were placed simultaneously in a neutral area; a novel standard shoebox cage**.** Behavior was observed in three 10-minute sessions during the light cycle (07:00-19:00) and three 10-minute sessions during the dark cycle (19:00-7:00). Between observations that occurred during the light cycle and those during the dark cycle, subjects were returned to the colony room. Subjects remained housed in triads until the termination of the colonization period. Subjects in the imposed (IMP) group remained colonized for 14 days before being singly housed. Subjects in the innate (INN) group were colonized for 16 hours before being separated and returned to singly housed conditions due to excessive aggression (and wounding and because 16 hr was determined to be sufficient

for the characterization of animals' social stratification). All behavioral interactions were recorded for offline measurement as detailed below.

Assessment of Social Dominance:

Three types of behavior were assessed in the colonized mice: 1) dominance-related behavior 2) submission-related behavior and 3) affiliative behavior.

Dominance-related behaviors:

1) Number of bites made: A bite by one conspecific directed toward any area of the body (i.e. head/face or back/flanks) of another conspecific in the triad. The average of the total bites made across all six observation periods was the measure used for subsequent statistical analyses.
2) Number of bites received: A bite received by an individual conspecific in the triad to any area of the body (head/face or back/flanks). The average of the total bites received across all six observation periods was the measure used for statistical analyses.
3) Latency to first attack: The time (in sec) that elapsed from when all three individuals were placed together on Day 1 until an individual subject attacked/bit another subject in the triad.
4) Total number of tail rattles: A tail rattle was defined as a "rapid lateral quivering or thrashing of the tail" (Schneider et al, 1992). Tail rattles have been reported to be correlated with aggression and social dominance (Grant and Mackintosh, 1963; Clark & Schein, 1966; Burg & Slotnick, 1983). The total number of tail rattles summed across all six observation periods was the measure used for statistical analyses.

5) Wounding: After the last observation session on Day 1, the severity of bodily wounds was ranked for each individual subject using a scale from 1-5 (1 being the least severe wounding and 5 being the most severe wounding). Typically, more serious wounding is seen in subordinate subjects while dominants are often spared extensive physical trauma.

Submission-related behavior:

1) The number of upright/sideways defensive rears: The defensive rear is considered a sign of retreat that occurs when a subject rises up on its hindlimbs with its "forearms limp, its head angled upward, and its ears retracted" (Miczek et al, 2001; Ginsburg & Allee, 1942; Miczek & O'Donnell ,1978; Miczek, Thompson, Shuster, 1982; Miczek, 1999, Grant and Mackintosh, 1963; Blanchard and Blanchard, 1977; Blanchard et al., 1979; Pellis and Pellis, 1988). We summed the total number of defensive rears across all six observation periods for statistical analyses.

Affiliative behavior:

1) Total time spent sniffing: Sniffing an "introductory/investigatory act" that was defined here as the time that a subjects' nose spent in contact with any area of the body of another conspecific in the triad (Grant & Mackintosh, 1963). The sum of the total time spent sniffing was used for statistical analyses.
2) Total time spent huddling: Huddling was defined as bodily contact between two or more mice in the triad during a period of rest that exceeded 60 sec in duration. The sum of the total time spent huddling was the measure used for statistical analyses.

Since the measures of behavioral dominance assessed here have been shown to be highly related (see below), we chose only a single dominance-related behavior (e.g. total average number of bites made) to determine social status in our subjects. Animals within each triad were categorized as "dominant" "mid submissive" or "low submissive" based on the total average number of bites made. Only the behavioral data from the "dominant" and "low submissive" animals from each triad was used for subsequent statistical analyses. In the two cases where the "low submissive" died, data from the "mid submissive" from the same triad was substituted for statistical analysis.

Learning Tasks:

The 48 CD-1 mice used in this experiment were assessed (in 2 independent replications) on the five learning tests which make up the core tasks used to evaluate general learning abilities (i.e. Lashley III maze, passive avoidance, spatial water maze, associative fear conditioning and odor guided discrimination). These tasks were chosen so that they place unique sensory, motor, motivational and information processing demands on the animals. The order of testing was designed so as to provide a temporal separation between any two tasks that are motivated by either food or water deprivation (to prevent excessive physical strain and to minimize any potential cross-task influences due to motivational factors). In addition, the testing order was designed to separate tasks based on similar processes or motor requirements (e.g. mazes of a similar nature, activity vs. passivity), again so as to minimize any potential transfer between tasks. All animals were run through the battery tasks in the following order: Lashley maze, passive avoidance, odor discrimination, fear conditioning and spatial water maze. In all learning

tasks, the animals' performance was assessed during the acquisition phase of learning (i.e., prior to reaching their stable, asymptotic level of performance). Thus the dependent measure for each task was analogous to the animals' *rate* of learning on that task, and these measures of each individual's performance could be ranked relative to other animals in the sample. We have consistently found that only the performance during acquisition of the various tasks correlate with each other. To quantify an animal's performance in tasks in which there were multiple training/test trials, performance during trials that fell within the acquisition phase were averaged. In tasks in which there was only one test trial (i.e. fear conditioning and passive avoidance), training parameters were used that were previously determined to result in sub-asymptotic responding by most animals

Spatial Water Maze:

This task requires animals to locate a submerged platform in a round pool of opaque water. Absent distinct intra-maze cues, animals' performance in this task is highly dependent on the interaction of extra-maze spatial cues. The animals are motivated by their aversion to the water. The latency and path length to locate the platform decreases over successive trials, despite entering the pool from different locations.

A round black pool (140 cm diameter, 56 cm deep) was filled to within 24 cm of the top with water made opaque by the addition of a nontoxic, water soluble black paint. A hidden 11 cm diameter perforated black platform was in a fixed location 1.5 cm below the surface of the water midway between the center and perimeter of the pool. The pool was enclosed in a ceiling-high black curtain on which five different shapes (landmark cues) were variously positioned at heights (relative to water surface) ranging from 24-150

cm. Four of these shapes were constructed of strings of white LEDs (spaced at 2.5 cm intervals) and include an "X" (66 cm arms crossing at angles 40^{o} from the pool surface), a vertical "spiral" (80 cm long, 7 cm diameter, 11 cm revolutions), a vertical line (31 cm) and a horizontal line (31 cm). The fifth cue was constructed of two adjacent 7-watt light bulbs (each 4 cm diameter). A video camera was mounted 180 cm above the center of the water surface. These cues provided the only illumination of the maze, totaling 172 lux at the water surface.

On the day prior to training, each animal was confined to the escape platform for 5 min. Training was conducted on the two subsequent days. On Day 1 of training, animals were started from one of three unique locations on each of five trials. The pool was conceptually divided into four quadrants, and one starting point was located in each of the three quadrants that did not contain the escape platform. The starting point on each trial alternated between the three available quadrants. An animal was judged to have escaped from the water (i.e., located the platform) at the moment at which all four paws were situated on the platform, provided that the animal remained on the platform for at least 5 sec. Each animal was left on the platform for a total of 20 sec, after which the trial was terminated. Trials were spaced at 10 min intervals, during which time the animals were held in their home cages. On each trial, a 90 sec limit on swimming was imposed, at which time any animal that had not located the escape platform was placed onto the platform by the experimenter, where it remained for 20 sec. The time it took for the animal to escape (latency) as well as the distance traveled (path length) to reach the platform were recorded.

Animals were observed from a remote (outside of the pool's enclosure) video monitor, and animals' performance was recorded on videotape for subsequent analysis. Day 2 of training proceeded, as did Day 1, albeit with four trials only. After the last training trial, a 90 min retention period began, after which animals were tested with a "probe" trial. On the probe test, the escape platform was removed from the pool, and all animals were started from the first position for that day. A 60 sec test was conducted and the animals' time searching in the target quadrant (that in which the escape platform was previously located) and non-target quadrants was recorded.

Lashley III Maze:

The Lashley III maze consisted of a start box, four interconnected alleys and a goal box containing a food reward. Previous studies have shown that over successive trials, the latency of rats to locate the goal box decrease, as does their number of errors (i.e., wrong turns or retracing). A Lashley III maze scaled for mice was constructed of black Plexiglas and a goal box marked by white electrical tape was located in the rear portion of the maze where 45 milligram BioServe (rodent grain) pellet served as a reinforcer. Illumination was 80 lux at the floor of the maze. The maze was isolated behind a shield of white Plexiglas to prevent the use of extra-maze landmark cues.

Food-deprived animals were acclimated and trained on two successive days. On the day prior to acclimation, all animals were provided with three food pellets in their home cages to familiarize them with the novel reinforcer. On the acclimation day, each mouse was placed in the four alleys of the maze, but the openings between the alleys were blocked so that the animals could not navigate the maze**.** Each animal was confined to the start and subsequent two alleys for 4 min, and for 6 min in the last (goal) alley, where

three food pellets were present in the goal box. This acclimation period promotes stable and high levels of activity on the subsequent training day. On the training day, each animal was placed in the start box and allowed to traverse the maze until it reached the goal box and consumed the single food pellet present in the cup. Upon consuming the food, the animal was returned to its home cage for a 20 min interval (ITI) during which the apparatus was cleaned. After the ITI, the mouse was returned to the start box to begin the next trial, and the sequence was repeated for five trials. Both the latency and errors (i.e., a turn in an incorrect direction, including those which result in path retracing) to enter the goal box were recorded on each trial.

Associative Fear Conditioning:

In this task animals received a tone (CS) paired with a mild foot shock (US). Two distinct experimental chambers (i.e., contexts;) were used, each of which was contained in a sound- and light attenuating enclosure. These boxes were designated as training and novel contexts, and differed as follows: The training chamber (16.5 x26.5 x 20 cm) was brightly illuminated (100 lx), had clear Plexiglas walls, and parallel stainless-steel rods (5mm, 10mm spacing) forming the floor. The novel chamber (23 x 21.5 x 19 cm) was dimly illuminated (6 lx) and all of the the walls and floor were constructed of clear plexiglass. In both boxes the one (60dB, 2.9 kHz) was delivered by a piezoelectric buzzer.

On Day 1 subjects were acclimated in both novel and training contexts for a 20 min period in each box. On Day 2 subjects received an 18 min training session in the training chamber. All training sessions were videotaped for subsequent offline scoring. Subjects received three tone/ shock presentations at 4, 10 and 16 min into the session.

The CS presentation consisted of a pulsed (.7 sec on .3 sec off) 20 sec tone. Immediately following the tone offset, the shock US (0.6-mA, constant-current footshock) was presented for 500 msec.

Freezing was measured during the 20 sec before (BASELINE FREEZING), during (TONE FREEZING) and after (POST SHOCK FREEZING) the 20 sec tone presentation. A measure for freezing during the training period (TRAINING FREEZING) was calculated by subtracting the time spent freezing in baseline from the time spent freezing during the tone.

On Day 3, freezing was measured during a 5 min session in the novel chamber during which time tone, but no shock was presented.

Odor Discrimination and Choice:

Rodents rapidly learn to use odors to guide appetitively-reinforced behaviors. In a procedure based on one designed for rats (Sara, Roullet, & Przybyslawski, 2001), mice learned to navigate a square field in which unique odor-marked (e.g., almond, lemon, mint) food cups were located in three corners. Although food was present in each cup, it was accessible to the animals in only one cup, the one marked by mint odor. An animal was placed in the empty corner of the field, after which it explored the field and eventually retrieved the single piece of available food. On subsequent trials, the location of the food cups was changed, but the accessible food was consistently marked by the same odor (mint). On successive trials, animals required less time to retrieve the food and made fewer approaches (i.e., "errors") to those food cups in which food was not available. Using this procedure, errorless performance was typically observed within three to four training trials.

A black Plexiglas 60 cm square field with 30 cm high walls was located in a dimly lit (10 fc) testing room with a high ventilation rate (3 min volume exchange). Three 4 x 4 x 2.0 cm (l, w, h) aluminum food cups were placed in three corners of the field. A food reinforcer (30 mg portions of chocolate flavored puffed rice) was placed in a 1.6 cm deep, 1 cm diameter depression in the center of each cup. The food in two of the cups was covered (1.0 cm below the surface of the cup) with a wire mesh so that it was not accessible to the animal, while in the third cup (the "target" cup), the food could be retrieved and consumed. A cotton-tipped laboratory swab, located between the center and rear corner of each cup, extended vertically 3 cm from the cups' surface.

Immediately prior to each trial, fresh swabs were loaded with 25 µl of either lemon, almond, or mint odorants (McCormick flavor extracts). The mint odor was always associated with the target food cup. It should be noted that in pilot studies, the odor associated with food was counterbalanced across animals and no discernible differences in performance could be detected in response to the different odors.

On the day prior to test animals were given 60 min of free feeding time at the same time of day they would receive have been acclimated. On test day, animals received four training trials in the field with three food cups present. On each trial, an animal is placed in the empty corner of the field. On Trial 1, the reinforcing food was available to the animal in the cup marked by mint odor. An additional portion of food was placed on the top surface of the same cup for the first trial only. The trial continued until the animal retrieved and consumed the food from the target cup, after which the animal was left in the chamber for an additional 20 sec and then returned to its home cage to begin a 6 min ITI. On Trials 2-4, the location of the food cups was rearranged, but the baited cup

remained consistently marked by the mint odor. Both the corner location of the mint odor and its position relative to the remaining odors was changed on each trial. On each trial, the latency to retrieve the food and errors was recorded. An error was recorded any time an animal made contact with an incorrect cup, or its nose crossed a plane parallel to the perimeter of an incorrect cup. Similarly, an error was recorded when an animal sampled (as above) the target cup but did not retrieve the available food.

One-Trial Passive Avoidance:

A chamber illuminated by dim (< 20 lux) red light was used for training and testing. Animals were confined to circular ("safe") chamber (10 cm diameter, 8 cm high). The walls and floor of this chamber were white, and the ceiling was translucent orange. The floor was comprised of plastic rods (2 mm diameter) arranged to form a pattern of 1 cm square grids. A clear exit door (3 cm square) was flush with the floor of the safe compartment, and the door was able to slide horizontally to open or close the compartment. The bottom of the exit door was located 4 cm above the floor of a second circular chamber (20 cm diameter, 12 cm high). This "unsafe" chamber had a clear ceiling and a floor comprised of 4 mm wide aluminum planks that formed a pattern of 1.5 cm square grids oriented at a 45^{o} angle relative to the grids in the safe compartment. When an animal stepped from the safe compartment through the exit door onto the floor of the unsafe compartment, a compound aversive stimulus comprised of a bright (550 Lux) white light and "siren" (60 dB above the 50 dB background) was initiated.

Animals learn to suppress movement to avoid contact with aversive stimuli. This "passive avoidance" response is exemplified in step-down avoidance procedures, where commonly, an animal is placed on a platform, whereupon stepping off of the platform it

encounters a footshock. Following just a single encounter with shock, animals are subsequently reluctant to step off of the safe platform. The animals' reluctance to leave the platform is believed to *not* reflect fear, because typical fear responses are not expressed in animals engaged in the avoidance response (Morris, 1974; Bolles, 1969). Upon stepping off the platform, animals here were exposed to a compound of bright light and loud oscillating noise rather than shock, so as not to duplicate stimuli between tasks (see fear conditioning, above). Like more common procedures, our variant of this task supports learning after only a single trial (i.e., subsequent step-down latencies will be markedly increased).

Animals were placed on the platform behind the exit blocked by the Plexiglas door. After 4 min of confinement, the door was retracted and the latency of the animal to leave the platform and make contact with the grid floor was recorded. Prior to training, baseline step-down latencies typically range from 8-20 sec. Upon contact with the floor, the door to the platform was closed and the aversive stimulus (light, noise, and vibration) was presented for 4 sec, at which time the platform door was opened to allow animals to return to the platform, where they were again confined for 5 min. At the end of this interval, the door was opened and the latency of the animal to exit the platform and step onto the grid floor (with no aversive stimulation) was recorded. The ratio of post-training to pre-training step-down latencies was calculated for each animal and this served to index learning. It has been determined that asymptotic performance is apparent in group averages following 2-3 training trials; thus performance after a single trial reflects, in most instances, sub-asymptotic learning.

<u>Exploratory and Sensory/Motor testing:</u>

All animals underwent seven assessments of physical characteristics and behavioral tendencies **(**Matzel et al, 2006; Kolata, Wu, Light, Schachner & Matzel, 2008**;** Kolata, Light & Matzel, 2008). These included activity in the unwalled areas of an open field (a common measure of exploration), exploration in the light/dark box and in an elevated plus maze (potential measures of anxiety), latency to lick a paw on a hotplate test (pain sensitivity), screen hanging (a measure of paw strength), latency to fall from a small elevated platform and separately to latency to fall from and ability to maneuver across a balance beam (measures of coordination).

Exploratory Tasks:

Open Field

A square field (46 × 46 cm) with 13 cm high walls was constructed of white Plexiglas and was located in a brightly lit room (400 lux) with a background noise of 65 dBc. The field was conceptually comprised of a 6 × 6 grid (7.65 cm quadrants), where 20 of the quadrants abutted the outer walls of the field (i.e., "wall" quadrants), and 16 quadrants were displaced from the walls and comprised the interior (i.e., "open" quadrants) of the field.

Animals were placed in the center of the field. After 20 s had elapsed (during which the animals self-selected a "starting" location), the animals' behavior was monitored for 4 min. Throughout this time the animal's entries into walled and open quadrants were recorded. An entry was recorded whenever both front paws crossed the border of a quadrant. Both total activity levels (i.e., quadrant entries regardless of category) as was the percentage of entries into unwalled (open) quadrants of the field were recorded. It

should be noted that a 4-min test was explicitly chosen because changes in exploratory behavior (not necessarily simple motor activity) were not detectable over time.

Elevated Plus Maze

The maze was constructed of grey Plexiglas with four arms in the form of a "plus." Each of these arms was 6 cm wide, and the entire maze was suspended 30 cm above a black surface. Two opposing arms of the maze were enclosed in 8 cm high, grey Plexiglas walls, while the two remaining arms were left open. The maze was located in a brightly lit room (300 lux).

Animals were placed in the center of the maze facing a closed arm, and their behavior in the maze was recorded in 1-min blocks for 4 min. Of particular interest was their total number of arm entries, their percent of total arm entries that were into open arms, closed and open arm entries as well as reentries into a previously occupied arm.

Light/Dark Discrimination Test

The rectangular box used in this task (56 x 15 x 10, l x w x h) was constructed from black Plexiglas and was located in a dimly lit room (<50 lux). The box was divided by a gray Plexiglas wall, creating two equal size compartments (28 cm l). The animals could travel between compartments by way of a small opening in the dividing wall (3 cm x 5 cm). The walls of one compartment were painted white while the other side remained black, resulting in a light and dark side. The lid on the apparatus was clear above the light side of the box and was opaque on the dark side. The lighting was arranged so that a 60 watt lamp was shined directly on the light side of the box, resulting in a differential illumination between the light side and the dark side (300 lux versus <50 lux).

The animals were placed in the dark side of the box and allowed to freely explore the apparatus for 5 min. During this time the latency to enter the light side (hind front and hind legs) fully pass through the door between compartments was recorded. In addition, a number of other exploratory measures were recorded including the percent of total time spent in the light side and the number of crossings between the light side and the dark side.

Sensory/Motor tasks:

Balance Beam

Animals were placed on a 40 × 0.7 × 2 cm (l × w × h) beam suspended 30 cm above the ground. The beam was explicitly designed so that animals do not typically fall from it. Instead, movement along the beam was the variable of interest, as movement is presumed to interact with balance. In a 4-min test, mice exhibit wide variability in the amount of movement along its length.

Hot Plate Test of Pain Sensitivity

The animals were placed on an aluminum plate which was maintained at a surface temperature of 52.6 °C. The animals' latency to raise a hind paw and to either lick or shake the paw served as the index of pain sensitivity.

Screen Hanging Test of Grip Strength

The animals were placed on the underside of a wire mesh screen (7 mm grids) tilted 40° from vertical and suspended 24 cm from ground. Both the latency to drop from the screen and the distance moved prior to dropping from the screen (cm/s; 180 maximum test duration) were recorded.

Balance Pole

Animals are placed on a platform atop a 4 mm rod coated with black rubber (shrink tubing). The rod was suspended 30 cm above ground. Latency to drop from the rod (an index of balance) was recorded.

Stress Procedure and Assessment of Plasma Corticosterone:

After colonization, subjects in the imposed (IMP) and innate (INN) groups underwent a mild stress procedure. For the imposed (IMP) group this procedure occurred 114 days after colonization and for the innate (INN) group this procedure took place 32 days after colonization. During the interim between the end of colonization and the initiation of the stress procedure, subjects in the imposed (IMP) and innate (INN) groups were singly housed.

To inflict the stress, animals were confined on a 10-cm diameter platform elevated 120 cm above the floor in a brightly lit, unfamiliar room for a 6 min period. Ten minutes after the procedure subjects were decapitated to collect trunk blood. Plasma corticosterone levels were quantified using the mouse corticosterone Enzyme Immunoassay (EIA) kit available from Cayman Chemicals (*Ann Arbor, MI*).

Results

Social Behavior data:

To examine the inter-relationships between the measures of social behavior quantified during Day 1 observations, a Pearson's product-moment correlation matrix was created (Table I). A negative correlation was observed between the total average number of bites made and the number of wounds [$r=-.30$, $n=46$, $p<.05$]. There was also a positive correlation between the total average bites made and total tail rattles [$r = .30$, $n = 46$, $p <$

.05] and a negative correlation between total tail rattles and wounding [r= -.44, n=46, p<.05]. Wounding and time spent huddling were positively correlated [r= .46, n=46, p<.05] while time spent huddling and total tail rattles were inversely correlated [r = -.29, n = 46, p =.05]. Lastly, there was a positive correlation between the latency to attack and bites received [r = .31, n = 46, p=.05].

These results indicate that both dominant and subordinate subjects consistently express dominance-related behaviors which are consistent with previously established behavioral phenotypes for submissive and dominant rodents.

Researchers have exhaustively observed and reported the social behaviors associated with social dominance and submission. However, little work has been done to systematically examine the relationships *between* these factors. We examined the correlations among eight previously identified markers of social dominance, subordination and affiliation (Miczek, 1999; Miczek, Maxson, Fish & Faccidomo, 2001; Blanchard, McKittrick, Hardy & Blanchard, 2002; Blanchard, Wall & Blanchard, 2003; Benton, Dalrymple-Alford & Brain, 1980; Benton, 1982; Uhrich, 1938; Ginsburg & Allee, 1942; Grant & Mackintosh, 1963; Brain & Hui, 2003). The results of this correlational analysis are in line with previous studies which have characterized the behavioral phenotype of dominant and subordinate mice.

The number of attacks (e.g. bites) and the number/severity of wounds received are widely used indicators of behavioral aggression/dominance and submission, respectively (Miczek, 1999; Miczek, Maxson, Fish & Faccidomo, 2001; Benton, Dalrymple-Alford & Brain, 1980; Benton, 1982; Blanchard, Wall & Blanchard, 2003). While these two measures have been anecdotally linked to one another, our study has provided formal

evidence that an inverse relationship exists between these factors. Tail rattling, another behavior that is reportedly more prevalent in mice deemed aggressive or socially dominant was also related to these measures of dominance. Specifically, mice that have increased expression of dominant behaviors (i.e. higher total average bites made and lower levels of wounding) tail rattled more than mice with low levels of social dominance (i.e. subordinates) (Grant & Mackintosh, 1963; Clark & Schein, 1966; Burg & Slotnick, 1983).

Mice with positive markers for social submission (i.e. high level of wounding and low levels of tail rattling) tended to huddle with other mice in the triad more than their dominant counterparts. These findings are in agreement with data from Uhrich (1938) who found that during periods of social instability, dominant mice rest away from the group while submissive mice huddle together. Subordinate mice with low levels of overt aggression, measured as a longer latency to attack, also received significantly more bites from conspecifics. It has been reported that submissive animals show behavioral passivity and predominantly defensive behavioral strategies (Blanchard, McKittrick, Hardy & Blanchard, 2002). It seems plausible that the lack of behavioral reactivity in these subordinates may have led to them being attacked more vigorously.

Although the behaviors measured in our study have often been reported to co-occur with one another, we have now confirmed statistically, through the use of correlational analysis, that these measures are in fact related. The high degree of relatedness among these measures in our study supports our use of the triadic colony model to study social subordination.

Exploratory Data:

In the open field test, there was a trend toward significance for between-groups differences for the percent of internal entries [F(3,26) = 2.67, p=.07]. Post-hoc comparisons revealed that IMP DOMS had a higher percentage of entries in the internal/unwalled areas of the open field than IMP SUBS (p=.01). Similarly, INN SUBS had a higher percentage of internal entries in the open field than IMP SUBS (p=.05) (Figure 2A). A separate ANOVA for the total number of entries made in the open field also showed a trend toward significance [F(3,26) = 1.57, p=.22]. Post-hoc comparison showed that IMP DOMS were more active (e.g. had more total entries) than IMP SUBS (p=.04) (Figure 2B). Run speed in the open field also showed a trend toward significance [(F(3,24)= 1.74, p=.19] with post-hoc comparisons indicating that IMP SUBS had a slower run speed (cm/sec) than IMP DOMS (p=.03) (Figure 2C).

In the elevated plus maze task, there was a trend toward significance for the percent of open entries [F(3,23) =1.95, p=.15]. A post-hoc test showed that IMP SUBS had a significantly lower percentage of open entries than did INN SUBS (p=.04) (Figure 2D). There were no other significant finding for measures in the elevated plus maze (closed entries: [F(3,23) = .12, n.s.], open entries: [F(3,23) = 1.41, n.s.], reentries: [F(3,23) = 1.32, n.s.], total entries: [F(3,23) = .65, n.s.]). In the Light/Dark discrimination task, there were no tendencies for group differences for the time spent in the dark [F(3,26) =1.42, n.s.] or the latency to enter lighted areas of the apparatus [F(3,26) = .54, n.s.].

Subjects were tested prior to the assessment of learning abilities to determine whether there were pre-existing differences in exploratory tendencies. As has been reported elsewhere, the percent of internal entries in the open field and the percent of open entries in the elevated plus maze were significantly lower in subjects that were made subordinate

prior to testing (IMP SUBS) (Ruis et al, 1999; Menzaghi et al, 1994; 1996; Heinrichs et al. 1992; 1994; Berton et al. 1998; 1999) and mice (Avgustinovich, Gorbach & Kudryavtseva, 1997; Rodgers & Cole, 1993; Haller & Halasz, 1999; Peres & Leite, 2002; mice: Kudryavtseva et al 1991 a,b). While these two measures are commonly used as indices of anxiety it is unclear whether the behavior documented here is the result of a change in the anxiety state of the imposed subordinates or if the data merely reflect a decrease in activity levels. Subjects in the imposed subordination group did have decreased total activity (number of total entries) and run speed in the open field, suggesting the latter assertion may be true. Further, there were no differences in light/dark box exploration or post-shock freezing (see data below); common measures of emotionality. To fully determine the nature of the impairment in the imposed subordinates, we would need to conduct a more in depth study including anxiety-like behavioral measures such as bolli (i.e. defecations) counts in an open field, exploration in and magnitude of escape responding in a straight alley as well as startle responses.

The lack of effects in the innate group implies that the impairments identified here are due to imposition of subordination and are not innately expressed in subjects with a tendency toward subordination.

Sensory/Motor data:

There were no significant between group differences for any of the tests of sensory or motor abilities (Balance beam: latency to fall [$F(3,26) = .33$, n.s.], distance traveled: [$F(3,26) = .94$, n.s.], Screen Hang: latency to fall: [$F(3,26) = 1.08$, n.s.], number of crossings: [$F(3,26) = .52$, n.s.]; Balance pole: latency to fall: [$F(3,26) = .80$, n.s.]; Hotplate: [$F(3,26) = 1.36$, n.s.]).

The lack of significant findings for these measures of sensory/motor function suggests that none of the animals, especially those that were colonized prior to assessment, suffered from gross motor impairments as a resulting of wounding.

Learning Battery Data:

In the Lashley III maze, mice learned the task as evidenced by a significant reduction of errors across trials [$F(4,104) = 11.97$, $p < .000001$]. However, there was no difference between groups [$F(3,26) = 1.13$, n.s.] nor was there an interaction between group and trial [$F(12,104) = .96$, n.s.]. Latencies to reach the goal box in the Lashley III maze also decreased across trials [$F(4,104) = 4.43$, $p<.05$], and group differences in latencies were also seen [$F(3,26) = 3.36$, $p<.05$] with IMP SUBS taking longer to arrive at the goal box than IMP DOMS ($p=.02$) There were no significant group by trial interaction effects [$F(12,104) = .62$, n.s.] (Figure 3A).

In the associative fear conditioning task, training levels of freezing (post-pre freezing in sec) increased across trials [$F(2,52) = 18.77$, $p < .000001$], indicating that the animals learned to freeze to the tone as expected. However there were no significant group [$F(3,26) = .31$, n.s.] or interaction [$F(6,52) = .33$, n.s.] effects, which suggests that the acquisition of learned fear witnessed here was unrelated to the subjects' social status. Similarly, separate repeated measures ANOVA's carried out for freezing assessed in the absence (baseline) and presence (tone) of the tone were significant for trial only (baseline: $F(3,26) = .64$, $p < .05$; tone: $F(3,26) = .55$, $p < .05$). A separate ANOVA for post-shock freezing was non-significant ($F(3,26) = .11$, n.s.).

For the passive avoidance task, ANOVA revealed a significant main effect for group for baseline step-down latencies [$F(3,26) = 17.52$, $p =< .000001$]. A planned comparison

demonstrated that IMP SUBS had significantly longer baseline step-down latencies compared to IMP DOMS ($p < .000001$) and INN SUBS ($p <. 000001$) (Figure 3B). A separate ANOVA for step-down latencies after stimulus presentation showed a trend towards significance [$F(3,26) = 2.62$, $p=.07$]. Subjects in the IMP SUB group had longer latencies to step down after exposure to the aversive stimulus than did subjects in the IMP DOM group ($p=.03$) (Figure 3C). The ratio of baseline step-down latencies to post-stimulus step-down latencies was also significant ($F(3,26) = 4.17$, $p < .05$). Planned comparisons confirmed that learning evidenced by the ratio of baseline step-down latencies to post-stimulus step-down latencies was impaired in IMP SUBS compared to IMP DOMS ($p=.002$) and INN SUBS ($p=.05$) (Figure 3D).

In the spatial water maze task, latencies to reach the platform decreased significantly across trials [$F(9,234) = 6.50$, $p<.000001$]. However, there were no significant group [$F(3,26) = 1.16$, ns] or interaction [$F(27,234) = .47$, n.s.] effects. Path length to the platform also decreased across the ten trials [$F(9,180) = 5.80$, $p<.000001$] and there were significant group differences for path length to the platform [$F(3,20) = 5.54$, $p<.05$], although planned comparisons revealed a significant difference between IMP SUBS and INN SUBS in Trial 5 only. There were no effects for interaction [$F(27,180) = .58$, n.s.] (Figure 3E).

In the odor discrimination task, a repeated-measures ANOVA for errors was significant for trial [($F(3,78) = 18.15$, $p<.000001$] but was non-significant for group [($F(3,26) = .42$, n.s.] or interaction [($F(9,78) = .72$, n.s.]. Repeated-measures ANOVA for latency was significant for trial [$F(3,78) = 25.21$, $p<.000001$], however there were no significant group [$F(3,26) = .67$, n.s.] or interaction effects[$F(9,78) = .83$, n.s.].

Data from the sensorimotor battery revealed that subsequent to subordination, subjects had expressed behavior consistent with a decrease in exploratory tendencies or increases in behavioral anxiety. Latency data from tasks in the learning battery support these findings. In the Lashley III maze, the mean latency to arrive at the goal box was significantly greater in imposed subordinates versus imposed dominants. While this finding could be interpreted as a learning effect; this seems unlikely since imposed subordinates learned at the same rate and to the same level as the other subjects, as evidenced by the number of errors made. Similar increases in latency were seen in the passive avoidance task. Both baseline and post-stimulus step-down latencies were significantly elevated in imposed (IMP) subordinates. Unlike what was seen in the Lashley III Maze, imposed subordinates had corresponding deficits in learning in the passive avoidance task evidenced by a significantly lower ratio of baseline step-down latencies to post-stimulus step-down latencies.

Factor score data:

An unrotated principal components factor analysis of the performance data for the five tasks that comprise the learning battery produced two factors which accounted for a total of 56% of the variance in performance. The first factor accounted for 29% of the total variance (eigenvalue = 1.46, n = 48) (Table 2). From this first factor, factor scores were extracted to represent animals' general learning scores. ANOVA revealed a main of effect of group for factor scores [$F(3,26) = 5.21$, $p = <.01$]. Post-hoc comparisons revealed that imposed subordinates (IMP SUBS) had significantly lower factor scores than imposed dominants (IMP DOMS) ($p = .005$), innate dominants (INN DOMS) ($p = .002$) and innate subordinates (INN SUBS) ($p = .003$) (FIGURE 4A).

Consistent with prior studies in our laboratory, a secondary factor was extracted which accounted for 27% of the variance (eigenvalue = 1.37, n=48) (Table 2). Upon examining the structure of this factor we concluded that it is not readily interpretable and will not be further considered here.

We conducted a second principal components analysis to determine the relationship between general learning and measures of exploration and sensory/motor function since general learning abilities have been shown to be related to exploratory tendencies but not sensory/ motor traits (Matzel et al, 2006). This unrotated principal components factor analysis produced a factor which accounted for a total of 24% of the variance in performance (eigenvalue = 4.42, n = 25) (Table 3). Open field (total and % internal entries) and elevated plus maze (closed, open, % open entries) measures loaded in the opposite direction of general learning scores.

Lastly, since we saw group differences in factor scores, we wanted to examine how the individual social behaviors were related to general learning scores. A final unrotated principal components factor analysis of general learning abilities and measures from the dominance social behaviors produced a factor which accounted for a total of 26% of the variance in performance (eigenvalue = 2.11, n = 25) (Table 4). Huddling and defensive rears loaded in the same direction as general learning on this factor.

We found that that the imposition of subordination led to decrements in general learning performance. Conversely, subjects that were innately subordinate showed no learning impairments. These findings illustrate that absent the imposition of

subordination, mice with a tendency towards subordination learn comparably to dominant animals. The finding that general learning abilities were significantly different between groups although only a few significant effects were seen on performance in individual tasks (see above) may at first seem puzzling. Yet, one must consider that although general learning/intelligence has an influence over domain-specific abilities, it is not necessarily the primary determinant of performance on individual tasks as it only accounts for 29% of the variance in performance across all of the tasks in the battery.

Here, as in previous studies in our laboratory, open field (OF) and elevated plus maze (EPM) exploration measures were inversely related to general learning scores (Matzel et al., 2006, Grossman et al., 2007, Light et al., 2008). Lastly, behavioral measures linked with submission (e.g. defensive rears and huddling) loaded in the same direction as general learning scores, which validates our finding that subordinates have impaired learning.

Stress-Induced Corticosterone Data:

A between-groups ANOVA showed a trend toward significance [$F(3,26) = 1.45$, $p>.25$] and a planned comparison post-hoc was significant with impossed subordinates (IMP SUBS) having significantly lower CORT levels than innate subjects (INN SUBS) ($p<.05$) (Figure 5A). A t-test for independent samples between subjects in the PRE group and subjects in the INN group subjects was significant [$t(1,28) = 2.08$, $p< .05$] whereby INN group subjects had higher levels of plasma corticosterone than subjects in the IMP group (Figure 4A). A t-test for independent samples between all subordinate and all dominant subjects (regardless of status) was non-significant [$t(1,28) = .46$, n.s.].

A Pearson's product-moment correlation matrix was constructed for stress-induced CORT and both general learning factors revealed no significant relationships between these factors (TABLE 5). A separate correlation matrix including stress-induced CORT and measures from the sensorimotor battery showed only one significant correlation between balance beam latency to fall and stress induced CORT [$r = -.39$, $n = 28$, $p = .05$]. It is more than likely that this effect is due to chance and has no meaningful implications.

A principal components analysis which included stress-induced CORT and dominance measures produced a factor which accounted for 52% of the variance (eigenvalue = 4.64, n= 29) (TABLE 6). Analysis of the structure of this factor suggests that dominance measures positive for agonstic involvement (e.g. sniffing, bites made, bites received, defensive rears, tail rattling, wounding) were highly related to stress induced CORT. One could conclude that the experience of taking part in an agonstic encounter regardless of whether an animal was subordinate or dominant is related to stress-induced CORT reactivity.

Another principal components analysis which included stress-induced CORT and sensorimotor measures produced a factor which accounted for 27% of the variance (eigenvalue = 4.53, n= 25) (TABLE 7). Open field (total and % internal entries) and elevated plus maze (closed, open, % open entries) measures loaded in the same direction of stress-induced CORT levels, a pattern of results that we have previously observed (Matzel et al, 2006).

Here we have shown that the experience of triadic colonization leads to an increase in stress reactivity (e.g. stress-induced CORT levels to a mild stressor) that persists for at least one month after removal from the social environment. This is line with prior studies

that show prolonged HPA axis activity in response to social subordination (for review see: Blanchard et al., 2003).

In our study, there was no relationship between stress-induced CORT levels and general learning performance. Taken together with prior studies from our laboratory, these findings support the disassociation between stress reactivity and general learning performance (Matzel et al., 2006; Grossman et al., 2007).

Our data also revealed that participating in an agonistic encounter is related to stress reactivity. Regardless of their social status (subordinate or dominant) it appears that involvement in a fighting experience is related to increases in stress-induced CORT subsequent to agonistic encounters.

Lastly, in agreement with a prior study from our laboratory, we found that elevated plus maze and open field measures were related to stress-induced CORT (Matzel et al, 2006).

General Discussion

The goal of the current experiment was to confirm and expand upon previous reports which detail the effects of social subordination on cognition. To that end, we established a triadic colony model to examine the impact of this type of social stress on both domain-specific and domain-general learning processes. We found that subordination under imposed, but not innate conditions led to alterations in some domain-specific learning tests. More importantly though we found that general learning abilities were severely impaired in subjects exposed to social subordination prior to the learning experience.

Ours is the first animal study to directly examine the effects of social subordination on general learning abilities making these findings particularly intriguing.

In addition to the learning data, we found that imposition of subordination resulted in a decrease in exploratory tendencies, although, we were unable to conclude whether these behaviors were indicative of increased anxiety. It is often difficult to distinguish differences in learning from differences in performance (e.g. exploration, motor differences etc.). While we believe that the imposed subordinates in our study exhibited deficits in both learning and performance, we must fully consider whether our battery data reflect modifications in learning processes or whether they are merely the consequence of variations in exploratory tendencies.

In the passive avoidance test, compared to the other subjects, imposed subordinates had significantly longer step-down latencies both before and after stimulus presentation, yet, they also failed to learn the operant association. We used a ratio to normalize for pre-existing differences in step-down latencies in order to isolate learning performance. The ratio data revealed that imposed subordinates did not increase their step-down latencies in response to stimulus presentation and therefore did not effectively learn the task. The inability to properly form associations between one's actions and negative consequences could prove detrimental and result in further exposure to stressful events.

Despite their differences in exploratory tendencies imposed subordinates showed no impairments in acquisition of the water maze task. This finding is in agreement with several prior studies (Francia et al., 2006; Moregrega et al., 2003). While some researchers have reported deficits in water maze performance in subordinate animals their studies utilized middle-aged subjects which were older than the subjects in our study

(Francia et al., 2006). It is possible that aged animals are more susceptible to the negative effects of subordination on spatial learning. Water maze impairments have also been documented in a subset of subordinates deemed highly stress reactive (Touyarot, Venero, Sandi, 2004). In our study, we did not distinguish between high and low stress reactive subordinates which may have led to the discrepancy between the findings in their study and ours.

In line with previous studies from our laboratory we saw an inverse relationship between measures of exploration and general learning abilities (Matzel et al, 2006; Light et al, 2010, Kolata et al, 2007, 2008; Grossman et al, 2007). The expression of exploratory behavior in animals is said to be regulated by at least two opposing biological functions, the tendency to seek novelty and neophobia, or anxiety (Montgomery, 1955; Whimbey & Denenberg, 1967). In the current study, we found that that stress-induced CORT levels (i.e. stress reactivity) were unrelated to general learning abilities, suggesting that differences in individual stress reactivity are not responsible for variations in general learning performance. An earlier study in our laboratory addressed the question of whether general learning abilities are mediated by stress reactivity by attempting to pharmacologically disassociate stress reactivity from exploration and general learning (Grossman et al., 2007). Mice were administered the anxiolytic chlorodiazepoxide (CDP) prior to being tested in the learning battery. CDP-treated mice exhibited reductions in stress-induced corticosterone levels and behavioral reactivity to mild stressors and a corresponding increase in exploration. However, CDP-treated mice did not show facilitated acquisition of any of the learning tasks and their general learning performance was comparable to controls. These results indicate that although reducing

stress reactivity increases exploration, this does not result in an enhancement of general learning abilities, suggesting that the relationship between general learning and exploration is not mediated by stress reactivity.

In a separate experiment we examined whether differences in the propensity to explore translate into differences in learning battery performance (Light et al., 2008). Specifically we asked if we could augment animals' general learning abilities by inducing an increase in exploratory behaviors. Young and adult mice were exposed to twelve novel environments after which exploratory tendencies and general learning abilities were assessed. Exposure to novel environments increased overall exploration however; there was no effect on general learning abilities when subjects were tested as adults. Therefore, while exposure to novel environments promote long-lasting increases in mice's exploratory tendencies, these increases in exploration did not appear to causally impact general learning abilities (Light, Kolata, Hale, Grossman, Matzel, 2008).

While it is unlikely that stress reactivity (i.e. stress-induced CORT) mediates general learning abilities, it is still possible that that increases in glucocorticoid (e.g. CORT) expression in response to subordination underlie the changes in general learning abilities seen in this study. To assess the relationship between CORT and learning abilities in socially stressed animals, we would need to quantify circulating CORT levels after colonization but prior to performance in learning tasks and determine if these measures were correlated. If they were, further studies would need to be conducted to establish whether glucocorticoids are necessary and sufficient to produce decrements in general learning abilities similar to those seen in our study.

Glucocorticoids exert direct effects in areas key to stress and learning by binding to glucocorticoid receptors in these regions. In the larger stress and learning literature, numerous brain areas (e.g. hippocampus, amygdala, prefrontal cortex, BNST etc.) have been implicated in cognitive stress effects (Bangasser & Shors, 2010; Shors, 2006; Buwalda et al, 2005; Gould, Tanapat, McEwen, Flügge, Fuchs, 1998; Magariños, McEwen, Flügge, Fuchs, 1996; McKitrick et al., 2000; Von Frijtag et al., 2000; McGaugh & Roozendaal, 2002; McIntyre, Power, Roozendaal, McGaugh, 2003). However, social stress studies have only looked at the effects of subordination on the hippocampal structure.

Deficits in hippocampal long-term potentiation have been reported in response to social subordination stress (Bodnoff et al., 1995; von Fritag, 2001). Long-term potentiation has been implicated in several learning and memory processes, particularly spatial learning. Although we saw no differences between subordinates and dominants in spatial learning it is still possible that other tasks in the battery have components which are mediated by hippocampal LTP and that decreases in this form of synaptic plasticity could contribute to the cognitive deficits we saw in the current study.

Reductions in hippocampal volume have also been reported in response to social subordination stress (Buwalda et al., 2005). The change in volume is thought to reflect corresponding alterations in dendritic structure (Buwalda et al., 2005). Specifically, chronic social stress results in the atrophy of apical dendritites of hippocampal CA3 pyramidal cells (Magarinos, McEwen, Flugge, Fuchs, 1996). Compared to controls, animals exposed to subordination stress have fewer CA3 neuron apical dendritic arbors, branch points and a smaller apical dendritic length (McKitrick et al., 2000; Magarinos,

McEwen, Flugge, Fuchs, 1996). There are conflicting opinions as to the functional significance of CA3 dendritic atrophy. It has been suggested that atrophy may signify the beginning of irreversible hippocampal damage, yet, it has also been proposed that dendritic atrophy could be a protective measure meant to prevent against through neuronal loss by retracting connections with excitatory neurons (Czeh et al, 2001). Clearly, further work must be done to examine the reasons for these structural changes.

Social subordination stress has also been shown to result in an inhibition of neurogenesis in the dentate gyrus (DG) area of the hippocampus (Gould, 1998; Buwalda et al, 2005; Czeh et al., 2001). While the functional implications of decreased neurogenesis are not entirely clear it is has been postulated that stabilizing DG granule cells may be necessary for contextual learning to take place during the course of the stressful experience (Gould, 1998).

The stress-related effects on dendrtric atrophy and neurogenesis can be prevented by pre-treatment with the drug tianeptine, a selective serotonin reuptake enhancer (Czeh et al, 2001). Selective serotonin reuptake enhancers are used as antidepressants and are as effective as the more traditional selective serotonin reuptake inhibitors (SSRI's) despite their different mechanism of action.

If the stress effects on hippocampal morphology and spatial learning are prevented by treatment with SSRE's, it is plausible that other stress-related cognitive deficits, including the effects on general learning seen here, could be alleviated by similar pharmacological treatments. In humans, previous experience of social stress (including subordination) is a risk factor for mental disorders such as anxiety and depression (Brown, 1998; Kendler, Karkowski, Prescott, 1999; Kessler, 1997; Pine, Cohen, Johnson,

Brook, 2002; Caspi et al., 2003). Likewise, animal studies have shown that social subordination stress can produce depression-like symptoms (Wood, Walker, Valentino, Bhatnagar, 2010; von Fritag et al., 2000; Malatynska & Knapp, 2005; Shivley, Laber-Laird,Anton, 1997). Cognitive deficits are widely documented in individuals with depression and related clinical psychopathologies (Elliott, Sahakian, McKay, Herrod, 1996; Purcell, Maruff, Kyrios, Pantelis,1997; Murphy, Michael, Robbins, Sahakian, 2003; Beaudreau; O'Hara, 2008; Stein, Hollander, O. Rothbaum, 2009; Gualtieri CT, Johnson LG, Benedict, 2006;Jeste DV, Heaton SC, Paulsen JS, Ercoli L, Harris J, Heaton RK, 1996). As such, several studies have examined the effects of antidepressant treatment on cognitive deficits in individuals with depression. On the whole they found that anti-depressant therapy improved but did not normalize cognitive functions (Doraiswamy et al., 2003; Nebes, et al., 2003; Butters et al., 2000; Frasch et al., 2000; Jeste et al., 1996). However, a recent study found that patients who suffer from depression and were being treated with the antidepressant bupropion performed at levels comparable to non-depressed controls across a wide range of neurocognitive tasks (Gualtieri & Johnson, 2007). Participants who were treated with the anti-depressant venlafaxin or other traditional selective serotonin reuptake inhibitors (SSRI's) showed cognitive deficits when compared to controls. The authors believe that bupropion was more effective at enhancing cognition due to the fact that, unlike the SSRI's, bupropion increases noreadrenergic activity. Increased norepenephrine metabolism has been associated with better cognitive performance (Ordway, Schwartz & Frazer, 2007). While these findings are promising they are also flawed. In this study researchers were unable to obtain baseline (i.e. non-drug) measures of learning performance. Further, they did not

randomly assign subjects to drug treatment groups. A more efficient way to study the effects of anti-depressants and other pharmacological treatments on stress-related cognitive deficits would be to employ an animal model of social stress wherein subjects co-express cognitive deficits and depression like behaviors. Under these conditions, the experimenter could control drug administration and obtain pre and post-drug measures of learning and memory function.

While drug treatments may be suitable for clinical populations with psychiatric disorders, such treatments may not be appropriate for subordinate individuals who are exposed to social stress but do not suffer from mental disorders per se. Equally, it does not seem feasible to treat children who are victims of bullying with medication simply to counteract the negative effects of subordination on academic performance. Data from the current study suggest that absent the imposition of subordination individuals who are innately disposed to subordination do not display cognitive deficits. It seems then that an efficient behavioral intervention would involve eliminating or limiting exposure to subordination stress. Individuals often suffer subordination in the form of bullying in the school or workplace setting. The introduction of anti-bullying programs may serve to reduce the occurrences of bullying and thereby decrease social stress and ameliorate the negative effects on learning seen in bullied individuals.

Cognitive therapies may also be useful in counteracting the negative effects of submission. In humans, working memory training has been shown to improve cognition. We have recently developed a working memory training regimen in mice which has been shown to successfully increase both selective attention and general learning performance (Light et al, 2010). Using the model of social stress established in the present study, we

could test whether working memory training administered either before or after social stress could prevent or alleviate the negative effects of learning which result from subordination.

Overall, the findings of our study underscore the detrimental consequences of social stress on cognition. The model we have established here is uniquely suited to probe the biological basis of the social stress-related learning deficits which are conserved across mammalian species. Further, our model can be utilized to examine how potential pharmacological and behavioral interventions may be instituted in order to improve the quality of life for individuals who suffer due to the stress of social subordination.

BIBLIOGRAPHY

Alzoubi, KH; Abdul-Razzak, KK; Khabour, OF; Al-Tuweiq, GM; Alzubi, MA and Alkadhi, KA. (2009). Adverse effect of combination of chronic psychosocial stress and high fat diet on hippocampus-dependent memory in rats. *Behavioural Brain Research*, 204, 1(1), 117-123.

Avgustinovich, DF; Gorbach, OV; Kudryavtseva, NN. (1997). Comparative analysis of anxiety-like behavior in partition and plus-maze tests after agonistic interactions in mice. *Physiol Behav*., 61(1), 37-43.

Bangasser, DA; Shors, TJ. (2010). Critical brain circuits at the intersection between stress and learning. *Neurosci Biobehav Rev*., Feb 11.

Barnard, CJ; Luo, N. (2002) Acquisition of dominance status affects maze learning in mice. *Behav Processes*, 60(1),53-59.

Bartolomucci, A; de Biurrun, G; Czéh, B; van Kampen, M; Fuchs, E. (2002). Selective enhancement of spatial learning under chronic psychosocial stress. *Eur J Neurosci.*,15(11),1863-6.

Bartolomucci, A; Palanza, P; Gaspani, L; Limiroli, E; Panerai, AE; Ceresini, G; Poli, MD; Parmigiani, S. (2001) Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiol Behav*.,73(3):401-10.

Baumeister, RF; Twenge, JM; Nuss, CK. (2002) Effects of social exclusion on cognitive processes: anticipated aloneness reduces intelligent thought. *J Pers Soc Psychol*, 83(4):817-27.

Benton, D. (1982). Is the concept of dominance useful in understanding rodent behavior. *Aggressive Behavior*, 8(2), 104 – 107.

Benton, D; Dalrymple-Alford, JC; Brain, PF. (1980). Comparisons of measures of dominance in the laboratory mouse. *Animal Behaviour*, 28(4), 1274-1279.

Berton, O; Aguerre, S; Sarrieau, A; Mormede, P; Chaouloff, F. (1998). Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience*, 82(1),147-59.

Berton, O; Durand, M; Aguerre, S; Mormède, P; Chaouloff, F. (1999). Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. N*euroscience*, 92(1),327-41.

Beylin, AV; Shors TJ. (2003). Glucocorticoids are necessary for enhancing the acquisition of associative memories after acute stressful experience. *Horm Behav*., 43(1),124-31.

Blanchard, DC; Sakai, RR; McEwen, B; Weiss, SM; Blanchard, RJ. Subordination stress: behavioral, brain, and neuroendocrine correlates. (1993) *Behav Brain Res.*,58(1-2):113-21.

Blanchard, DC; Spencer, RL; Weiss, SM; Blanchard, RJ; McEwen, B; Sakai, RR. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology*., 20(2), 117-34.

Blanchard, RJ; Wall, PM; Blanchard, DC. (2003). Problems in the study of rodent aggression. *Horm Behav*., 44(3), 161-70.

Blanchard DC, McKittrick CR, Hardy MP, and Blanchard RJ. 2002, Effects of Social Stress on Hormones, Brain and Behavior. In: DW Pfaff, AP Arnold, AM Etgen, SE Fahrbach, and RT Rubin (Eds.) Hormones, Brain and Behavior, volume 1, pp. 735-772. San Diego: Academic Press.

Blanchard, R.J., Takahashi, L.K., Fukunaga, K.K., Blanchard, D.C. (1977). Functions of the vibrissae in the defensive and aggressive behavior of the rat. *Aggress. Behav*., 3, 231–240.

Bliss, T; Collingridge G; Richard Morris, R. (2004) Long-term Potentiation: Enhancing Neuroscience for 30 Years. Oxford University Press:UK.

Bodnoff, SR; Humphreys, AG; Lehman, JC; Diamond, DM; Rose, GM; Meaney, MJ. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J Neurosci*.,15(1), 61-9.

Bolles, RC. Avoidance and escape learning: simultaneous acquisition of different responses. (1969*) J Comp Physiol Psychol.*, 68(3):355-8.

Brain, PF; Hui, SE (2003). Variability in patterns of intra-specific biting attack in commonly used genetic lines of laboratory mice. *Scand. J. Lab. Anim. Sci*, 3(30), 113-127.

Brown G W (1998). Genetic and population perspectives on life events and depression. *oc. Psychiatry Psychiatr. Epidemiol.*,33(8), 363-72.

Burg, RD; Slotnick, BM (1983). Response of colony mice to intruders with different fighting experience. *Aggressive Behavior***,** 9(1), 49-58**.**

Butters, MA; Becker, JT; Nebes (2000). Changes in cognitive functioning following treatment of late-life depression. *Am J Psychiatry*, 157, 1949–1954.

Buwalda, B; Kole, MH; Veenema, AH; Huininga, M; de Boer, SF; Korte, SM; Koolhaas, JM. (2005). Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. *Neuroscience & Biobehavioral Rev.*, 29(1), 83-97.

Buwalda, B; de Boer, SF; Schmidt, ED; Felszeghy, K; Nyakas, C; Sgoifo, A; Van der Vegt, BJ; Tilders, FJ; Bohus, B; Koolhaas, JM. (1999). Long-lasting deficient dexamethasone suppression of hypothalamic-pituitary-adrenocortical activation following peripheral CRF challenge in socially defeated rats. *J Neuroendocrinol.*,11(7), 513-20.

Caspi, A; Sugden, K; Moffitt, TE; Taylor, A; Craig, IW; Harrington, HL; McClay, J; Mill, J; Martin, J; Braithwaite, A; Poulton, R. (2003). Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. *Science*, 301(5631), pp. 386 – 389.

Chao, HM; Blanchard, DC; Blanchard, RJ; McEwen, BS; Sakai RR. (1993) The effect of social stress on hippocampal gene expression. *Mol Cell Neurosci.*, 4(6):543-8.

Clark, LH; Schein, MW (1966). Activities associated with conflict behavior in mice. *Animal Behaviour*, 14(1), 44-49.

Coe, C; Mendoza, SP; Levine, S. (1979). Social stastus constrains the stress response in the squirrel monkey. *Physiol Behav.*, 23(4), 633-8.

Czéh, B; Michaelis, T; Watanabe, T; Frahms, J; De Biurrun, G; Van Kampen, M; Bartolomucci, A; Fuchs, E (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proc Natl Acad Sci U S A.*, 98(22), 12796–12801.

de Kloet, ER; Oitzl, MS; Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends Neurosci.*, 22(10), 422-6.

de Kloet, ER; Schmidt, M; Meijer, OC. (2005) Corticosteroid receptors and HPA axis regulation. In Handbook of stress and the brain. 1. The neurobiology of stress By N. H. Kalin, J. M. H. M. Reul. NY: Elsevier Science & Technology Books.

Der-Avakian, A; Will, MJ; Bland, ST; Deak, T; Nguyen, KT; Schmid, MJ; Spencer, RL; Watkins, LR; Maier, SF. (2005). Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats. *Psychopharmacology;* 179(2), 409-17.

Dijkstra, H; Tilders, FJ; Hiehle, MA; Smelik, PG. (1992). Hormonal reactions to fighting in rat colonies: prolactin rises during defence, not during offence. *Physiol Behav.*, 51(5), 961-8.

Dong, Q; Salva, A; Sottas, CM; Niu, E; Holmes, M; Hardy, MP. (2004) Rapid glucocorticoid mediation of suppressed testosterone biosynthesis in male mice subjected to immobilization stress. *J Androl.*, 25(6), 973-81.

Doraiswamy PM, Krishnan KR, Oxman T (2003). Does antidepressant therapy improve cognition in elderly depressed patients? *J Gerontol A Biol Sci Med Sci*., 58, M1137–M1144.

Elliott, R; Sahakian, BJ; McKay, AP; Herrod, JJ (1996). Neuropsychological impairments in unipolar depression: The influence of perceived failure on subsequent performance. Psychological Medicine: A Journal of Research in Psychiatry and the Allied Sciences, 26(5), 975-989.

Ely, DL; Henry JP. (1978). Neuroendocrine response patterns in dominant and subordinate mice. *Horm Behav.*, 10(2):156-69.

Fitchett, AE; Collins, SA; Barnard, CJ; Cassaday, HJ. (2005) Subordinate male mice show long-lasting differences in spatial learning that persist when housed alone. *Neurobiol Learn Mem.*, Nov; 84(3):247-51.

Fitchett, A.E.; Barnard, C.J.; Cassaday, H.J. (2009). Corticosterone differences rather than social housing predict performance of T-maze alternation in male CD-1 mice. *Animal Welfare*, 18(1), 21-31(11).

Francia, N; Cirulli, F; Chiarotti, F; Antonelli, A; Aloe, L; Alleva, E. (2006) Spatial memory deficits in middle-aged mice correlate with lower exploratory activity and a subordinate status: role of hippocampal neurotrophins. *Eur J Neurosci.*, 23(3):711-28.

Frasch, K; Bretschneider, S; Bullacher, C; Hess, R; Wittek, R; Neumann, NU. (2000). Do cognitive deficits in depressive disorders remit? *Psychiatr Prax*, 27, 291–295.

Giammanco, M; Tabacchi, G; Giammanco, S; Di Majo, D; La Guardia, M. (2005) Testosterone and aggressiveness. *Med Sci Moni,*. 11(4), RA136-45.

Ginsburg, B; Allee, W. C. (1942). Some Effects of Conditioning on Social Dominance and Subordination in Inbred Strains of Mice. *Physiological Zoology*, 15(4), 485-506.

Gottfredson, LS (1998).The General Intelligence Factor. Exploring Intelligence, Scientific American Presents, Winter, 1998.

Gould, E; Tanapat, P; McEwen, BS; Flügge, G; Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA, 17;95(6), 3168-71.

Gould, E; Tanapat, P.(1999). Stress and hippocampal neurogenesis. *Biol Psychiatry*, 46(11), 1472-9.

Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A. & Fuchs, E. (1997). Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.*, 17, 2492–2498.

Grant, EC; Mackintosh, JH. (1963). A comparison of the social postures of some common laboratory rodents. *Behaviour*, 21(3-4), 246-259.

Grossman, HC; Hale, G; Light, K; Kolata, S; Townsend, DA; Goldfarb, Y; Kusnecov, A; Matzel, LD. (2007). Pharmacological modulation of stress reactivity dissociates general learning ability from the propensity for exploration. *Behav Neurosci.*, 121(5), 949-64.

Gualtieri, CT; Johnson, LG (2007). Bupropion Normalizes Cognitive Performance in Patients With Depression. *Med Gen Med*., 9(1), 22.

Gualtieri CT, Johnson LG, Benedict KB. (2006). Neurocognition in depression: patients on and off medication versus healthy comparison subjects. *J Neuropsychiatry Clin Neurosci*., 18(2), 217-25.

Haemisch, A.(1990). Coping with social conflict, and short-term changes of plasma cortisol titers in familiar and unfamiliar environments. *Physiol Behav*., 47(6), 1265-70.

Haller, J; Halász, J. (1999). Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity.*Psychopharmacology*,144(4), 311-5.

Hardy, MP; Sottas, CM; Ge, R; McKittrick, CR; Tamashiro, KL; McEwen, BS; Haider, SG; Markham, CM; Blanchard, RJ; Blanchard, DC; Sakai, RR.(2002). Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioral dominance. *Biol Reprod*.,67(6), 1750-5.

Heinrichs, SC; Menzaghi, F; Pich, EM; Baldwin, HA; Rassnick, S; Britton, KT; Koob, GF. (1994). Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology*, 111(3), 179-86.

Heinrichs, SC; Pich, EM; Miczek, KA; Britton, KT; Koob, GF. (1992). Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res.*, 29, 581(2), 190-7.

Herrero, A; Sandi, C; Venero, C. (2006). Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralcorticoid receptors. *Neurobio. of Learn. & Mem., 86*, 150-159.

Holmes, A; Wellman, CL. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neurosci Biobehav Rev.,* 33(6), 773-83.

Hoover, H.; Oliver, R; Hazler, RJ. (1992). Bullying: Perceptions of adolescent victims in the Midwestern USA. *School Psychology International*, 13(1), 5-16.

Horwood, J; Salvi, G; Thomas, K; Duffy, L; Gunnell, D; Hollis, C; Lewis, G; Menezes, P; Thompson, A; Wolke, D; Zammit, S; Harrison, G. (2008). IQ and non-clinical psychotic symptoms in 12-year-olds: results from the ALSPAC birth Cohort. The British Journal of Psychiatry, 193, 185–191.

Huhman, KL; Moore, TO; Mougey, EH; Meyerhoff, JL. (1992). Hormonal responses to fighting in hamsters: separation of physical and psychological causes. *Physiol Behav.*,51(5), 1083-6.

Jeste DV, Heaton SC, Paulsen JS, Ercoli L, Harris J, Heaton RK. (1996). Clinical and neuropsychological comparison of psychotic depression with nonpsychotic depression and schizophrenia. *Am J Psychiatry*,153(4),490-6.

Joëls, M; Pu, Z; Wiegert, O; Oitzl, MS; Krugers, HJ. (2006). Learning under stress: how does it work? *Trends Cogn Sci.*,10(4), 152-8.

Joëls, M; Verkuyl, JM; Van Riel, E. (2003). Hippocampal and hypothalamic function after chronic stress. *Ann N Y Acad Sci.*, 1007, 367-78.

Johnson, GM. (2005). Student Alienation, Academic Achievement and WebCT Use. *Educational Technology & Society*, 8(2), 179-189.

Jöhren, O; Flügge, G; Fuchs, E. (1994a). Regulation of hippocampal glucocorticoid receptor gene expression by psychosocial conflict. *Ann N Y Acad Sci.*, 746, 429-30.

Jöhren, O; Flügge, G; Fuchs, E. (1994b). Hippocampal glucocorticoid receptor expression in the tree shrew: regulation by psychosocial conflict. *Cell Mol Neurobiol.,* 14(3), 281-96.

Keeney, AJ; Hogg, S. (1999). Behavioural consequences of repeated social defeat in the mouse: preliminary evaluation of a potential animal model of depression. *Behav Pharmacol.,* 10(8), 753-64.

Kendler KS, Karkowski LM, Prescott CA. (1999). Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry,* 156(6), 837-41.

Kessler RC. (1997) The effects of stressful life events on depression. *Annu Rev Psychol.,* 48:191-214.

Kim, JJ; Diamond, DM. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Reviews Neuroscience,* 3, 453-462.

Kim, JJ; Haller, J. (2007). Glucocorticoid hyper-and hypofunction: stress effects on cognition and aggression. *Ann N Y Acad Sci*., 1113, 291-303.

Kim, JJ; Song, EY; Kosten, TA. (2006). Stress effects in the hippocampus: synaptic plasticity and memory. *Stress,* 9(1), 1-11.

Knox, E; Conti-Ramsden, G. (2003). Bullying risks of 11-year-old children with specific language impairment (SLI): does school placement matter? *Int J Lang Commun Disord,* 38(1), 1-12.

Kolata, S; Light, K; Grossman, HC; Hale, G; Matzel, LD. (2007) Selective attention is a primary determinant of the relationship between working memory and general learning ability in outbred mice. *Learn Mem*., 14(1), 22-8.

Kolata, S; Light, K; Matzel, LD. (2008) Domain specific and domain general learning factors are expressed in genetically heterogeneous CD-1 mice. *Intelligence,* 36(6), 619-629.

Kolata, S; Wu, J; Light, K; Schachner, M; Matzel, LD. (2008). Impaired working memory duration but normal learning abilities found in mice that are conditionally deficient in the close homolog of L1. J Neurosci.,28(50), 13505-10.

Koolhaas, JM; Schuurman, T; Wiepkema, PR. (1980) The organization of intraspecific agonistic behaviour in the rat. *Prog Neurobiol;* 15(3), 247-68.

Korte, SM; De Boer, SF. (2003). A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. *European Journal of Pharmacology,* 463(1-3), 163-175.

Krugers, HJ; Douma, BR; Andringa, G; Bohus, B; Korf, J; Luiten, PG. (1997) Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus.,* 7(4), 427-36.

Kudryavtseva, NN; Bakshtanovskaya, IV; Koryakina, LA. (1991a). Social model of depression in mice of C57BL/6J strain. *Pharmacol Biochem Behav*., 38(2), 315-20.

Kudryavtseva, NN; Madorskaya, IA; Bakshtanovskaya, IV. (1991b) Social success and voluntary ethanol consumption in mice of C57BL/6J and CBA/Lac strains. *Physiol Behav*., 50(1), 143-6.

Kvist, B. (1989) Learning in mice selectively bred for high and low aggressiveness. *Psychol Rep*., 64(1):127-30.

Light, KR; Kolata, S; Hale, G; Grossman, H; Matzel, LD. (2008) Up-regulation of exploratory tendencies does not enhance general learning abilities in juvenile or young-adult outbred mice. *Neurobiol Learn Mem*., 90(2):317-29.

Light, KR; Kolata, S; Wass, C; Denman-Brice, A; Zagalsky, R; Matzel, LD. (2010). Working Memory Training Promotes General Cognitive Abilities in Genetically Heterogeneous Mice. *Curr Biol.*, 777-782.

Lupien, SJ; McEwen, BS; Gunnar, MR; Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci., 10*(6), 434-45.

Magariños, AM; McEwen, BS; Flügge, G; Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J Neurosci.*, 16(10), 3534-40.

Manogue, KR ; Leshner, AI; Candland, DK (1975). Dominance status and adrenocortical reactivity to stress in squirrel monkeys (*Saimiri sciureus*). *Primates*, 16 (4), 457-463.

Martinez , M; Calvo-Torrent, A; Pico-Alfonso, MA (1998). Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggressive Behavior*,24(4), 241 – 256.

Matzel, LD; Han, YR; Grossman, H; Karnik, MS; Patel, D; Scott, N; Specht, SM; Gandhi, CC.(2003) Individual differences in the expression of a "general" learning ability in mice. *J. Neurosci,*. 23(16), 6423-33.

Matzel, LD; Townsend, DA; Grossman, H; Han, YR; Hale, G; Zappulla, M; Light, K; Kolata, S. (2006) Exploration in outbred mice covaries with general learning abilities irrespective of stress reactivity, emotionality, and physical attributes. *Neurobiol Learn Mem*., 86(2), 228-40.

McEwen, BS; Sapolsky, RM. (1995). Stress and cognitive function. *Curr Opin Neurobiol*., 5(2):,5-16.

McGaugh JL, Roozendaal B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Curr Opin Neurobiol*.,12(2),205-10.

McIntyre CK, Power AE, Roozendaal B, McGaugh JL. (2003). Role of the basolateral amygdala in memory consolidation. *Ann N Y Acad Sci.*, 985:273-93

McKittrick, CR; Magariños, AM; Blanchard, DC; Blanchard, RJ; McEwen, BS; Sakai, RR. (2000). Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. Synapse, 36(2), 85-94.

Menzaghi, F; Heinrichs, S.C.; Vargas-Cortes, M.; Goldstein, G.; Koob, G.F. (1996).IRI-514, a synthetic peptide analogue of thymopentin, reduces the behavioral response to social stress in rats. *Physiology and Behavior*, 60(2), 397-401(5).

Menzaghi, F; Howard, RL; Heinrichs, SC; Vale, W; Rivier, J; Koob, GF (1994). Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. *JPET*, 269(2), 564-572.

Miczek, KA; DeBold, JF; Thompson, ML. (1984). Pharmacological, hormonal, and behavioral manipulations in analysis of aggressive behavior. *Prog Clin Biol Res.*, 167, 1-26.

Miczek, KA; Maxson, SC; Fish, EW; Faccidomo, S. (2001). Aggressive behavioral phenotypes in mice. *Behav Brain Res.*, 125(1-2), 167-81.

Miczek, KA; O'Donnell, JM. (1978). Intruder-evoked aggression in isolated and nonisolated mice: effects of psychomotor stimulants and L-dopa. *Psychopharmacology.*, 14;57(1):47-55.

Miczek, KA; Thompson, ML; Shuster, L. (1982). Opioid-like analgesia in defeated mice. *Science.*,215(4539), 1520-2.

Miczek, KA. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology.*, Feb 28;60(3), 253-9.

Miczek, KA. (1999). Aggressive and social stress responses in genetically modified mice: from horizontal to vertical strategy. *Psychopharmacology.*,147(1), 17-9.

Miczek, KA. (1979). Chronic delta9-tetrahydrocannabinol in rats: effect on social interactions, mouse killing, motor activity, consummatory behavior, and body temperature. (1979) *Psychopharmacology*, 31, 60(2),137-46.

Miczek, KA.; O'Donnell, JM. (1978). Intruder-evoked aggression in isolated and nonisolated mice: Effects of psychomotor stimulants and {l}-dopa. *Psychopharmacology*, 57(1), 47-55.

Miczek, KA.; Thompson, ML.; Shuster, L. (1982). Opioid-like analgesia in defeated mice. *Science*, 215(4539), 1520-1522.

Monder, C; Sakai, RR; Miroff, Y; Blanchard, DC; Blanchard, RJ. (1994). Reciprocal changes in plasma corticosterone and testosterone in stressed male rats maintained

in a visible burrow system: evidence for a mediating role of testicular 11 beta-hydroxysteroid dehydrogenase. *Endocrinology.*, 134(3), 1193-8.

Montgomery, K. C. (1955).The relation between fear induced by novel stimulation and exploratory drive. *Journal of Comparative and Physiological Psychology*, 48(4).

Moragrega, I; Carrasco, MC; Vicens, P; Redolat, R. (2003). Spatial learning in male mice with different levels of aggressiveness: effects of housing conditions and nicotine administration. *Behav Brain Res.*, 17, 147(1-2), 1-8.

Morris, RGM (1974) .Pavlovian conditioned inhibition of fear during shuttlebox avoidance behavior. *Learn Motiv*, 5, 424–447.

Morris, RGM (1981). Spatial localization does not require the presence oflocal cues. *Learn Motiv*., 12:239–260.

Moyer, TR; Motta, RW. (1982). Alienation and school adjustment among black and white adolescents. *J Psychol.*, 112, 21-8.

Murphy, FC; Michael, A; Robbins, TW; Sahakian, BJ. (2003). Neuropsychological impairment in patients with major depressive disorder: the effects of feedback on task performance. *Psychological Medicine,* 33(3), 455-467.

Nebes RD, Pollock BG, Houck P, et al. Persistence of cognitive impairment in geriatric patients following antidepressant treatment: a randomized, double-blind clinical trial with nortriptyline and paroxetine. J Psychiatr Res. 2003;37:99–108.

Ohl, F; Fuchs, E. (1998). Memory performance in tree shrews: effects of stressful experiences. *Neurosci Biobehav Rev*., 23(2), 319-23.

Ohl, F; Fuchs, E. (1999). Differential effects of chronic stress on memory processes in the tree shrew**.** *Cognitive Brain Res.*,7, 1379–387

Olweus, D. Aggressors and their victims: Bullying at school. (1984). Disruptive behaviours in schools. New York: Wiley.

Ordway, GA; Schwartz, MA; Frazeer, A. (2007). Brain Norepinephrine: Neurobiology and Therapeutics. New York: Cambridge University Press.

Ormerod, BK; Galea, LA. (2003). Reproductive status influences the survival of new cells in the dentate gyrus of adult male meadow voles. *Neurosci Lett.*,346(1-2), 25-8.

Park, CR; Campbell, AM; Diamond, DM. (2001). Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. *Biol Psychiatry*,50(12), 994-1004.

Pellis, SM; Pellis,VC; Bekoff, Marc (Ed); Byers, John Alexander (Ed). (1998). Animal play: Evolutionary, comparative, and ecological perspectives. Structure-function interface in the analysis of play fighting. Animal play: Evolutionary, comparative, and ecological perspectives. (pp. 115-140). New York, NY, US: Cambridge University Press. xvi, 274 pp.

Pellis, SM; Pellis,VC. Structure-function interface in the analysis of play fighting. Animal play: Evolutionary, comparative, and ecological perspectives. Bekoff, Marc (Ed); Byers, John Alexander (Ed). (1998). Animal play: Evolutionary, comparative, and ecological perspectives. (pp. 115-140). New York, NY, US: Cambridge University Press. xvi, 274 pp.

Peres, RC; Leite, JR (2002). The Influence of Competitive Status (Winner/Loser) on the Behavior of Male Rats in Three Models of Anxiety. *Aggressive Behavior*, 28, 164–171.

Perry, DG; Kusel, SJ; Perry, LC (1988). Victims of peer aggression. *Developmental Psychology*, 24(6), 807-814.

Pine DS, Cohen P, Johnson JG, Brook JS. (2002). Adolescent life events as predictors of adult depression. *J Affect Disord.*, 68(1):49-57.

Plomin R. (1999). Genetics and general cognitive ability. Nature, 402(6761 Suppl):C25-9.

Purcell, R; Maruff, P; Kyrios, M; Pantelis, C. (1997). *Psychological Medicine*, 27,1277-1285.

Rodgers, RJ; Cole, JC. (1993). Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors. *Physiol Behav.*, 53(2), 383-8.

Rodgers RJ. (1997) Animal models of 'anxiety': where next? *Behav Pharmacol*, 8(6-7):477-96.

Roozendaal, B. (2003). Systems mediating acute glucocorticoid effects on memory consolidation and retrieval. *Prog Neuropsychopharmacol Biol Psychiatry*, 27(8), 1213-23.

Roozendaal, B. (2000). Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology*, 25(3), 213-238.

Roozendaal, B. (2002). Stress and Memory: Opposing Effects of Glucocorticoids on Memory Consolidation and Memory Retrieval. *Neurobio. of Learn. and Mem.*, 78, 578–595.

Ruis, MAW; te Brake, JHA; Buwalda, B; de Boer, SF; Meerlo, P; Korte, SM; Blokhuis, HJ; Koolhaas, JH. (1999). Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology,* 24, 285-300.

Sandi, C; Pinelo-Nava, MT. (2007). Stress and Memory: Behavioral Effects and Neurobiological Mechanisms. *Neural Plasticity,* 78970.

Sapolsky, RM. (1983). Individual differences in cortisol secretory patterns in the wild baboon: role of negative feedback sensitivity. *Endocrinology,* 113(6):2263-7.

Sara, SJ; Roullet, P; Przybyslawski, J. (1999). Consolidation of memory for odor-reward association:β-adrenergic receptor involvement in late phase. *Learning and Memory;* 6, 88–96.

Schmader, T; Johns, M; Forbes, C. (2080). An integrated process model of stereotype threat effects on performance. *Psychological Review*, 115(2), 336-356.

Schneider, R; Hoffmann, H.J; Schicknick, H; Moutier, R. (1992). Genetic analysis of isolation-induced aggression. I. Comparison between closely related inbred mouse strains. *Behavioral and Neural Biology,* 57, 198-204.

Spritzer, MD; Meikle, DB; Solomon, NG. (2004). The relationship between dominance rank and spatial ability among male meadow voles (Microtus pennsylvanicus). *J Comp Psychol.,*118(3), 332-9.

Strozik, E; Festing, MF. (1981). Whisker trimming in mice. *Lab Anim.*, 15(4), 309-12.

Tornatzky, W; Miczek, KA. (1994). Behavioral and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol.*Psychopharmacology*, 116(3), 346-56.

Touyarot, K; Venero, C; Sandi, C. (2004). Spatial learning impairment induced by chronic stress is related to individual differences in novelty reactivity: search for neurobiological correlates. *Psychoneuroendocrinology*, 29(2), 290-305.

Uhrich, J. (1938). The social hierarchy in albino mice. *Journal of Comparative Psychology*, 25(2), 1938, 373-413.

van der Poel, AM; Noach, EJ; Miczek, KA. (1989). Temporal patterning of ultrasonic distress calls in the adult rat: effects of morphine and benzodiazepines. *Psychopharmacology.*, 97(2), 147-8.

Veenema, AH; Meijer, OC; de Kloet, ER; Koolhaas, JM; Bohus, BG. (2003). Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. *Horm Behav.*, 43(1):197-204.

Vivian, JA; Miczek, KA. (1993a) Morphine attenuates ultrasonic vocalization during agonistic encounters in adult male rats. *Psychopharmacology*., 111(3),367-75.

Vivian, JA; Miczek, KA (1993b). Diazepam and gepirone selectively attenuate either 20-32 or 32-64 kHz ultrasonic vocalizations during aggressive encounters. *Psychopharmacology*., 112(1):66-73.

Vivian JA, Miczek KA. (1998). Effects of mu and delta opioid agonists and antagonists on affective vocal and reflexive pain responses during social stress in rats. *Psychopharmacology*, 139(4):364-75.

Vivian JA, Miczek KA. (1999). Interactions between social stress and morphine in the periaqueductal gray: effects on affective vocal and reflexive pain responses in rats. *Psychopharmacology*,146(2), 153-61.

Von Frijtag JC, Kamal A, Reijmers LG, Schrama LH, van den Bos R, Spruijt BM. (2001). Chronic imipramine treatment partially reverses the long-term changes of hippocampal synaptic plasticity in socially stressed rats. *Neurosci Lett*., 309(3),153-6.

von Holst, D. (1977) Social stress in tree shrews: problems, results and goals. *Journal of Comparative Physiology,* 120, 71-86.

Whimbey. AE; D enenberg, VH. (1967). Two independent behavioral dimensions in open field performance. *Journal of Comparative and Physiological Psychology*, 63(3), 500-504.

Wirth, MM; Welsh, KM; Schultheiss, OC. (2006). Salivary cortisol changes in humans after winning or losing a dominance contest depend on implicit power motivation. *Horm Behav*., 49(3), 346-52.

Wood, SK; Walker, HE; Valentino, RJ; Bhatnagar, S. (2010). Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology*, 151(4), 1795-805.

Zook, JM; Adams, DB. (1975). Competitive fighting in the rat. *Journal of Comparative and Physiological Psychology;* 88(1) 418-423**.**

Zoladz, PR; Diamond, DM. (2009). Linear and non-linear dose-response functions reveal a hormetic relationship between stress and learning. *Dose Response*, 7(2), 132–148.

TABLE 1

	Huddling	Sniffing	Bites made	Attack Latency	Bites received	Defensive rearing	Tail rattling	Wounds
Huddling	1.00	-0.03	-0.25	-0.17	-0.10	-0.09	**-0.29**	**0.46**
Sniffing	-0.03	1.00	0.28	-0.07	0.07	0.07	-0.09	-0.12
Bites made	-0.25	0.28	1.00	0.00	0.26	-0.08	0.30	**-0.30**
Attack Latency	-0.17	-0.07	0.00	1.00	**0.31**	0.28	-0.20	0.10
Bites received	-0.10	0.07	0.26	**0.31**	1.00	0.18	-0.03	0.06
Defensive rearing	-0.09	0.07	-0.08	0.28	0.18	1.00	-0.19	0.23
Tail rattling	**-0.29**	-0.09	**0.30**	-0.20	-0.03	-0.19	1.00	**-0.44**
Wounds	**0.46**	-0.12	**-0.30**	0.10	0.06	0.23	**-0.44**	1.00

TABLE 2

	G Factor 1	**G Factor 2**
Passive Avoidance	0.75	-0.01
Lashley Maze	0.76	0.36
Morris Water Maze	0.09	0.83
Odor Discrimination	0.15	-0.66
Fear Conditioning	-0.54	0.32
eiganvalue	1.46	1.37
% variance explained	29%	27%

TABLE 3

	Factor 1	Factor 2
Open field: total entries	0.68	-0.15
Open field: % internal entries	0.46	-0.11
Open field: run speed	-0.62	-0.01
Light/Dark discrimination: time in dark	-0.24	0.41
Light/Dark discrimination: latency to enter light	-0.46	0.004
Balance beam: latency to fall	-0.13	0.63
Balance beam: distance travelled	0.16	0.80
Screen Hang: latency to fall	-0.06	0.78
Screen Hang: # of crossings	-0.11	0.78
Balance Pole: latency to fall	0.26	0.098
Hotplate	0.10	0.01
EPM: closed entries	0.79	-0.04
EPM: open entries	0.84	0.14
EPM: reentries	0.10	0.20
EPM: total entries	0.89	0.07
EPM: % open entries	0.82	0.14
G Factor 1	-0.29	-0.21
G Factor 2	-0.27	0.13
eiganvalue	4.42	2.60
% Variance Explained	25%	15%

TABLE 4

	Factor 1	Factor 2
G Factor 1	0.40	0.15
G Factor 2	0.52	0.27
Huddling	0.57	-0.10
Defensive Rears	0.12	0.85
Sniffing	-0.42	0.001
Bites made	-0.80	-0.23
Bites received	-0.67	0.37
Latency to attack	-0.29	0.64
eiganvalue	2.11	1.42
% Variance explained	26%	18%

TABLE 5

	CORT	"g" Factor 1	"g" Factor 2	Dom. Factor 1	Dom. Factor 2
CORT	1.00	-0.11	0.08	-0.08	**-0.40**
"g" Factor 1	-0.11	1.00	0.01	0.08	0.26

TABLE 6

	Dominance Factor 1	Dominance Factor 2
Huddling	-0.31	0.06
Sniffing	0.42	-0.33
Bites Made	0.93	-0.02
Latency	-0.03	-0.79
Bites Received	0.10	-0.11
Defensive Rears	0.82	-0.33
Tail rattling	0.87	0.29
Wounding	0.95	-0.05
CORT	0.51	0.62
eiganvalue	4.64	1.33
% Variance Explained	52%	15%

TABLE 7

	Factor 1	Factor 2
Open field: total entries	0.65	-0.16
Open field: % internal entries	0.42	-0.14
Open field: run speed	-0.58	-0.09
Light/Dark discrimination: time in dark	-0.18	0.36
Light/Dark discrimination: latency to enter light	-0.45	0.01
Balance beam: latency to fall	-0.21	0.64
Balance beam: distance travelled	0.05	0.82
Screen Hang: latency to fall	-0.07	0.78
Screen Hang: # of crossings	-0.12	0.76
Balance Pole: latency to fall	0.33	0.03
Hotplate	0.12	-0.03
EPM: closed entries	0.80	0.06
EPM: open entries	0.86	0.20
EPM: reentries	0.11	0.09
EPM: total entries	0.91	0.14
EPM: % open entries	0.82	0.20
CORT	0.54	-0.18
eiganvalue	4.53	2.60
% Variance Explained	27%	15%

FIGURE 1A

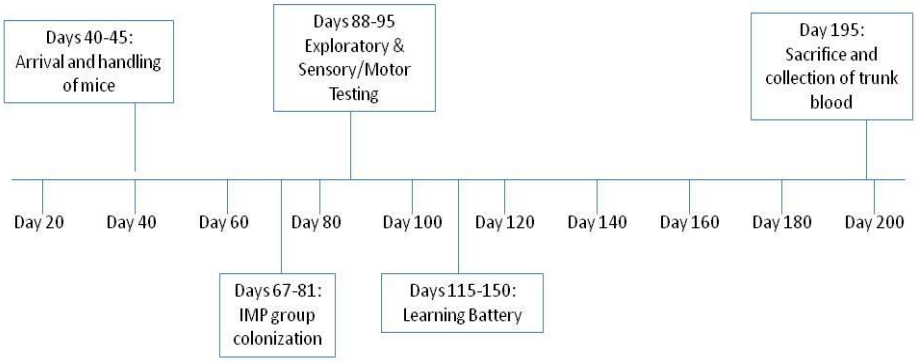
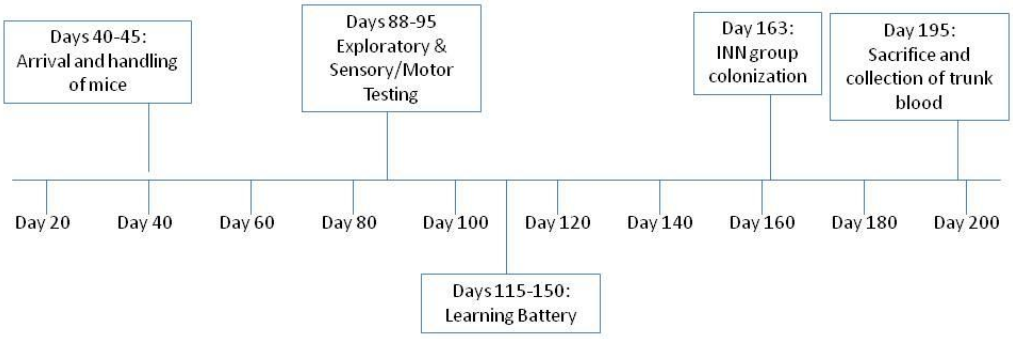
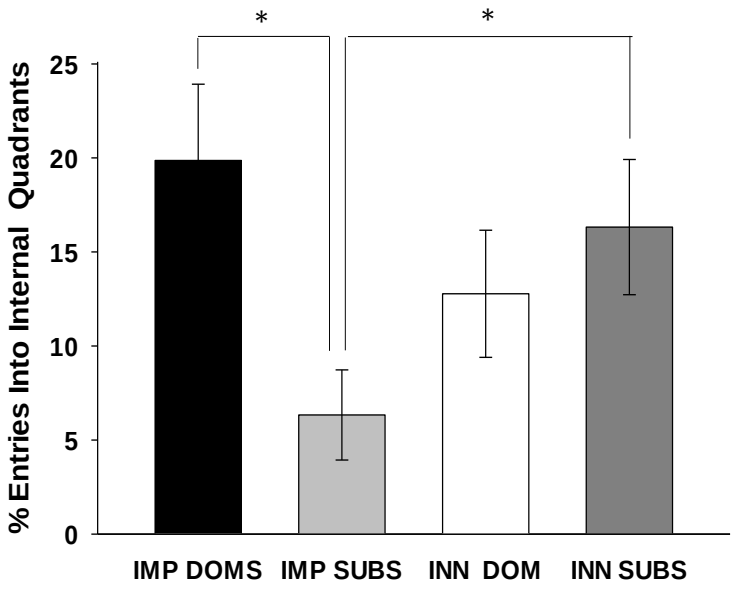


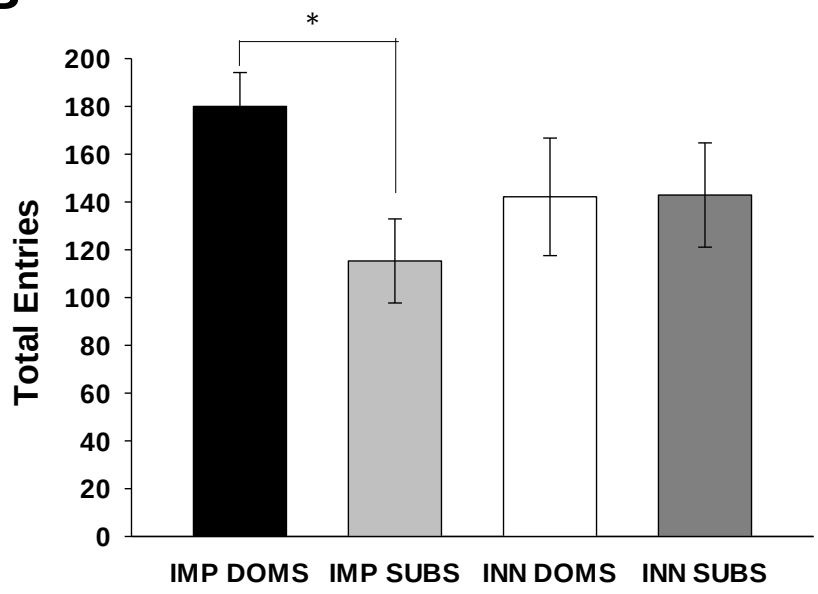
FIGURE 1B.



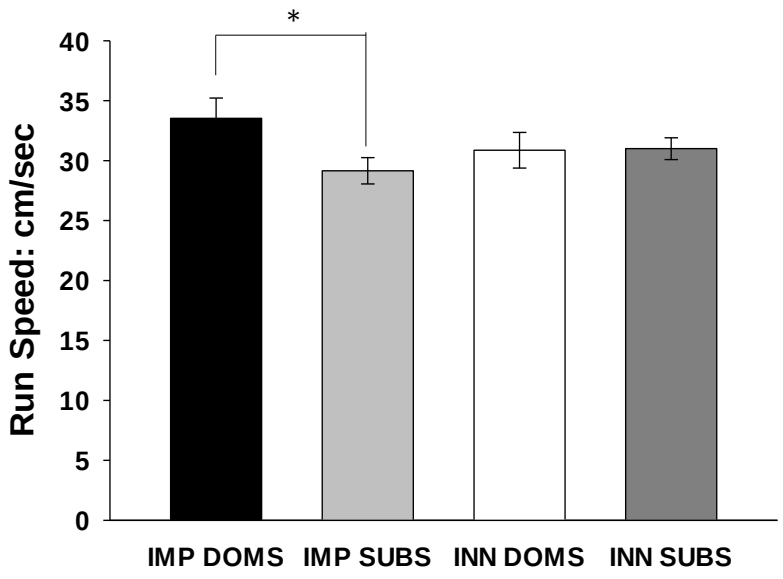
2A


*
*
% Entries Into Internal Quadrants
25
20
15
10
5
0
IMP DOMS
IMP SUBS
INN DOM
INN SUBS

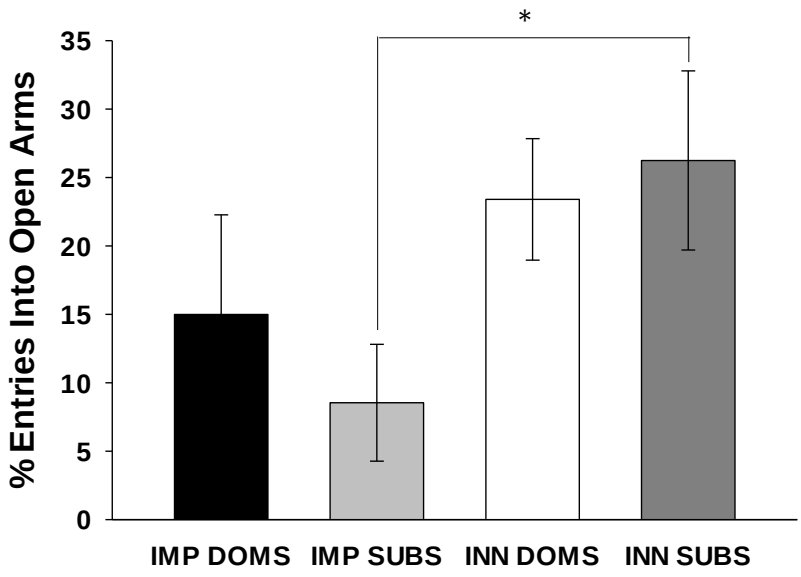
2B

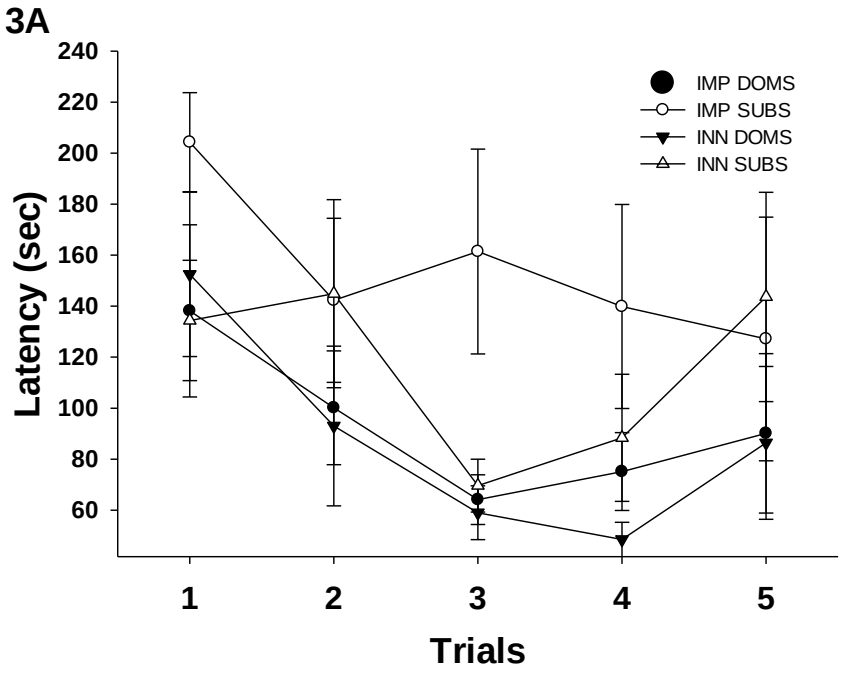

*
200
180
160
140
120
100
80
60
40
20
0
Total Entries
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

2C

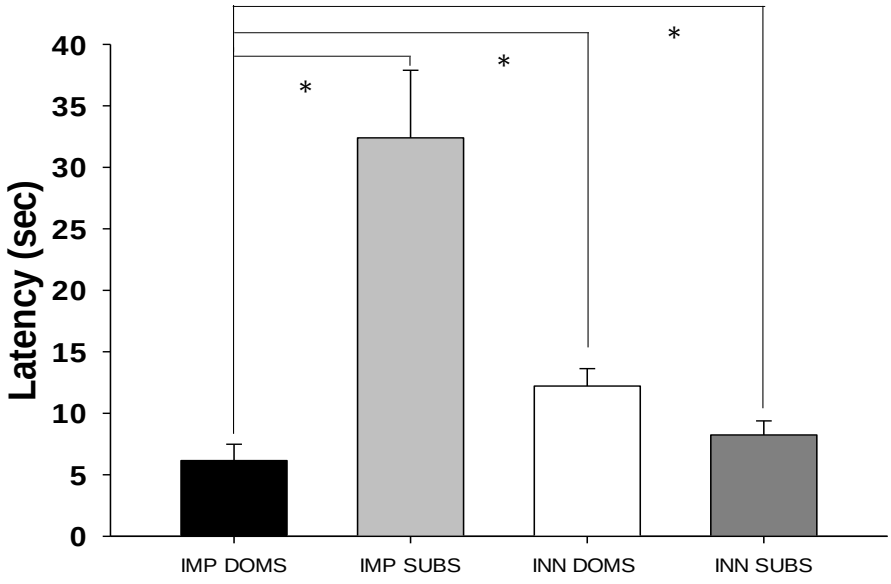

*
40
35
30
25
20
15
10
5
0
Run Speed: cm/sec
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

2D

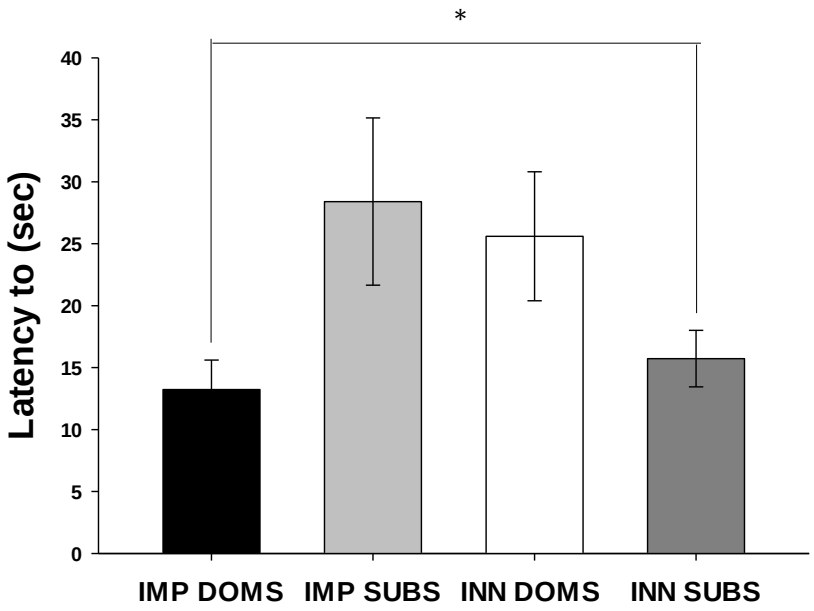

*
35
30
25
20
15
10
5
0
% Entries Into Open Arms
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

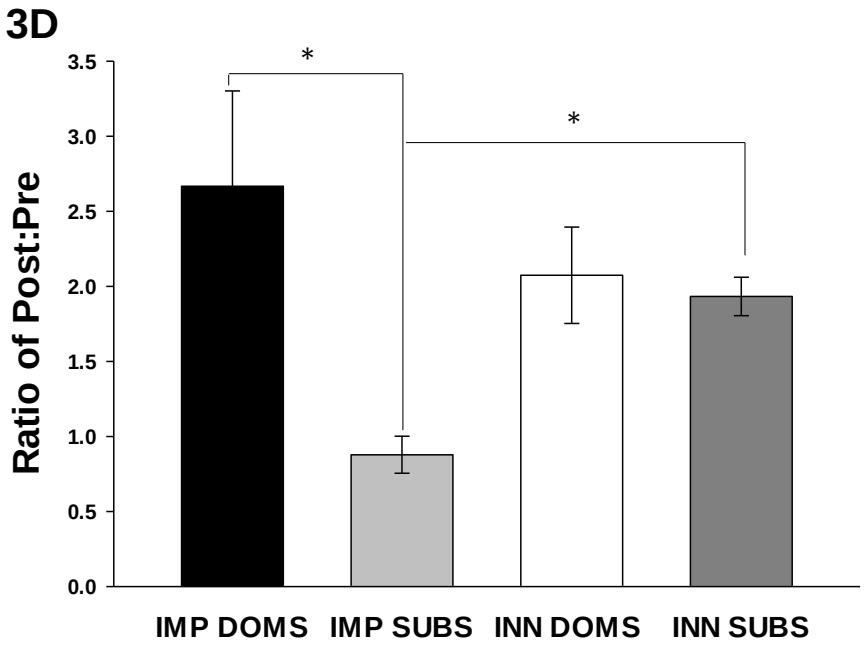

3A
240
220
200
180
160
140
120
100
80
60
Latency (sec)
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS
1
2
3
4
5
Trials

3B

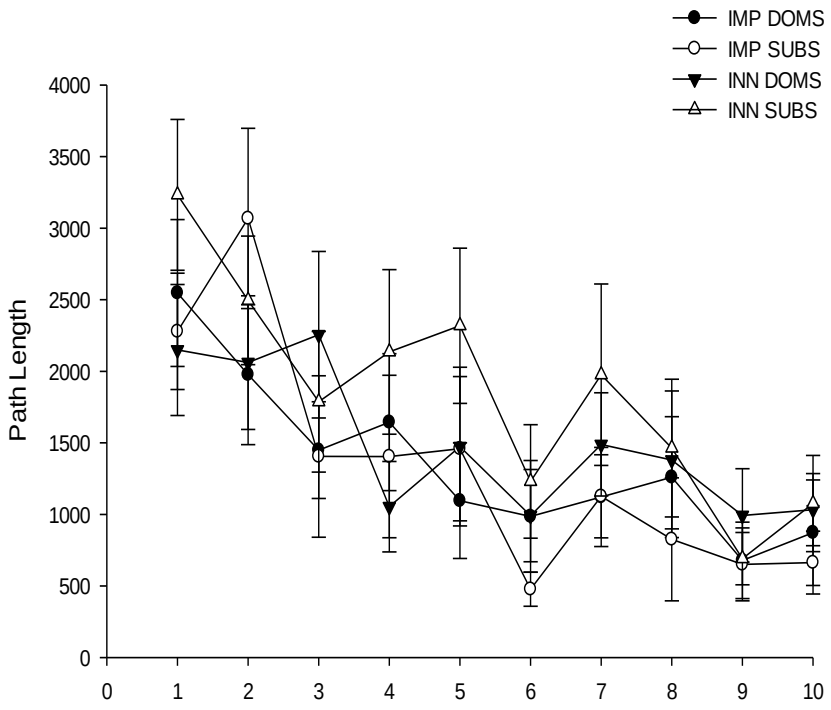

Latency (sec)
40
35
30
25
20
15
10
5
0
*
*
*
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

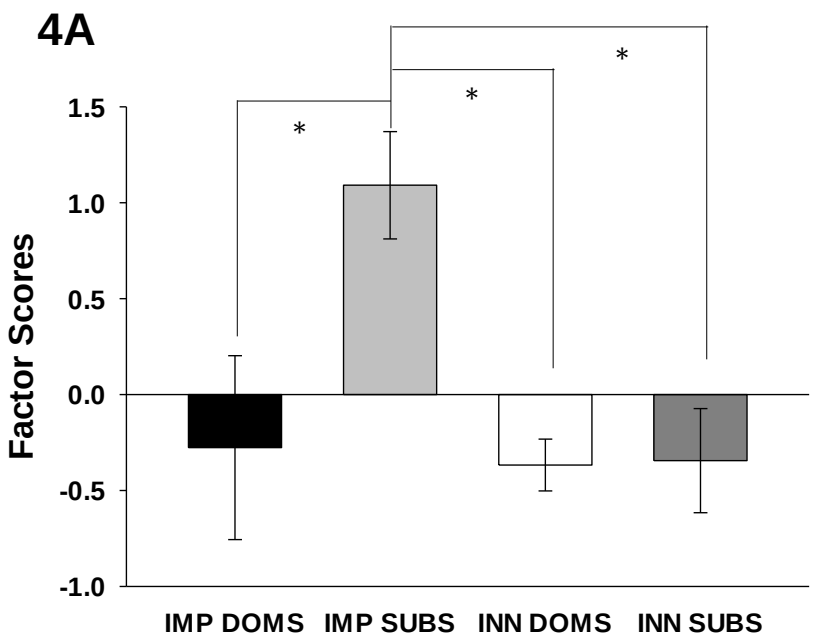
3C


*
Latency to (sec)
40
35
30
25
20
15
10
5
0
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

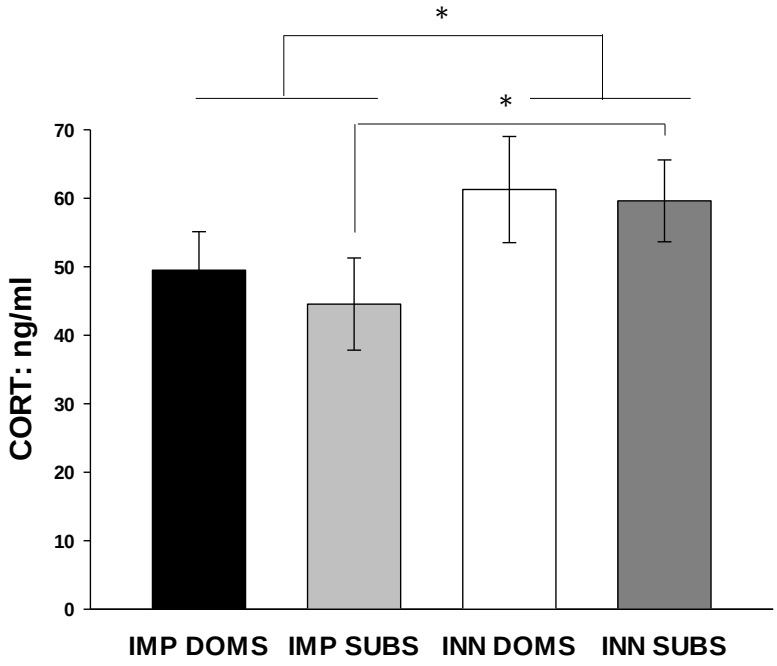

3D
Ratio of Post:Pre
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

3E


IMP DOMS
IMP SUBS
INN DOMS
INN SUBS
Path Length
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
8
9
10


4A
Factor Scores
1.5
1.0
0.5
0.0
-0.5
-1.0
*
*
*
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

5A


*
*
70
60
50
40
30
20
10
0
CORT: ng/ml
IMP DOMS
IMP SUBS
INN DOMS
INN SUBS

Curriculum Vitae

Danielle C. Colas

EDUCATION:

Degree	Institution	Date	Department
B.A.	William Paterson University	2003	Psychology
M.S.	Rutgers University	2005	Psychology

TEACHING:

6/08-8/08; 8/09-5/10	*Lecturer/Coadjunct*	Rutgers University, NJ
9/03 – 5/08	*Teaching Assistant*	Rutgers University, NJ

RESEARCH PUBLICATIONS:

PEER-REVIEWED JOURNAL ARTICLES:

Wass, C.; Denman-Brice, A.; Colas-Zelin, D.; Rios, C.; Kolata, S. and Matzel, L.D. (2010) Co-Variation of Learning and Reasoning in Genetically Heterogeneous Mice Suggests an Evolutionary Conservation of the Operations of 'Intelligence'. In submission.

Kolata, S.; Light, K.; Wass, C.; Colas, D.; Roy D.; Matzel, L.D. (2009) Gene expression analysis of general learning abilities in mice implicates working memory related genes. In submission.

Light, K.; Grossman, H.; Kolata, S., Colas, D; Wass, C. (2009) Rates of information processing underlie the relationship between general learning abilities and exploration in CD-1 outbred mice. In preparation.

Matzel, L.D.; Wass, C.; Kolata, S.; Light, K. and Colas, D. (2009) Age-related impairments of new memories reflect failures of learning, not retention. *Learning & Memory, 16(10),* 590-4.

Rossi-George, A.; Urbach, D.; Colas, D.; Goldfarb, Y.; Kusnecov, A.W. (2005) Neuronal, endocrine, and anorexic responses to the T-cell superantigen staphylococcal enterotoxin A : Dependence on tumor necrosis factor-α. *The Journal of Neuroscience, 22(25),* 5314-5322.

BOOK CHAPTERS:

Colas, D. and Matzel, L.D. (2009) Learning Mutants. In N.M. Seel (Ed.), *The Encyclopedia of Learning Sciences*. New York: Springer Publishing. In press.