THE EFFECTS OF ENRICHMENT ON COGNITION IN RATS

RATTUS NORVEGICUS

by

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Abstract

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Animal models play an integral role in pharmaceutical research when developing drugs for human use. It is therefore imperative that animal models accurately represent human systems. In an attempt to reduce variability of test results, animals are often kept in barren, non-natural conditions. There is, however, a growing awareness that environmental enrichment will increase the validity of test results. The aim of the present study was to allow animals to control their environment using operant conditioning procedures, and to assess the effect of control on cognitive tasks. Four predictions were tested: 1. Rats (*Rattus norvegicus*) will control three stimuli (light, sound and a running wheel). 2. Animals will exhibit preferences for particular stimulus strengths. 3. Animals that exert control over the environmental stimuli will show increased performance in cognitive tasks compared to animals that lack control.4. Animals that can control environmental stimuli will have lower corticosterone levels than animals that lack such control, where corticosterone levels are used as an assessment of stress. Experimental subjects in both experiments did show control over a light stimulus, and performed significantly better in a discrimination task as compared with subjects that could not

control their environment. There was no difference in corticosterone levels between control and experimental subjects. These results will contribute to an understanding how enrichment and control of environmental stimuli, in particular, affect the welfare of animals in captive environments, and aid in designing experimental conditions that will produce animal models that will increase validity and reliability in research.

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Introduction: animal models in research

Animals are an integral part of experimental research. They have been examined in studies that test comparative, developmental, psychophysical, cognitive, and medical questions. In particular, pharmaceutical research has relied on data from animal studies because of the similarities between humans and laboratory subjects. These similarities have made it possible to test potentially harmful drugs and procedures before their use on humans. While we depend on similarities for valid and reliable results, there is a double standard of using animals in research. While we use them as their bodily systems are similar to ours, the standard care often provided is not sensitive to their physiological and behavioral needs. The standard impoverished environments that animals are housed in have the potential to interfere with their normal physiological and behavioral development (Garner, 2005), which then raises the question of these animals being valid models. Also, a problematic consequence of animals kept in barren environments is that experimental procedures often neglect the behavioral needs of laboratory subjects, yet behavioral data are still gathered. Although standard care has led to increased convenience in the husbandry of laboratory animals, the customary procedures have led to poor science (Garner, 2005).

In 1966, the Animal Welfare Act was passed with amendments (1970, 1976, 1985, and 1990, 2003) to promote standardized care for laboratory animals (http://www.nal.usda.gov/awic/legislat/usdaleg1.htm). Many species commonly used in laboratory research were left out of the document's guidelines (e.g., rats, mice, etc.), and only minimal care was stipulated for covered subjects. In general, the guidelines reflect only the physical needs of subjects, and do not address behavioral necessities for many of

the species used. For example, is a clean cage and adequate food sufficient environmental stimuli for the normal development of laboratory animals? The lack of concern for behavioral needs is reflected in the standard operating procedures used in numerous animal facilities, which maintain that the normal development of laboratory subjects solely relies on whether or not the animals' physical needs are met (Garner, 2005). It is important to consider whether or not the basic regulations set forth by the federal government are sufficient to take care of the subjects properly, or whether restricting the subjects' normal range of behaviors through implementation of standardized care protocols should also be considered a welfare issue that has further consequences for the validity of research (Dawkins, 1990).

Current practice requires that animal models resemble human systems, either in general terms (high-fidelity models) or in some specific property (high discrimination models) (Russell & Burch, 1959) allowing inference about cause and effect of pharmaceutical and surgical practices. Improved standards for laboratory animal, such as increasing the complexity of their cage environments, may have the effect of changing the behavioral and physiological attributes of laboratory subjects to better mimic those of human systems. In addition, there is also a growing concern about the ethics of procedures that are used in animal facilities. Consumers are interested in making sure that surgical procedures and drug testing are not unnecessarily harming animals, and pharmaceutical companies are encouraged to employ better techniques in their procedures, and are now complying in order to stay competitive with other companies.

There is a wealth of data that show that current housing conditions do not provide sufficient stimulation for a laboratory animal to develop normally (Benefiel & Greenough, 1998; Rosenzweig & Bennett 1996). Valzelli (1973) noted that mice that

were singly housed, differed from communally housed mice in hormone levels, brain structure/weight, immune response, and cognitive ability. Lack of environmental complexity, which has also been labeled as a form of sensory deprivation (Würbel, 2001) has been associated with behavioral retardation in laboratory rodents, with non-enriched animals showing a decreased performance on cognitive tasks (Mohanty & Behera, 1997). It is intuitive that allowing animals to express normal biologically relevant behaviors, which may be brought about by matching the complexity of the environment to the behaviour and physical needs of the animals, will yield optimal research models.

Past research on improving captive housing systems has focused giving laboratory animals items to interact with such as tubes, boxes or nesting material. A more current approach to addressing the lack of behavioural opportunities in laboratory animal environments includes allowing animals to indicate their preference for certain environmental conditions. Allowing animals to exhibit preference or control over stimuli mimics important natural behaviors shown by animals in their environment. For example, animals in their natural environments control nesting locations, choice of mates and the use of foraging sites. Animals in laboratory settings lack such control, potentially leading to frustration and subsequently to abnormal behaviors (Bassett & Buchanan-Smith, 2007). Research on preference and control will provide additional information on techniques that can be used for laboratory animals. Further, applying this paradigm will allow caretakers of animal maintenance facilities to understand their subjects' normal preferences, and when applied, ultimately result in improved husbandry practices.

Laboratory research often relies on behaviors that are performed by animals subjected to cognitive tasks following specified treatments (e.g., brain lesions, hormonal intervention, etc.) (Gerlai & Clayton, 1999). Animals that are allowed control over their

housing environment show improved cognitive performance compared with animals that lack control, resulting in increased external validity of scientific research (Gerlai & Clayton, 1999). It has therefore been suggested that standard housing for laboratory subjects should no longer be used (Würbel, 2001). Two important considerations must guide the use of cognitive tasks in laboratory research: 1.The animal must be able to perceive the stimulus presented, and 2. the expected response must be species-typical, i.e., the subject cannot display a behavior that is difficult or impossible to perform.

What is enrichment

Enrichment is any physical or social addition to an animal's vital requirements (Garner, 2005). This definition, however, is not generally agreed upon, and more general descriptions, such as procedures that increase welfare for confined animals by presenting appropriate stimulation have been used (Shepherdson, 1998). In general terms, enrichment is concerned with improving the outcome of an animal's behavior (Newberry, 1995). The animal's natural behaviors should be considered when giving enrichment, but keeping in mind that not all behaviors are equally functionally significant (Dawkins, 1998). Preference and control tests might aid in deciding on an enrichment regime that optimizes the animals' environment.

In order to understand how enrichment affects an animal's welfare measures of stereotypies or other abnormal behaviors, structure and functions of the hypothalamic-pituitary-adrenal axis (HPA), hormones, weight loss/gain, body temperature and immunocompetence can be measured (Manser, 1992; Broom & Johnson, 1993; Mench & Mason 1997; Terlouw, Schooten, & Ladewig, 1997; Clark, Rager, & Calpin, 1997). Testing several of the listed parameters is generally necessary, as a single criterion is not

sufficiently informative, but several taken together may give a better picture of the welfare of the subject (Olsson & Dahlborn, 2002).

An important consideration is to evaluate and verify what we think is enriching to animals. That is, are animals interacting with the given enrichment that benefits them, and also to consider if and how enrichment used outside of experimental procedures may actually affect the results of an experiment (Olsson & Dahlborn, 2002). Comparisons with behaviors shown by animals in their natural habitat may be helpful in assessing whether enrichment has a positive influence (i.e., improved health). There is a caveat, however, as making such comparisons may not always be valid because of possible physiological or behavioral differences between wild and domesticated animals (Dawkins, 1980). For example, a bird may often perform dust-bathing behaviors to clean itself in natural conditions but the same behavior may not be necessary when a bird is in a clean cage.

History of research performed using enrichment

The theory that physical exercise can affect the physiology of the brain was first suggested by Charles Bonnet (1720-1791). He discussed his theory with M. V. Malcarne (1744-1816) that the brain was like a muscle that when exercised would increase in size (Renner & Rosenzweig, 1987). It was Malcarne who was the first to test the hypothesis that experience would alter the brain in birds. While his conclusions were not fully developed, he did find changes in the physiological system due to augmented environments.

Enrichment approaches used for laboratory rodents date back to 1949 when neuroscientist D. O. Hebb adopted a few of his research rats, adding environmental enrichment to the animals' cages. He noticed a difference in behavioral traits including

increased activity and maze performance in the adopted rats that differed dramatically from the behavior of the non-enriched laboratory animals. He also found that these changes were permanent, and proposed that enrichment should be studied and introduced to laboratory environments (Hebb, 1949).

In 1958, Harlow performed several landmark studies testing environmental and social effects. In one such study, he tested the effects of social deprivation in young rhesus macaques and found dramatic behavioral consequences when young animals were isolated for various periods of time. These tests included observing the reactions of socially deprived infants to various environmental stimuli, where their responses were noticeably different from those of infants raised in more normal social circumstances. Harlow found that impoverished environments were deleterious to the animal's normal development, and thus concluded that a socially and physically complex environment was important for the animals' normal physiological and behavioral development (Harlow & Zimmerman, 1958).

Further studies of how social or environmental enrichment affect physiological attributes in mammals continued in the 1960's, when virtually all aspects of brain and body were measured in research studies. Many of these studies have shown that the brain requires physical and/or social stimulation for normal development. For example, experiments performed on the visual system in cats have shown that stimulation is necessary for normal development of the visual system, as adult cats did not respond to visual stimuli if the subject's eyes were blocked from stimulation during early development (Hubel & Wiesel, 1970). Further, when the kittens' visual system was restricted to specific stimuli, such as bars of light, this would be the only pattern that they responded to in their adult life. Associated with these behavioral findings, was evidence

of a reduced number of neurons in the occipital lobe, where the lack of use of the visual system may have led to synaptic pruning (Hubel & Wiesel, 1970). Thus, the lack of a complex environment seemed to have led to an altered neurophysiological development in the brains of these animals and associated altered behavioral responses (e.g., cats not responding to certain stimuli in their environment), that were different to animals raised in more natural, complex environments.

Rosenzweig, in 1960's, performed the first systematic studies to prove that both social and environmental enrichment causes neurophysiological differences in the brain (Bailey & Kandel, 1993). Rosenzweig et al. also demonstrated that enriched animals outperformed impoverished animals as the difficulty of a task increased (Rosenzweig & Bennett, 1996).

Enrichment studies continued through the 1960's (Wallace, 1982), but not until recently has a more detailed approach to improving welfare of laboratory subjects utilizing enrichment and enriched environments been undertaken.

Numerous studies have shown the effects of environmental and social enrichment on laboratory animals. Though the effects on physiological systems are well documented, the behavioral consequences are not as abundantly reported. In the following section, some physiological and behavioral consequences of enrichment approaches are investigated, and the connection between physiological changes and behavioural responses related to the use of enrichment are described.

How does enrichment affect animals: environmental, social and handling enrichment

Data collected from animals kept under enriched regimens have indicated that the nervous system is sensitive to social, environmental and handling enrichment, but there is

a lack of consensus of the exact effect of each (Olsson & Dahlborn, 2002). Renner and Rosenzweig (1987) found that enrichment has the potential to have profound effects on all mammals tested to date, and also noted that small changes produced by enrichment are combinatorial so that enriched animals exhibit a long-term increase in learning abilities. They further concluded that the brain, if not actively used, will atrophy (Renner & Rosenzweig, 1987). It may be that this "use it or lose it" concept can be applied to many aspects of the nervous system. Several studies have found that enrichment reverses cognitive abilities of older animals to be like those of younger animals (Meaney, Aitken, Berkel, Bhatngar, & Sapolsky, 1988; Bodnoff et al., 1995; Fernández-Teruel, Escorihuel, Castellano, González, & Tobeña, 1997; Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999). It may be that animals that are enriched may have never "lost" neurons or the ability to perform behaviors, but enrichment has kept appropriate brain regions active, where these regions resemble those of younger animals in shape and function (Rosenzweig & Bennett, 1996). The following section reviews findings on the effects of environmental and social enrichment, as well as human handling, on the behaviour and physiology of laboratory subjects.

Changes due to environmental enrichment

Several physiological changes have been noted to come about when animals are subjected to environmental enrichment. The following modifications are found generally throughout the brain when animals are housed in environments that utilize enrichment to provide opportunities for animals to perform species-appropriate behaviors: increased neuronal count (Renner & Rosenzweig, 1987), dendritic branching including spine count (Renner & Rosenzweig, 1987; Fiala, Joyce, & Greenough, 1978), increased RNA and nerve growth factors (Renner & Rosenzweig, 1987), increased dendritic fields leading to

increased synaptic firing (Volkmar & Greenough, 1972), and glial cell increase (Walsh & Cummins, 1979). Specific areas of the brain that change when animals are given environmental enrichment are: increased thickening of the hippocampus (Rosenzweig, 1966), increase of progenitor-derived cells in the dentate gyrus (Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999), a four percent increase in dorsal, sensory (visual and somatic) cortex and total cortex (Rosenzweig, Krech, Bennett, & Diamond, 1962), and a significant increase in nerve growth factor in hippocampus, visual and entorhinal cortices (Pham et al., 1999).

Behavioral differences that have been noted when animals were subjected to environmental enrichment include: a reduction in overall fearfulness in tests that require open field activity (Chamove, 1989; Prior & Sachser, 1994; van de Weerd, Baumans, Koolhaas, & Zupthen, 1994), increased performance on the Morris water maze and the nose poke task (Pham et al., 1999), faster and increased accurate learning (Juraska, Henderson, & Muller, 1984), and increased performance in discrimination tasks with decreased attention to unimportant cues (Van Woerden, 1986).

Changes due to social enrichment and handling

Though usually mentioned in the methods of research papers, the importance of whether or not laboratory subjects are multi-housed or single-housed is often overlooked. More consideration may be needed surrounding this issue, as there are both physiological and behavioral ramifications to social housing conditions that may potentially increase or decrease welfare for laboratory subjects. The social environment can act as a powerful form of enrichment, if used appropriate for the species in question, and especially when animals retain an element of control over social conditions (e.g., able to avoid aggressive conspecifics). For example, Hurst, Barnard, Nevison, & West (1997) reported that

animals in the presence of additional littermates had significantly lower levels of corticosterone than single-housed subjects, and singly housed animals were found to be less mobile than multi-housed subjects (Hurst et al., 1997).

Humans also play an important role in the physical and social environments of captive animals, and especially for laboratory animals living in certain standardized conditions. The interactions between humans and animals can be a form of enrichment, or a potential stressor. Handling is any type of holding of subjects performed by laboratory personnel that constitutes more than removing the animal from its cage for routine maintenance. Handling can last anywhere from a few seconds to minutes in order to habituate subjects to human touch and procedures such as injections. It is important to consider the nature and form of this handling in order to determine the short and long-term effects it can have on the welfare on animals, and their physiological/neurological development.

Behavioral effects that are seen when animals are subjected handling enrichment include decrease in emotionality, decrease in excreting fecal boli and an increase in exploratory behavior (Fernández-Teruel, Escorihuel, Driscoll, Tobeña & Bättig, 1991), decrease in self-grooming (Tobeña et al., 1996), and improved learning in the Morris water maze (Fernández-Teruel, Escorihuel, Castellano, González, & Tobeña, 1997).

Control

Under natural conditions animals have control of their behaviors within their environment, including choice of nest site location, nesting material, foodstuff and mates (Kavanau, 1963). Laboratory settings have taken much of this control out of the animal's daily routines. This lack of control may manifest itself in deleterious effects on physiological and/or behavioral patterns. Davis and Levine (1982) found that regardless

of the consequences of their action, giving animals some form of control of their environment is desirable. For example, a lack of control of an electric shock affects the production of corticoids in laboratory animals. But allowing the animal control (where behaviors performed by the animals had the effect of turning off the shock) was associated with decreased levels of corticosterone in rats (Davis & Levine 1982). Control results in predictability of environmental conditions, which ultimately might reduce the intensity and length of stress responses (Davis & Levine, 1982) and increase animal welfare (Bassett & Buchanan-Smith, 2006).

In a carefully designed experiment, giving the animal control over its environment using certain preference testing methodologies can let the animal "tell us what it wants" (Dawkins, 1980). Several experiments have been performed allowing subjects to control or display preference over various environmental features. Specific paradigms include manipulation of bedding (Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997), sleeping sites (Van de Weerd & Baumans, 1999), social partners (Van Loo, Van de Weerd, Van Zutphen, & Baumans, 2004), access to a running wheel (Sherwin, 1998) and light (Kish, 1955; Lockard, 1963; Kavanau & Havenhill, 1975).

Such experiments may also allow us to gage an animal's motivation to gain access to a particular stimulus by having it work under various imposed difficulties to attain the desired stimulus. This consumer demand model has been used when testing for preferences in several species (Dawkins, 1980). For example, van de Weerd et al. (1991) performed an experiment in which mice had to experience an electric shock in order to gain access to nesting materials. Results from preference studies will aid in deciding which stimuli should be added to the environment for the subject to control for enhanced enrichment.

Results from preference/control studies must be viewed with a caveat as preferences can change with several factors. An animal might choose item A over B, but might not choose either when a more preferred item C is introduced (Dawkins, 1980; Collier, Johnson, CyBulski, & McHale, 1990). Environmental factors can also change preferences. An animal might choose access to water when the ambient temperature is high, but may not prefer water when the ambient temperature is lower. Bradshaw and Poling (1991) introduced rats to a set-up where two cages were attached to each other, where one side had an enrichment item (wood, chips, plastic pipe, paper towels, or squares (20 x 20 cm) of plywood and the adjoining cage contained no item. They found that the rats significantly preferred the side of the cage that included enrichment, but the authors suggested that rats exhibited individual variation in their preferences for enrichment items that depended on their motivational state. Use of a particular enrichment item depended on what behavior the animal was performing at the time of choice, and the researchers also noted individual differences of preferences between subjects. Other factors that influence preference are age, social contacts (van Loo, van de Weerd, Zutphen, & Baumans, 2004) and length of exposure to environmental enrichment (Dawkins, 1980).

Although there are well-documented issues with animals showing changing preferences for stimuli within their environment, it is possible that the nature of the stimulus might not matter, but controlling it is what is important to the animal (Davis & Levine, 1982). For example, Kavanau (1962) found that deer mice would only use a motor driven running wheel if the subjects had turned the motor on themselves. If the experimenter turned on the motor, the subject would subsequently turn it off, even though it stopped the animal's access to running. Another issue regarding control is that animals

should only take control of primary reinforcers such as food and water. This is not always the case as Kish (1955) found that mice would press levers in order to turn on lights. These results were important because for the first time it was shown that an animal exerted control over its environment in the absence of primary reinforcers.

Control of the environment affects an animal physiologically and behaviorally (Weiss, 1971). Benefiel and Greenough (1998) found increased neural arborization in animals that were housed in enriched environments They hypothesized that this increase lead to better cognitive skills in the animals such as improved learning and memory, or alternatively, that enrichment increases the efficacy of neurons, which helps facilitate improved performance within cognitive tasks (Benefiel & Greenough, 1998). Therefore, using control as a form of enrichment for laboratory animals may help to normalize an animal's environment in allowing subjects to perform species appropriate behaviors, and subsequent cognitive behavior thus increasing the validity of the research (Garner, 2005).

Cognition

Cognitive tasks have been used on laboratory subjects for testing behavioral differences following pharmacological and other treatments (i.e., drug administration, toxicity tests). Several types of cognitive tasks are being used in laboratory research. It is important to realize that the animal's performance on these cognitive tasks may be processed by different areas of the subjects' brain, and that an appropriate task should be chosen to reflect the involvement of these areas. Most cognitive tasks rely on the animal's ability to make associations between aspects of their environment, and memorize these associations (Crawley, 2007).

As valid research results rely on the appropriate choice of cognitive tasks; it has been suggested that laboratory subjects be subjected to several tasks in order to assess

potential changes resulting from experimental treatments (Ennaceur, Michalikova, Bradford & Ahmed, 2005). The Morris water maze is the most widely used memory test (Crawley, 2007) requiring the subject to utilize spatial navigation to find a hidden platform in an opaque water bath. The ability that individual animals can successfully learn where the platform is located appears to be dependent on the species strain, with some strains performing better than others. Other maze types can be used to ask similar questions. The radial arm task and T-maze task (finding a food reward in a particular arm of a maze), and the Barnes maze that requires the subject to find an escape compartment in order to not be exposed on an open platform (Crawley, 2007). These memory tasks involve the hippocampus and are utilized when experimental treatments are expected to affect changes in this brain structure (Crawley, 2007). Gould, Beylin, Tanapat, Reeves and Shors (1999) found an increase in hippocampal neurons when rats were subjected to use navigation as a facility in the Morris water maze task.

Other cognitive tasks such as the discrimination task and set shifting requires subjects to differentiate between two or more stimuli (e.g., objects, graphics or food) and repeatedly chose one over a number of trials. The obvious benefit of such tasks is that similar paradigms can be used when studying learning and memory abilities in humans, and thus valid comparisons can be made between laboratory subjects and humans (Bussey, Saksida & Rothblat, 2001). In a study on monkeys, Dias, Robbins and Roberts (1996) found that the animal's ability to pay attention to changes in the configuration of the visual stimulus of the stimulus in a discrimination task decreased when lesions were performed to the subject's prefrontal cortex. Thus, the authors concluded that stimulus discrimination was processed in this area of the brain. As neurophysiological development (neurogenesis and cell death) (Gage, 2002) is affected by both social and

environmental enrichment, these effects are also reflected in the subject's performance on cognitive tasks (Nilsson, Perfilieva Johansson, Orwar & Eriksson, 1999). Nilsson et al. (1999) found that when animals were socially enriched in a large cage that also contained environmental stimuli to manipulate, enriched subjects developed an increased number of neurons in the dentate gyrus, and at the same time increased their performance on a Morris water maze compared with controls animals that did not receive the enriched conditions. Although it was not possible to distinguish the effects of environmental or social enrichment in this study, the findings suggest that a combination of the two approaches has a significant effect on neurological development, and thus on learning and memory.

Cognitive performance has been shown to increase in laboratory subjects when housed in enriched environments, as demonstrated by Forgays and Forgays (1952) who tested subjects' performance when maintained in 1) a large cage with two levels with stimuli rats could manipulate, 2) a large space with two levels, 3) small mesh cages, and 4) small cages with metal sides (controls). Animals housed in the large cage with items to manipulate performed significantly better on a Hebb-Williams maze than the three other groups. Krech, Rosenzweig, and Bennett (1962) found that rats kept in both socially and environmentally enriched environments for 30 days performed better on a reversal discrimination task than rats kept in isolation after weaning for 21 days. Mohanty and Behera (1997) subjected rats to three conditions for 61 days: the first enriched group had several enrichment items such as wooden blocks and mirrors attached to the cage; subjects in the second group could only see enrichment items but had no physical contact with these objects; animals in the third group were prevented from seeing and making contact with the enrichment items. Results from a discrimination and a passive avoidance

learning task revealed that those rats that had physical contact with stimuli performed better on the cognitive tasks than the two other groups. These studies highlight the fact that results obtained in cognitive research are influenced by the environment subjects are raised in (Diamond, 2001; Schrijver, Bahr, Weiss & Würbel, 2002). It is, therefore, important to carefully control environmental factors to allow comparisons across studies, and to design experimental procedures using enrichment to increase external validity of the research results allowing an extension of the findings to human conditions (Garner, 2005; Gould & Gottesman, 2006).

Still another factor that affects cognitive performance in laboratory animals is the secretion of corticosteroids. As aforementioned, several cognitive tests used in laboratory research, such as spatial memory tests (Morris water maze and novel object placement tasks), are based on the involvement of the hippocampus. Thus, when circulating corticosteroids negatively affect the hippocampus (e.g., loss of neurons), it is possible that high levels of corticosterone will decrease memory functions in rats (Bardgett, Newcomer, & Taylor, 1996) and thereby decrease the subjects' performance in cognitive tasks. A three-month chronic corticosterone treatment significantly decreased performance on a water maze spatial memory task as compared to non-treated controls (Bodnoff et al., 1995). Similarly, Conrad, Galea, Kuroda, & McEwen, (1996) found that chronic stress produced by physical restraint decreased spatial memory ability on a Y-maze test in rats. These researchers stated that physical restraint produced structural changes (e.g., decrease of the number of neurons) in the hippocampus, which resulted in decreased cognitive ability. It is therefore important to understand the effects of enrichment on corticoid levels, and how this hormone affects the cognitive performance of laboratory animals.

Effects of corticoids

Suppression of the immune system is one of the known roles that corticoids have in mammals, but these hormones appear to have more than one function (Davis & Levine, 1982), which include: supplying energy to exhausted cells, firing activity of neuronal pathways and influence learning and memory processes (de Kloet, Oitzl, & Joëls, 1999). It also remains unknown what exactly activates the hypothalamic-pituitary-adrenal axis (HPA) to produce corticoids. Historically, the hormone has been associated with negative or maladaptive stimuli, although it is realized that both negative and positive stimuli have similar effects in activating the adrenal complex system (de Kloet, Oitzl, & Joëls, 1999). Two behavioral attributes that are affected by corticoids are fear and memory (de Kloet et al., 1999). Therefore, understanding the role of corticoids and their secretion is important because fear and memory are often tested in cognitive tasks (Dawkins, 1998).

***Physiological changes**:*

Corticoid receptors are found in several brain structures, including the hippocampus where circulating corticoids can affect differential activation of corticoid receptors (Kloet et al. 1999). Wantanabe, Gould, & McEwen, (1992) found that increased levels of corticoids decreased dendritic branching, and also decreased the length of dendrites in the hippocampus of rats. Aged subjects that had been handled as neonates showed a decrease in corticoid secretion when subjected to stressors, (Levine, Haltmeyer, Karas, & Denenberg, 1967). This resulted in a significant decrease in the loss of neurons in the hippocampus of rats (Meaney et al., 1988; Sapolsky, 1992; Fernández-Teruel, Escorihuel, Castellano, González, & Tobeña, 1997).

Behavioral changes:

There are conflicting reviews about whether high levels of chronic corticoids impair cognitive functions. There are two kinds of corticoid receptors in the brain, mineral corticoid receptors (MR) found in the hippocampus and glucocorticoid receptors (GR) found in the hippocampus and in other brain structures (de Kloet et al., 1999). Both MR's and GR's seem to play a role in memory formation and recall in cognitive tasks, where small amounts of corticoid agonists can produce increased memory ability. The activation of these receptors seems to preclude long-term potentiation (LTP), and it has been shown that corticoids may increase deleterious effects on short-term memory more so than on long-term memory (Bardgett, Newcomer, & Taylor, 1996).

McLay, Freeman and Zadina (1998) found that when rats where treated with corticosterone for three months (the version of corticoid found naturally occurring in rats), the subject's cognitive performance decreased when tested on the Barnes maze. Another study performed by Bodnoff et al. (1995) showed that middle-aged rats that were given chronic treatment of corticosterone displayed a decreased performance on the Morris water maze, while the same hormone treatment did not affect young rats. This study clearly demonstrated that developmental age is an important factor to consider when assessing cognitive abilities in animals.

Enrichment brought about by the animal's control of its environment (as reviewed earlier) may have an important role in the expression of corticosterone. The HPA axis responds to control in that corticosterone levels are significantly lowered when rhesus monkeys were allowed to control the termination of a shock (Hanson, Larson, & Snowdon 1976; Davis et al., 1977). Consequently, low levels of corticosterone may

increase cognitive skills, or prevent cognitive impairment (Weiss, 1972; de Kloet et al., 1999).

This review has shown that while there is an abundance of information on the effect that enrichment has on animal welfare, there is much less information addressing the consequences of control on an animal's cognitive abilities. Therefore, this thesis will address these issues and test the following predictions.

1. Animals will control their environment provided they can manipulate the means (a lever in an operant cage) allowing them access to one of three adjustable stimuli (light, sound and a running wheel).
2. Animals will exhibit selected preferences for these stimuli.
3. Animals that have control over their environment will show improved performance in cognitive tasks compared to animals that lack such control.
4. Animals that have control over their environment will have lower corticosterone levels than animals that lack such control.

Experiment 1

Cognitive effects of control over environment: light, sound and running wheel access

Methods

Subjects

Eighteen Long-Evans Hooded male rats at the age of 21 days were gentled for a week before testing began by handling each subject approximately two minutes every day. All subjects were singly housed and kept on a 12 hour light cycle with lights on at 1 pm. Animals were purchased from Harlan Laboratories, fed Harlan 2001 Lab chow™, and had water available *ad libitum.* Subjects were kept on paper bedding; cages were cleaned every five days. For consistency, only one person cleaned, handled and tested all

subjects throughout the experiment. The animals' homeroom was lit by fluorescent lighting and was 400 lx (measured at a height of one meter off the ground), temperature remained between 18 to 23° C, and humidity remained between 45 and 85 relative humidity units.

Apparatus

There were two components to this experiment in which operant conditioning sessions were followed by a cognitive test.

Operant conditioning

Individual subjects were placed in an operant cage (30.5 W x 33 H x 25.4 cm^3 D) in which by pressing one of three levers the animal had control over three stimuli, light, sound and the functioning of a running wheel (Figure 1). All instruments were manufactured by Coulbourn Instruments™.

Figure 1 – Operant cage with light, speaker and running wheel attached to cage

The light and speaker were located centrally in the ceiling of the operant cage and the wheel could be entered from a door that was centrally located on one of the sidewalls of the cage. The light and sound-activating levers were installed on the sidewall opposite to the wheel and the lever that released the brake for the running wheel was installed directly next to its entrance. Light was provided by a tungsten light bulb (illumination was 15 lx, measured at grid level). Two pure tones were used as a stimulus, both of 10,000 Hz. The first tone consisted of an intensity of 73 dBa (human auditory threshold curve), 74 dBc (flat curve) and the intensity of the second tone was 63 dBa (human auditory threshold curve), 64 dBc (flat curve) were produced by a frequency generator (BK Precision™, model 4011). The sound intensity between the two stimuli was modulated by two attenuators (Hewlett Packard™, 350 D Attenuator set).

Stimuli were controlled by a program written by the staff of Hunter College in Visual Basic™ (version 6) and a Switch and Sense™ control box that was connected to a Dell Optiplex™ computer.

Cognitive/Barnes maze task

A cognitive task was conducted using a Barnes maze (diameter: 122 cm), 18 holes (diameter: 10.2 cm) (Figure 2). Light from a 60 W tungsten light bulb, installed 61 cm centrally above the maze. Three of the walls of the experimental room were fitted with one of three visual landmarks which consisted of either a circle, a square or a triangle, made of black plastic, each measuring 30.5 cm in diameter, width and height, respectively. These landmarks were located 30 cm from the outer border of the maze.

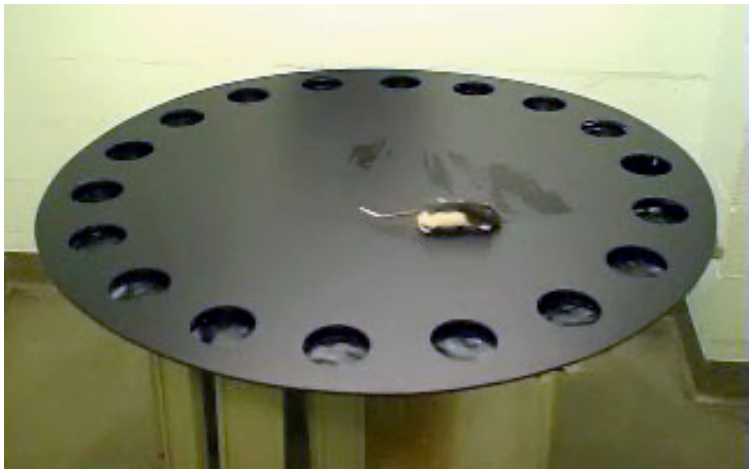

Figure 2 – Subject on a Barnes maze

Procedure

Operant conditioning

By pressing one of the levers in the operant cage, experimental subjects could change the stimuli to a particular setting (from here on in referred to as change-presses), i.e., when pressed, the lever controlling light would dim the light, the sound lever would attenuate the sound, and the brake on the running wheel would be released when the corresponding lever was activated. The chosen setting would last 20 seconds before reverting to its original status, when the subject would have to press the lever again to obtain the preferred setting. The experimental animal could also prolong the dimmed condition (an additional 20 seconds with each lever press) by pressing the lever during the time period when the stimulus was already on the changed setting (from here on in referred to as prolong-presses). Total lever presses included all lever presses produced by either experimental or control animals (total-presses). All three stimuli were set to the non-preferred setting as the starting condition for each session (the light was on, the louder sound was being emitted, and the brake was applied to the running wheel).

Animals were randomly selected to run in either an experimental (n = 9) or control group (n = 9). Control animals were tested in identical cages as the experimental animals; lever pressing, however, had no consequence on the stimulus status. Variables that were measured were number of lever presses for each stimulus, and total time spent in the chosen status.

Subjects were placed in the operant cages for one hour per day for a total of 27 consecutive days. Each cage was isolated in a separate room in order to minimize sound and light interference from other cages, as two experimental and two control cages were used simultaneously in each session. Cages were cleaned with Nolvasan™ between sessions.

Cognitive/Barnes maze task

At the completion of the operant conditioning phase, subjects ran four trials in the Barnes maze per day for three days with a trial not lasting longer then three minutes, and an inter-trial-interval of two minutes. The escape hole was kept in the same location for all trials. On the fourth day, subjects ran one trial in the Barnes maze, again with the escape hole kept in the same location as on the previous days. On the second through fourth trials, the maze was turned 45° counter-clockwise, changing the orientation of the escape hole to the visual references. The reason for this change in maze orientation was to add a cognitive component to the task to be able to distinguish orientation and navigation strategies used by the subjects. If the subjects return to the incorrect location where the hole was originally situated in the first 13 trials, the animal may be considered performing a less demanding motor task, i.e., path integration (Benhamou, 1996), but if the subject takes less time and makes fewer errors in finding the displaced hole over the

last three trials they are most likely using a cognitive map for navigating on the maze (O'Keefe & Nadel, 1978).

Latency to enter the escape hole and number of incorrect choices (dipping head in wrong hole) were recorded for all trials. The maze was cleaned with Nolvasan™ between trials and sessions. All tests were run in the evening, on average three hours prior to the end of the homeroom's light cycle that correlated with the beginning of the animal's activity cycle.

Data analysis and statistics

Statistical analysis for this experiment was performed using SPSS™ (version 17) and Microsoft Excel™.

IACUC

This experiment was approved by the Institutional Animal Care and Use Committee of Hunter College (PM-control 6/10-01).

Results

Operant Conditioning

Three sets of results are reported for lever pressing in the operant conditioning regimen (total-presses, change-presses and prolong-presses). Table 1 illustrates means and SEM's of lever presses by experimental and control animals for all three conditions: light, sound and running wheel access.

Table 1 – Mean ± 1 SEM for number of lever presses for light, sound, and for access to a running wheel

Light stimulus	**Total press**		**Change press**		**Prolong press**	
Animal	Mean	SEM	Mean	SEM	Mean	SEM
Experimental	266.55	30.10	152.89	20.47	114.33	14.71
Control	63.11	13.44	---	---	---	---
Sound stimulus						
Animal	Mean	SEM	Mean	SEM	Mean	SEM
Experimental	70.33	13.47	46.78	7.07	23.56	7.86
Control	71.78	13.06	---	---	---	---
Running wheel						
Animal	Mean	SEM	Mean	SEM	Mean	SEM
Experimental	70.22	9.92	46.44	6.52	23.78	4.07
Control	56.11	12.45	---	---	---	---

The total number of lever presses for the light stimulus was significantly higher in the experimental than the control groups (independent t-test) [$t(16) = 6.17$, $p < .01$] (Figure 3).

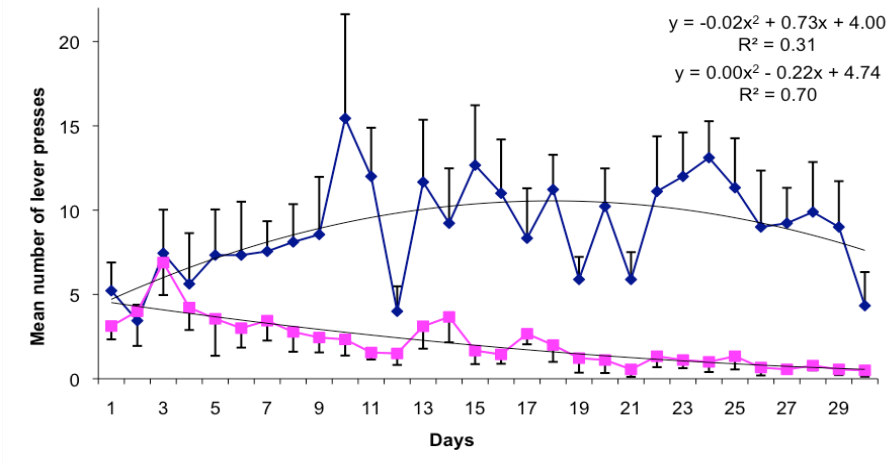

Figure 3 – Mean number and ± 1 SEM of lever presses for turning off the light stimulus over days for both experimental (♦) and control subjects (■). Binomial trend lines added with best-fit equations for both groups (top equation is for experimental subjects and bottom equation for the control subjects).

Figures 4 and 5 show the activity levels of lever presses over days with binomial trend lines for experimental and control groups. Table 2 shows the binomial regression functions including R^2 and r values for each trend line, revealing that subject number 10 in the experimental group displayed a significant increase in lever presses over days, whereas subjects 5 and 7 in the control group exhibited a significant decrease in lever pressing activity. Seven of nine experimental subjects showed a positive slope indicating that these animals increase the number of lever presses over time, when the opposite held for the control animals, where seven of nine animals decreased the lever pressing over time.

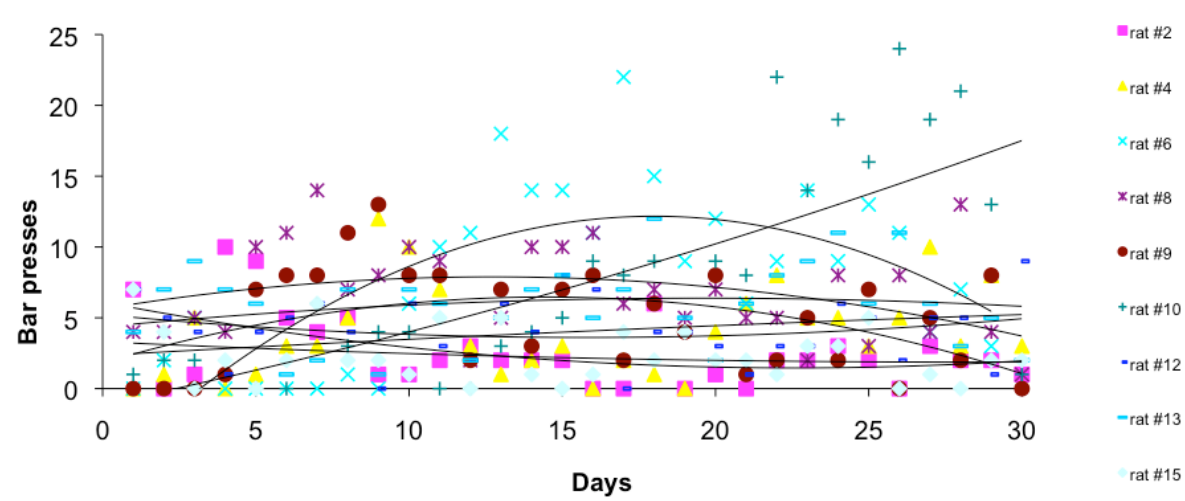

Figure 4 – Mean number of bar presses for individual subjects in the experimental group over days with best-fit binomial trendlines for each individual

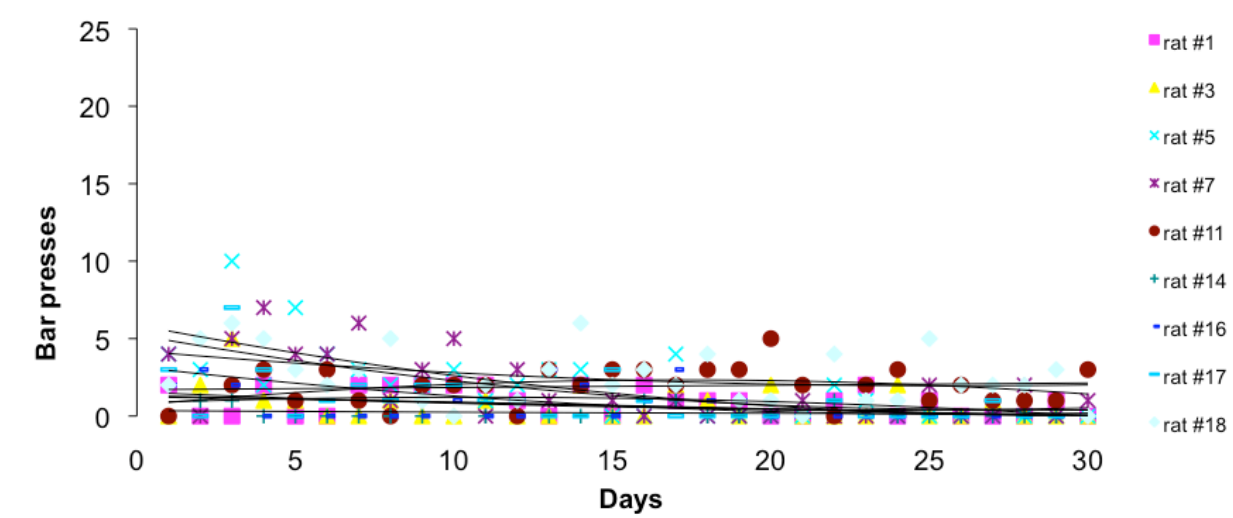

Figure 5 – Mean number of bar presses for individual subjects in the control group over days with best-fit binomial trendlines for each individual

Table 2 – Best-fit equations (binomial) for bar pressing activity (total presses) over days for both experimental and control subjects (* denotes a significant value at an alpha level of p = .05)

Experimental

Subject	intercept	R^2	r	
2	$y = 0.0309x^2 - 1.1326x + 13.178$	.18	.42	
4	$y = -0.0233x^2 + 0.7942x + 3.5473$	.02	.15	
6	$y = -0.0307x^2 + 0.9415x + 4.8734$	.11	.33	
8	$y = -0.0434x^2 + 1.0349x + 10.971$	.25	.50	
9	$y = -0.0488x^2 + 1.525x + 1.4956$	.17	.41	
10	$y = 0.0128x^2 + 0.4877x - 0.7751$	.55	.74	*
12	$y = 0.0099x^2 - 0.2651x + 8.0773$	.02	.15	
13	$y = -0.0307x^2 + 0.9415x + 4.8734$	.11	.33	
15	$y = 0.001x^2 + 0.0121x + 3.3635$	.01	.08	

Control

Subject	intercept	R^2	r	
1	$y = 0.0004x^2 - 0.0996x + 2.7374$	.14	.37	
3	$y = 0.0057x^2 - 0.2396x + 2.9246$	.12	.35	
5	$y = 0.0113x^2 - 0.6959x + 10.457$	.45	.67	*
7	$y = 0.0052x^2 - 0.4231x + 7.8498$	.46	.68	*
11	$y = -0.0161x^2 + 0.5124x + 0.2492$	.21	.46	
14	$y = -0.0014x^2 + 0.0429x + 0.1088$	.02	.13	
16	$y = 0.0002x^2 - 0.0865x + 2.4803$	.09	.30	
17	$y = 0.007x^2 - 0.3707x + 4.9956$	.26	.51	
18	$y = 0.0124x^2 - 0.5793x + 9.5698$	.20	.44	

The total number of lever presses for the sound stimulus did not significantly differ between experimental and control groups, [t (16) = 0.06, p >.05] (Figure 6). There was also no significant difference in the total number of lever presses for the running wheel stimulus between experimental and control groups [t (16) = 0.89, p >.05] (Figure 7). From here on, only the data obtained from lever presses that affected changes in the light stimulus will be reported. The reasons for excluding data from the sound and running wheel stimulus conditions will be discussed further below.

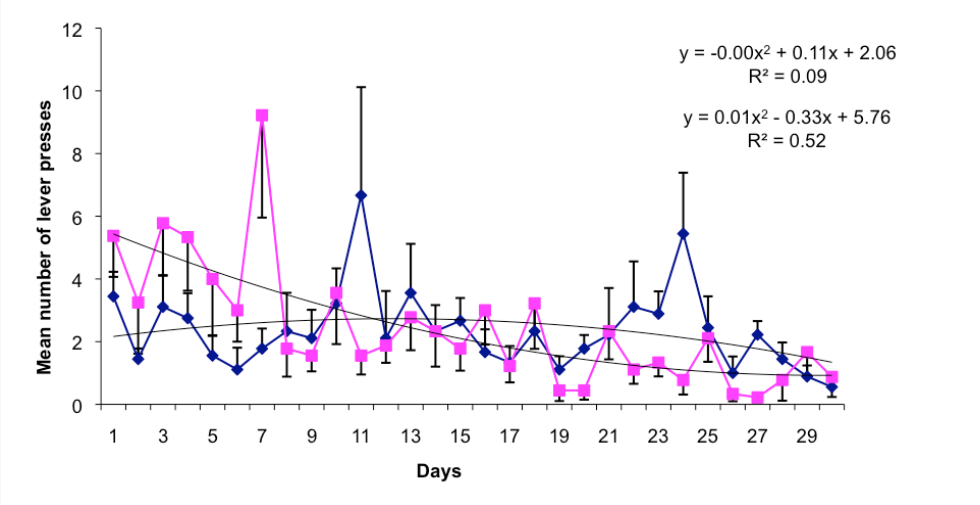

Figure 6 – Mean number and ± 1 SEM of lever presses affecting sound stimulus over days for both (♦) and control subjects (■). Binomial trend lines were added with best-fit equations for both groups (top equation: experimental subjects; bottom equation control subjects)

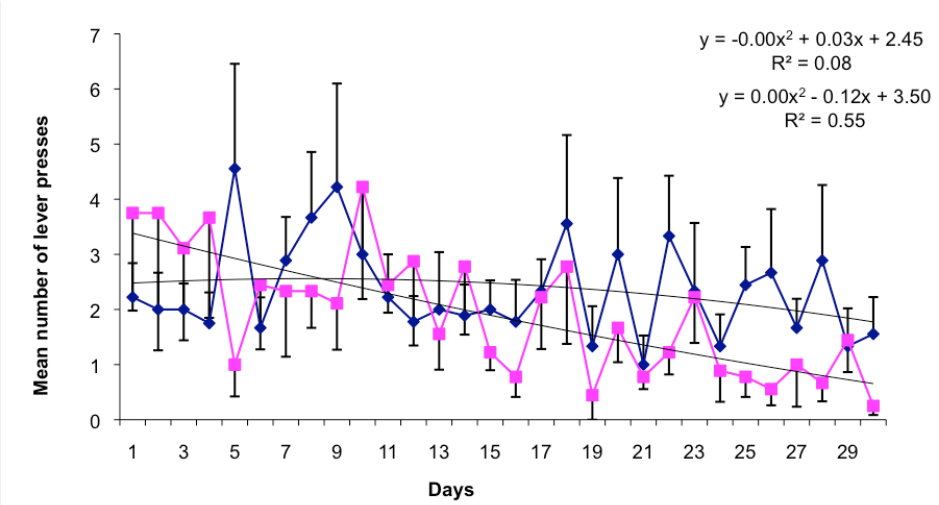

Figure 7 – Mean number and ± 1 SEM of lever presses affecting wheel stimulus over days for both (♦) and control subjects (■). Binomial trend lines were added with best-fit equations for both groups (top equation: experimental subjects; bottom equation control subjects)

Though the number of change-presses performed by the experimental subjects were not significantly different from prolong-presses [t (16) = 1.50, $p > .05$], there was a trend for the animals to press the lever more often to change the condition rather than to prolong the dark status affected by the stimulus. There was also less variance in the change-press data over the prolong-press data (Figures 8, and 9). This is further illustrated in Figure 10 that shows that there was no trend in experimental animals preferring to remain in dark condition throughout all sessions.

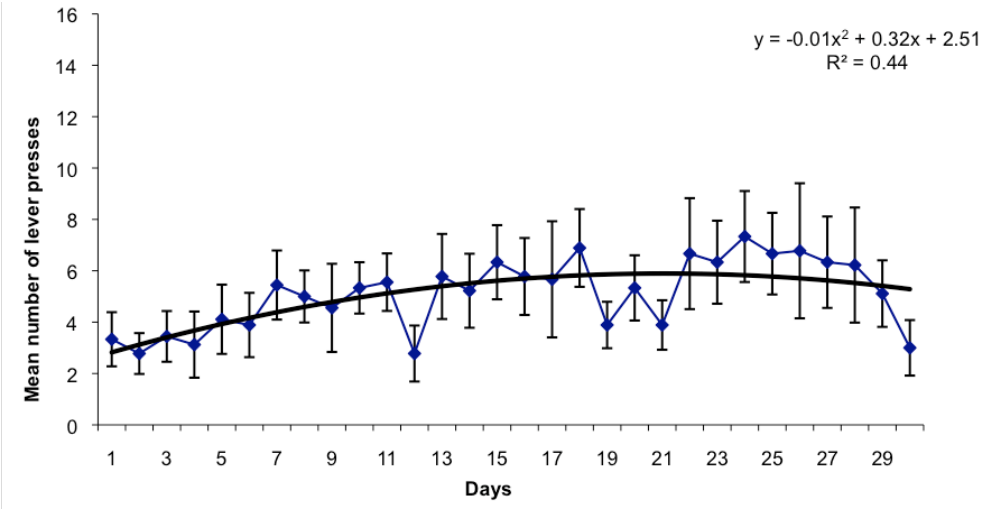

Figure 8 – Mean number and ± 1 SEM of change lever presses made by experimental subjects for the light stimulus over days. A best-fit binomial trendline added including equation and R^2

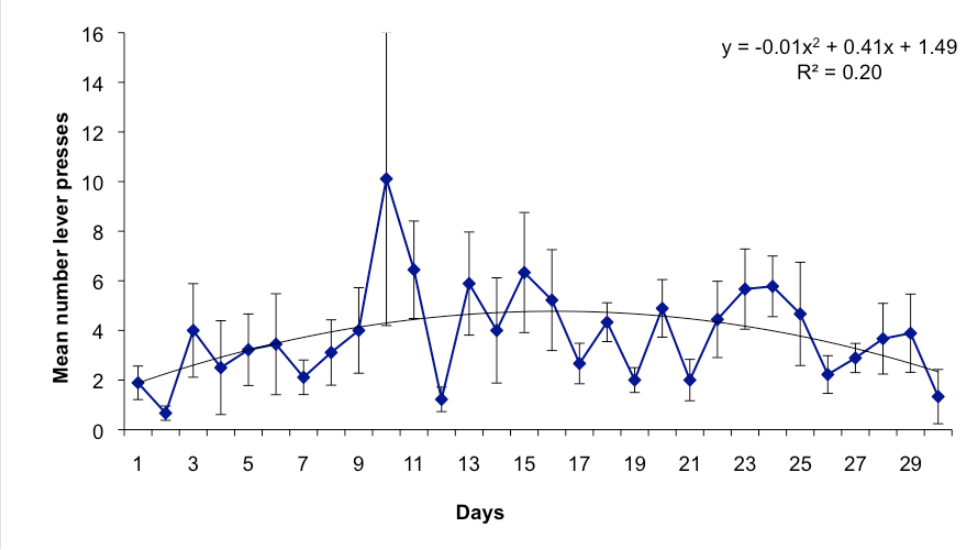

Figure 9 – Mean number and ± 1 SEM of prolong lever presses made by experimental subjects for the light stimulus over days. A best-fit binomial trendline added including equation and R^2

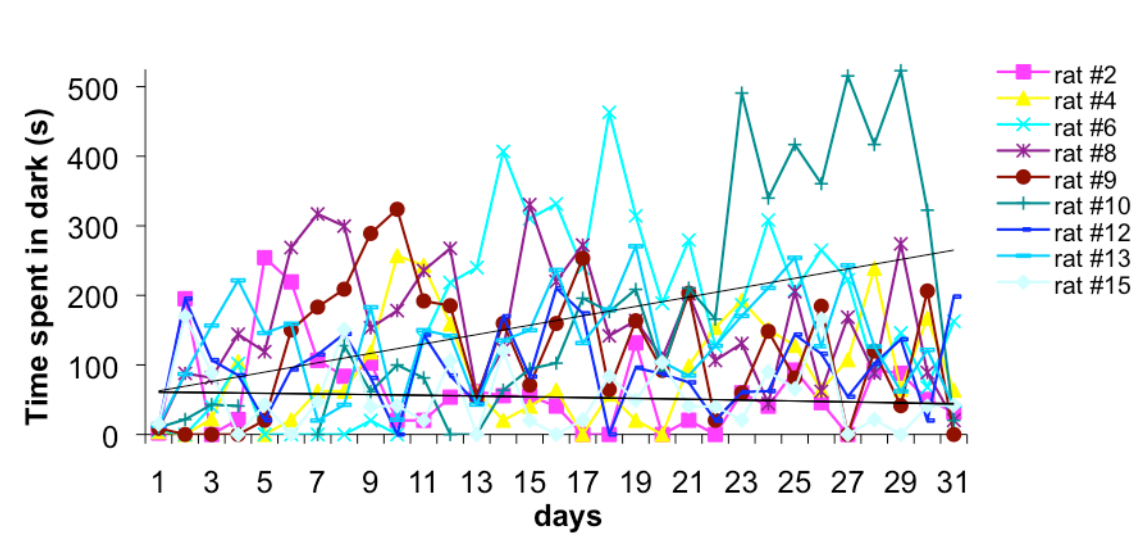

Figure 10 – Time spent in dark conditions by experimental subjects with best-fit trend lines shown for two animals (6 and 15)

Barnes Maze

Two sets of results are reported for the Barnes maze. The first set reports the initial 13 trials of the preliminary three days of the experiment when the escape hole was located in the same position for every trial. The second set of data is from trials 14 through 16 when the maze was turned 90 degrees for the final three trials on the fourth day. Data (time) from two subjects from the experimental group were removed because they never completed the maze within the set time limit.

Barnes maze trials 1-13

There was no significant difference in latency between groups [z = -0.08, p =.937]. Because a few of the subjects did not complete the maze in the allotted time a Wilcoxon non-parametric test was used allowing for animals to be grouped for statistical analysis. Figure 11 shows the mean time for all animals to locate the escape hole over trials. The time to complete the maze decreased in both groups. Data also showed that experimental animals took more time than control animals in finding the goal. Figure 12

displays the mean number of errors made over trials for experimental and control subjects (Table 3). The number of errors made significantly decreased over trials for both groups (two-way mixed ANOVA) [F (1,12) = 4.67, p = .00], but was not significant between groups at [F (1,12) = 0.31, p =.58].

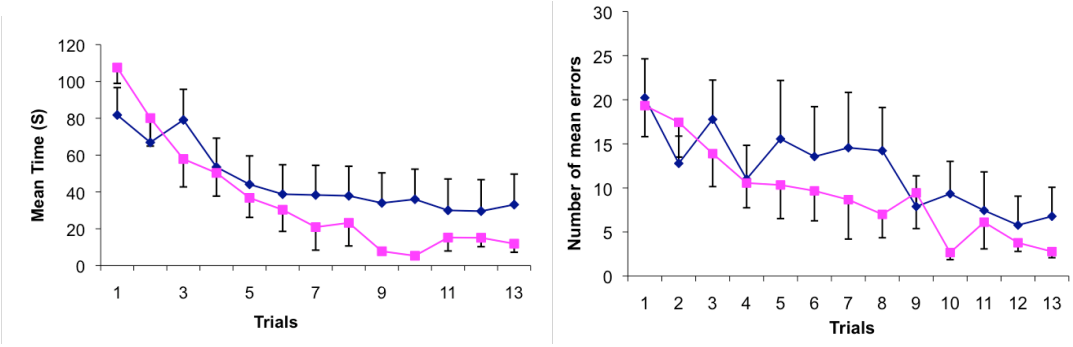

Figure 11 and figure 12 – Mean and ± 1 SEM for experimental (♦) and control (■) subjects to reach goal and number of errors made in trials 1-13

Table 3 - Means and ± 1 SEM for mean time and errors for the Barnes maze for both experimental and control groups for trials 1-13

Group	Mean (time, s)	SEM	Mean (errors)	SEM
Experimental	46.39	16.06	12.07	4.41
Control	35.57	9.07	9.36	2.92

Barnes maze trials 14-16

There was no significance between groups in latency to goal, [z = -0.23, p =.82] (Figure 13). Error scores significantly decreased over trials in both groups [F (1,2) = 4.44, p = .02], but there was no significant difference between groups on error scores at [F (1,1) = 0.32, p = .58] (Figure 14). The mean number of errors made and time to find goal for experimental and control subjects are displayed in Table 4. A comparison of time and error data between 1-13 & 13-16 trials in control and experimental animals showed no significant differences on either measure which suggests that rats used mapping (see discussion).

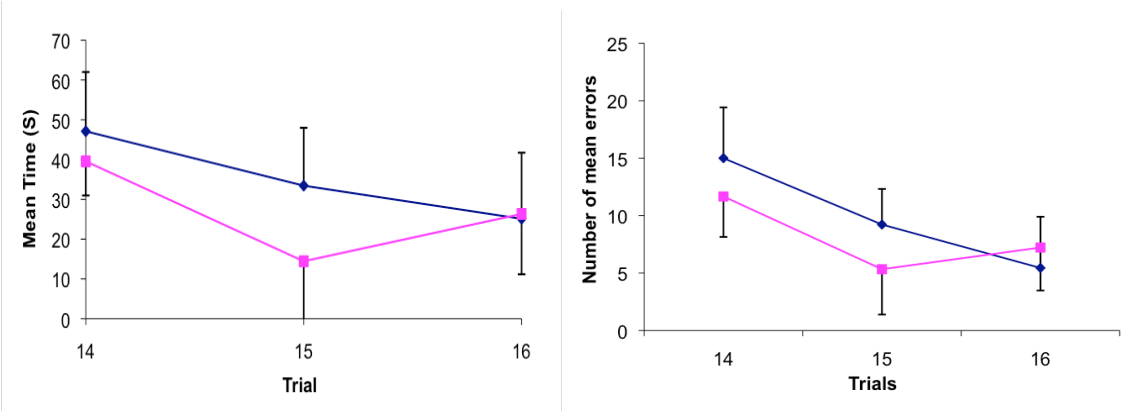

Figure 13 and figure 14 – Mean and ± 1 SEM for time and number of errors made by experimental (♦) and control (■) to reach goal in trials 14-16

Table 4 – Means ± 1 SEM for mean time and errors for the Barnes maze for both experimental and control groups for trials 14-16

Group	Mean (time, s)	SEM	Mean (errors)	SEM
Experimental	35.22	28.88	9.88	3.51
Control	26.78	7.37	8.07	2.00

Correlations between operant and Barnes maze performance

The number of lever presses performed on the operant conditioning task was rank-ordered by animal. The number of lever presses was then correlated with the animals' performance on the Barnes maze task (Table 5). Correlations were made using Pearson product-moment tests between total lever press activity with both time finding the goal and errors made on the Barnes maze for both experimental and control groups (Table 6). None of these correlations were significant.

Table 5 – Rank order by total number of bar presses for light in experimental and control groups

Group	**Experimental**		**Control**
Subject ID	bar presses	Subject ID	bar presses
15	116	14	9
2	161	3	30
12	213	16	36
4	255	1	40
9	293	17	44
13	294	7	88
10	323	11	92
6	344	5	97
8	400	18	132

Table 6 – Correlations between total number of lever presses in an operant chamber and performance on a Barnes maze (time and error) for experimental and control groups, and for the high performance (HPG) and low performance group (LPG) separately.

Group	**Experimental**		**Control**	
Subgroup	Time	Errors	Time	Errors
Total	.11	.09	-.35	-.17
HPG	.08	-.05	-.23	.62
LPG	-.37	.84	-.21	-.88

To test the prediction that high levels of lever-press performance on the operant task correlated with increased performance on the Barnes maze, both experimental and control groups were divided into two subgroups based on their lever press activity. The high performance group (HPG) was formed by selecting the four most active animals; the low performance group (LPG) comprised of the four animals that pressed the lever the least amount of times (Figures 15, 16). As shown in Table 6, all correlations between number of lever presses and maze performance in the experimental groups were not significant.

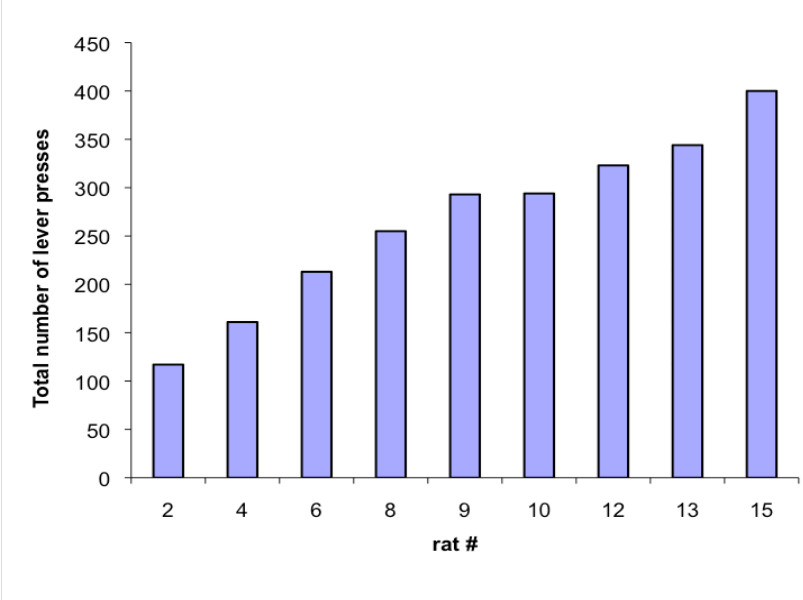

Figure 15 – Experimental animals in rank order according to the activity level of lever pressing for the light stimulus.

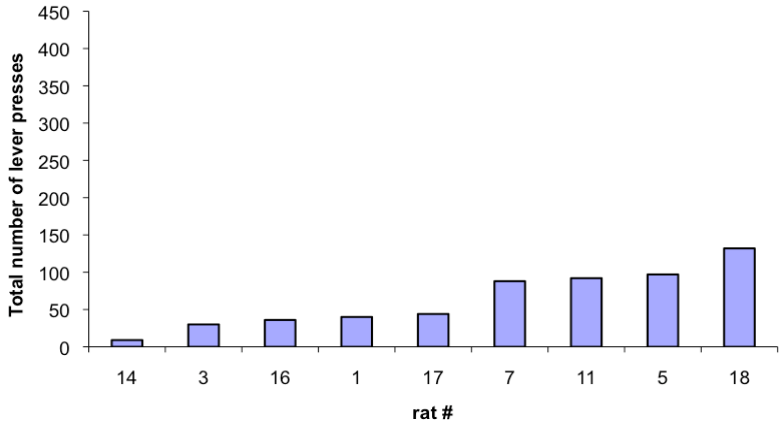

Figure 16 – Control animals in rank order according to the activity level of lever pressing for the light stimulus.

There was a positive trend between number of lever presses and the number of errors made on the Barnes maze performed by the animals in the HPG control group. The LPG control group exhibited a negative but not significant trend between lever presses and errors made on the maze (Table 6).

Correlations between the number of change-presses and prolong-presses and the animals' performance on the Barnes maze (time and error) showed trends in the experimental groups (HPG, LPG, and both combined), but none of them were significant. The HPG subjects displayed a positive trend in making more errors and taking more time on the maze. The experimental LPG that prolonged the dark conditions in the operant task displayed a trend to taking more time and making more errors on the maze. All other correlations between change-presses and prolong-presses were not significantly correlated with the results of the animals' performance on the Barnes maze (Table 7).

Table 7- Correlation between changed and prolong number of lever presses in the operant chamber and performance in Barnes maze (time and error) for experimental subjects, and for the high performance and low performance group separately.

Press type	**Change**		**Prolong**	
SubGroup	Time	Errors	Time	Errors
Total	.06	.20	.12	-.11
HPG	.61	.69	-.26	-.44
LPG	.45	.47	.86	.89

Discussion

This study showed that given a limited choice to control the consequence of lever presses, animals only controlled the lever that affected lighting conditions. No such preference was found for changing intensity levels of sound or access to the running wheel. Having control over environmental features did not lead to improved cognitive performance on the Barnes maze. Further, there was no significant correlation between operant activity and maze performance that would have supported the prediction that cognitive performance improves following stimulus control in the operant task.

The first aim of this study was to test if rats could control their environment given the opportunity to manipulate access to three different stimulus conditions. Results from the operant conditioning task did support the prediction that experimental subjects would manipulate levers for changing the stimulus status in the operant chamber. This was indicated by the fact that experimental animals pressed the lever more often than control animals. These results highlight the importance of animals having a similar degree of behavioral control that they would normally experience under more natural conditions. Factors such as nest site location and nesting materials, social interactions, and forging strategies are examples of environmental aspects animals do control (Kavanau, 1962). Animals kept in impoverished conditions, e.g., housed in laboratories that lack these opportunities, are prevented from displaying species-typical behaviors. Allowing laboratory animals to control aspects of their environment may aid in normal psychological and physiological development (Kavanau, 1962).

There was no consistency for light or dark illumination preference (Figure 9), although it was predicted that subjects would prefer the stimulus in the "off" position (as rats in a study performed by Blom, van Tintelen, Baumans, van den Broek and Beynen (1995) preferred cages with illumination of lower intensities) i.e., adjusting the illumination level at a lesser intensity for the duration of each session. There was also no significant difference between the number of change-presses and prolong-presses, although there was a trend in the experimental animals pressing the lever more often to change the status of the light from on to off rather than to prolong the dark condition. There was also an increased variance in lever pressing for prolong-presses. This may reflect a preference for control of the stimulus rather than a preference for a particular consequence, i.e., dark or light (McCall, 1965).

The second aim of the experiment tested the hypothesis that lever pressing activity, which enabled subjects to have control over the environment, would promote increased performance on cognitive tasks such as the Barnes maze. The results obtained from the Barnes maze study did not support the prediction that animals that had control over their environment would perform better than the control group that lacked such control in the operant cage. The experimental group made more errors and took longer finding the escape hole than the control group. It is possible that the assumed physiological changes (e.g., neurogenesis) promoting behavioral differences were not initiated while the experimental subjects had control in the operant task. Alternatively, physiological changes might still have occurred, affecting areas that were not tested appropriately by the Barnes maze (Renner & Rosenzweig, 1987). The Barnes maze is a similar task to the radial arm maze, which tests memory that is processed in the hippocampus (Crawley, 2007). Brain regions that utilize the prefrontal cortex may have been more sensitive to tasks that involve control over the environment such as the tests used in this experiment. Lesioning the dentate gyrus of the hippocampus impaired the performance of mice in spatial discrimination tasks, but the same subjects were able to successfully discriminate graphics with spatial parameters, suggesting that processing of this kind of information might be facilitated by subsequent neurogenesis in the hippocampus (Clelland et al., 2009), or the processing of the information used in discrimination tasks is happening elsewhere in the brain.

The fact that the experimental animals in the current experiment spent more time exploring the maze and made more errors might have been due to the fact that animals that have had control over their environment may have a cognitive advantage promoting exploratory behaviors. Several studies have found that enriched rats display decreased

emotionality to stimuli that promote non-fearful and more exploratory behavior (Larsson, Winblad, & Mohammed, 2002). The experimental group may have experienced such decreased emotionality to the stressful conditions of the maze. Where the non-enriched control animals may have searched for an area to escape the adverse conditions, the experimental subjects may have preferred exploring the environment resulting in an increased number of errors and time spent on the maze.

On trial 14 the Barnes maze was turned 45º in order to add another cognitive component to the task. In order to find the location of the hole after the maze was turned, subjects would have needed to rely on cognitive maps (O'Keefe & Nadel, 1978). It was predicted that experimental animals might have acquired this ability due to their experience during the operant phase of the experiment. This prediction was not confirmed as there was no significant difference in experimental animals before and after the maze was turned. This lends support to Benhamou's (1996) findings that rats do not have cognitive maps but negotiate the maze by path integration.

Two interesting trends developed within the HPG subgroup (Tables 6, and 7). The first was a correlation between change-press/prolong-presses and Barnes maze performance in experimental subjects. The second was a correlation between lever presses and maze performance between experimental and control groups. The amount of change-presses was positively correlated with time and error scores, meaning that animals that pressed the lever more often affecting the illumination took longer and made more errors finding the escape hole on the maze. Subjects in this group that prolonged the darkened condition displayed a negative trend with maze performance, i.e., taking less time and making fewer errors on the Barnes maze. One possible reason why subjects that produced more prolong-presses showed improved maze performance is that subjects that

learned the consequence of their prolonged-presses may have experienced a decrease in emotionality. Animals that only learned the consequence of a change-press may not have experienced this effect. Another explanation may be that animals that learned to prolong the darkened conditions underwent beneficial neurogenesis, which could have increased the rat's cognitive abilities resulting in enhanced performance on the Barnes maze.

A second interesting trend in the correlation analysis was between control and experimental HPG subjects. Although both experimental and control animals were from the high activity group, the experimental subjects that performed more prolong-presses, showed a negative correlation in maze performance (fewer errors and less time), whereas the control animals that pressed the lever most made significantly more errors and took more time on the maze. The results may indicate that simply pressing the lever with no consequence was not sufficient for improved maze performance, but that learning the association of lever pressing with a consequence may increase performance on the Barnes maze.

There was no significance between groups with respect to lever pressing for the sound stimulus. This may have been due to the fact that subjects did not have a preference for either sound level. Experimental subjects may not have detected a difference between the sound intensities of the two stimuli presented, or that subjects may not have been motivated to press the levers to lower the sound intensity because the more intense sound was not sufficiently aversive. The sound levels used in the operant chamber were based on the audibility curves obtained from psychophysical experiments on rats, and were within the optimal hearing range of the subjects determined in previous studies (Blackwell & Schlosberg, 1943).

Previous research found robust results in lever pressing in rats when using access to a running wheel as a stimulus (Kavanau, 1969). The lack of significant results in this study was most likely due to the malfunctioning of the equipment, which was detected after the completion of the operant phase. However, data of lever presses for both sound and access to the running wheel were reported as they most likely affected the number of lever presses controlling the light condition.

The results of this study may have been different if all three stimuli had functioned properly, thereby creating choice in a multi-modal environment (Bradshaw & Poling, 1991), as the nature and number of features, such as sound, food, mates and shelter will vary what animals prefer to control within their environment. Also, preferences are not fixed, but can vary as choices over certain stimuli can affect each other (Bateson, 2004). For example, if a sound stimulus was found more noxious, animals may have preferred extinguishing all stimuli more frequently or, alternatively, chosen less noxious stimulus conditions. The fact that preferences will develop depending on the animals' behavioral and physiological state is adaptive in that preferences will help attain homeostasis leading to increased fitness.

Preferences may also change over time as shown in Figure 3. Experimental animals pressed the lever controlling light most often midway through the operant phase and then slowed down their activity by the end of the trials. The change of preference may have been due to habituation to the operant cage, i.e., experiencing less fear of the stimulus (light/dark) changes.

The goal of this study was to test the effects of environmental control on cognitive abilities and by inference of physiological processes. This research may have important implications for current scientific standards. Current conditions under which laboratory

animals are often kept may not be adequate in order to maintain the animals' physiological and behavioral well-being. Improvement is called for to comply with humane scientific conduct and to guarantee the validity of scientific research if laboratory animals are to represent accurate models for humans. Scientific research should maintain the highest standards by using the healthiest animals, which is a necessity for obtaining maximum validity. This may be accomplished by understanding how best to accommodate laboratory animals and also understanding the aspects of their environment that affect their physiological and behavioral requirements.

Experiment 2

Changes in cognition and corticosteroids levels due to control of ambient cage light

Experiment 1 showed that experimental rats increased the rate of lever presses irrespective of the nature of the consequence. To quantify the animal's response to a single stimulus modality, this experiment presented one stimulus (light) over which subjects had control. Further, subjects were able to control different illumination levels in an operant paradigm so that preference for stimulus strength could be determined. A yoked cage was added to control for possible outcomes due to stimulus variance while keeping cage conditions identical.

Methods

Subjects

Forty-five Long-Evans Hooded male rats at the age of 21 days were gentled for a week before testing began by handling each subject approximately two minutes every day. All subjects were singly caged and kept on a 12 hour light cycle with lights on at 1 p.m. The vendor for all animals was Harlan Laboratories and fed Harlan 2001 Lab chow™ and had water available *ad libitum*. Selected subjects (n = 24) were food

restricted to 90% body weight for the cognitive testing section of the experiment for 17 days.

All tests were run in the evening, close to the time when lights would turn off in the homeroom, i.e., coinciding with the animals' active phase. As in experiment 1, for consistency of handling, only one person cleaned, handled and tested all subjects throughout the experiment. Homerooms were lit with fluorescent light and were measured at 400 lx (one meter off the ground), temperature remained between 18 to 23° C, and humidity was kept between 45 and 85 relative humidity units. Animals were kept on paper bedding and cages were cleaned every five days.

Apparatus

There were five components to this experiment: 1) an object-approach test, 2) Barnes maze, 3) operant conditioning, 4) a discrimination task, and 5) a corticosterone assay (Table 8).

Table 8 – Time line for experiment

Day	**Task**
1	Gentling
2 – 3	Habituation
4	Object-approach test
5	Barnes maze
6-33	Operant conditioning
34-35	Barnes maze
36-63	Operant conditioning
64-78	Discrimination

Object approach test

The open field was constructed of black Plexiglas (61 W x 61 H x 20 D cm^3). A novel object, a soda can with contrasting coloration, was placed in the center of the open

field. The room where the animals were tested was free of all extraneous sounds and the ambient light level of the room was 60 lx.

Barnes maze

The same Barnes maze used in Experiment 1 (Figure 2) was also used in this experiment with the difference that one wall of the room was fitted with a visual landmark, a 30 cm isosceles triangle.

Operant conditioning

Operant conditioning trials were performed in an operant cage equipped with five light-emitting diodes (LED's) located in the ceiling of the chamber (Figure 17). The LED's could be sequentially turned on or off by two different levers that were located on one of the walls of the chamber. The average ambient light levels were 26.6, 69, 88, 122 and 140 lx for any one, two, three, four and five LED's respectively (measured from the center of the floor of the cage). Lights were activated by a program written by the staff at Hunter College in Visual Basic™ (version 6) using a Switch and Sense™ control box connected to a Dell Optiplex™ computer. The operant cages were manufactured by Coulbourn Instruments.

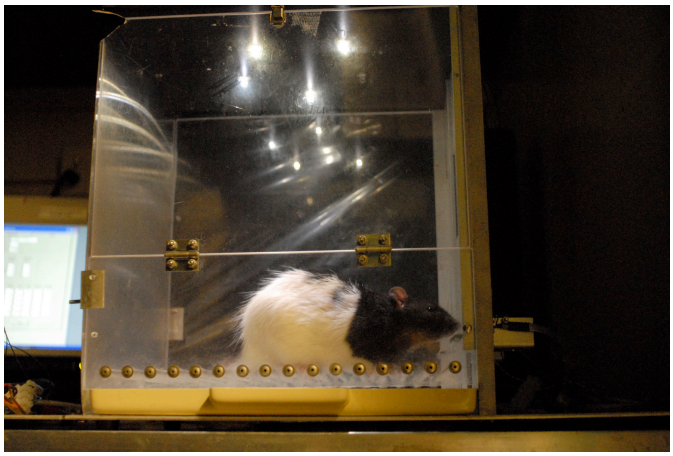

Figure 17 – Rat in operant cage positioned in front of the "on" lever with four of the five lights illuminated.

Cognitive/discrimination test

The same open field that was used for the object approach test was used for the discrimination test with the addition of a divider fitted with a starting gate operated by a sliding door (Figure 18). Two 15 x 18 cm cards with a 4.4 cm white graphic (a five pointed star or square) on a black background were used as stimuli. The starting gate was located 46 cm away from the cards, which were located 30.5 cm from each other.

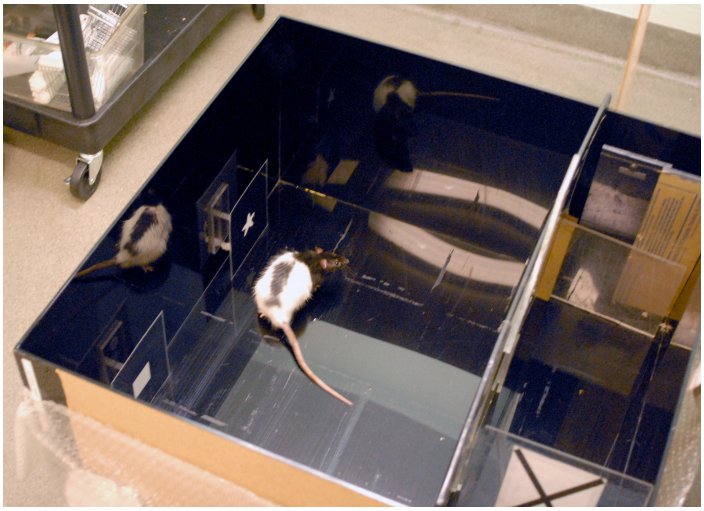

Figure 18 – Discrimination test box including the stimulus of the two graphics (star and square).

Corticosterone testing

The diagnostic kit Corticosterone HS EIA was used to measure levels of corticosterone and was manufactured by IDS Diagnostics. Instructions that were supplied with the kit were followed for all assays. The sensitivity that the kit was able to detect was 0.17 ng/ml of corticosterone.

Procedure

Object approach test

For two days, prior to testing, individual rats (n = 45) were habituated to the open field for five minutes. This analysis was included to test for possible correlations between activity levels and inspection time with a novel object and the animals' performance on cognitive tasks. The results may serve as a future indicator for choosing animals that would perform well in subsequent research. The following measurements were taken for the test: latency to approach object, time spent at object, and number of times touching object. As a backup, all behaviors were recorded using an overhead Sony Mini DV™ video camera. The open field box was cleaned with Nolvasan™ between sessions.

Barnes maze

Subjects (n = 45) ran three trials in the Barnes maze with the escape hole kept in the same location in reference to the visual landmark (maximum trial duration: three minutes, inter-trial-interval: two minutes). The following behaviors were recorded: latency to enter the escape hole and number of incorrect choices. As a backup, all behaviors were recorded using an overhead Sony Mini DV™ video camera. The maze was cleaned with Nolvasan™ between each trial to remove possible chemical markers left by the subjects.

Operant conditioning

Following the first Barnes maze test, on the next day, rats performed the operant conditioning task. Subjects could control the level of environmental illumination by the use of two levers. One lever would increase illumination while the second lever would decrease it incrementally by affecting on- or offset of zero to five LED's. Three conditions were tested in identically equipped cages (Figure 19): 1) experimental subjects

had control over illumination levels (n = 15), 2) yoked subjects experienced the same light levels as the experimental animals in a yoked cage, i.e., without having control over illumination levels (n = 15), and 3) control subjects that experienced a constant illumination from four LED's for the duration of the session (n = 15) (both yoked and control animals had levers, but when pressed had no consequences). Each session started with four LED's illuminated in all cages. LED patterns for a given illumination were randomized but identical for the experimental and yoked cages. The number of bar presses, the time the lever was pressed, number of LED's in use, and the status (on/off) of each LED was automatically recorded for experimental, yoked and control subjects. The three cages (experimental, yoked and control cages) were isolated in a room to reduce light and sound interference from other apparatus. Three complete set-ups ran at the same time (total of nine cages). Each session ran for half an hour, and the task was completed in 53 days (Table 8).

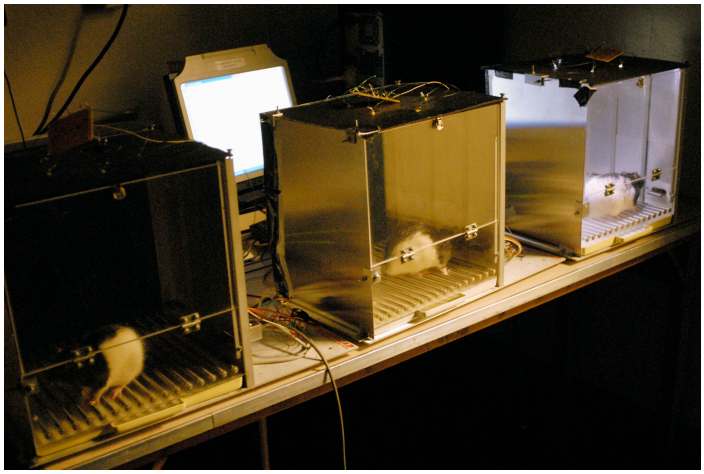

Figure 19 – Position of operant cages: experimental, yoked and control

Cognitive/discrimination test

Subjects used in this task were selected based on their activity levels (lever pressing) in the operant conditioning task. The most active animals were chosen from each of the three groups (n = 8 each). This selection was based on the assumption that experience in the operant phase increased physiological changes. Therefore, animals that pressed the lever more in the operant phase presumably would perform better on the discrimination task. The selected animals from each group were tested in the discrimination task for 15 consecutive days following completion of the conditioning trials.

Animals were target-trained (primed) to a graphic that was different from those used in the following discrimination trials. Target training consisted of having each subject touch the graphic with its nose to receive a drop of Nutracal™ for a total of 10 touches.

Following the priming sessions, individual animals were placed in the starting area of the open field and given two minutes to acclimate. Upon opening the gate, subjects could choose from one of the two graphics. Each trial lasted no longer than three minutes with an inter-trial-interval of two minutes. Subjects had to first learn to chose, then remember a particular graphic (star or square) and touch it with their nose in order to get a drop of Nutracal™ reward for a total of ten trials per session. The cards were pseudo-randomly presented in either the left or right position of the open field to insure a balanced number of presentations of the graphics.

Corticosterone testing

Fecal boli were collected from each subject at the same time of day, for each week of the operant phase of the experiment and kept frozen at -80° C, but only the first, fourth and eighth week collections were assayed.

To validate the assay, a corticosterone challenge test was performed. A total of six animals were used where three subjects received an ACTH (500 µl/100g) subcutaneous injection and three subjects received a saline subcutaneous injection (500 µl/100g). Fecal boli were collected at time 0, 5, 10, 15, 20, 25 and 30 hours after injection, but only collections 0, 10, 15, and 20 hours were assayed.

Data analysis

Statistical analysis for this experiment was performed using SPSS™ (version 17) and Microsoft Excel™.

IACUC

The experiment was approved by the Institutional Animal Care and Use Committee of Hunter College (AA/PM 6/09-01).

Results

Object approach test

On the object approach test, rats (n = 45) took an average of 67.2 s (SD = 59.9 s) to approach the object, 5.8 (SD = 4.7) number of times to approach the object, and 15.0 s (SD = 10.8 s) total time spent at the object. These statistics will serve in comparing the animals' performance on the operant conditioning task and the discrimination task, as well as the corticosterone data.

Operant conditioning (0-55 days)

To test for differences in activity in bar pressing over time the sessions in the operant chamber were split into segments of 0-55, 0-27 and 28-55 days. These data will show whether animals remained consistent in their activity over time. Table 9 summarizes the means and SEMs of lever presses made by experimental (n = 15), yoked (n = 15), and control animals (n = 15) for these blocks of time.

Table 9 – Mean number of bar presses with ± 1 SEM for all time periods for operant conditioning made by experimental, yoked and control subjects

	0-55 days		0-27 days		27-55 days	
Group	Mean	SEM	Mean	SEM	Mean	SEM
Experimental	773.13	140.42	476.27	87.27	296.87	61.98
Yoked	688.87	129.03	512.33	96.60	176.53	39.70
Control	204.40	26.50	156.13	16.38	48.27	11.47

There was a significant difference in lever pressing across the three groups for light (repeated measures ANOVA) [F (1,2) = 10. 70, p =.00] (Figure 20). An LSD post-hoc test showed a significant decrease in the amount of lever pressing for the control group and both the experimental and yoked groups for the light stimulus. Figure 21 illustrates the mean number of lever presses for the three groups.

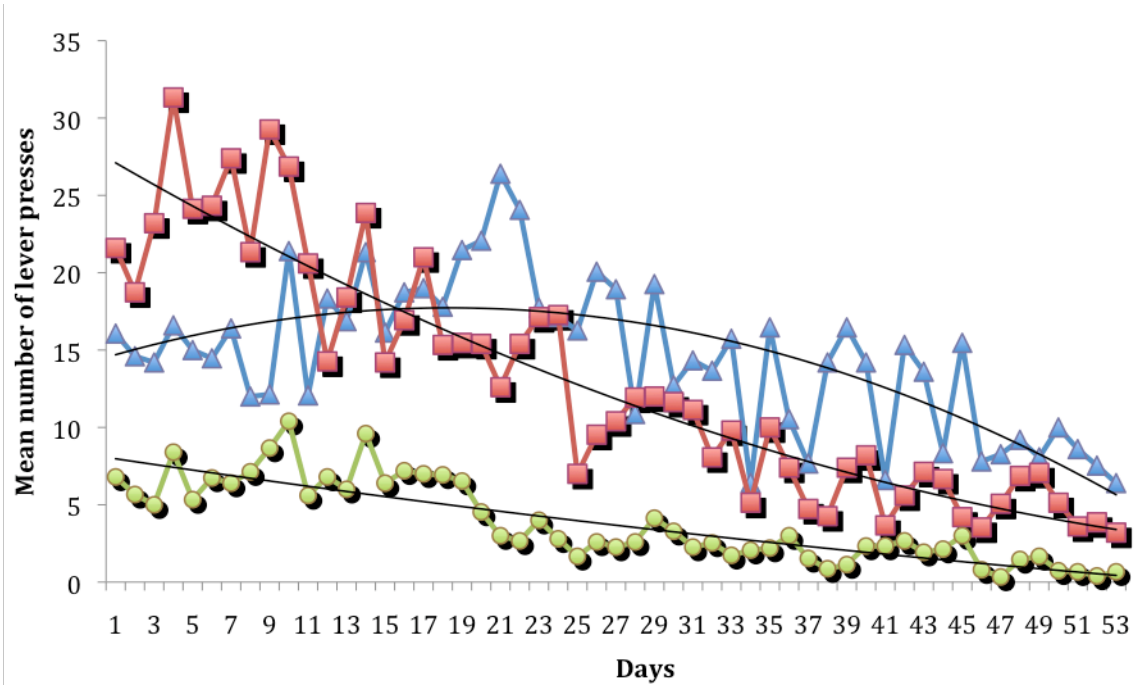

Figure 20 – Mean number of lever presses in experimental (▲), yoked (■) and control (●) animals for all sessions in the operant cage with best-fit trendlines

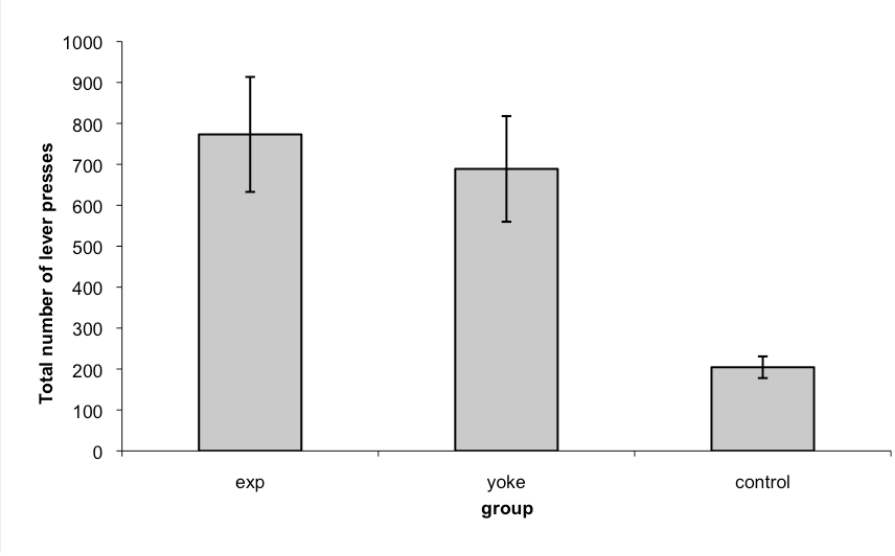

Figure 21 – Mean and ± 1 SEM for total number of lever presses in experimental, yoked and control groups for light for 0-55 days

There was a significant increase in the number of change-presses (presses that changed the light to either brighter or darker illuminations) over the number of prolong-

presses (lever presses that kept the light in either the brightest or darkest condition) [t (104) = 5.32, p <.01].

Operant conditioning (0 – 27 days)

There was a significant difference in lever pressing across the three groups on the number of lever presses for the light stimulus made in the first half of the operant conditioning phase [F (1,2) = 11.55, p =.00] using a repeated measures test. An LSD post-hoc test showed a significant decrease in lever pressing between the control group and both the experimental and yoked groups (Figure 22).

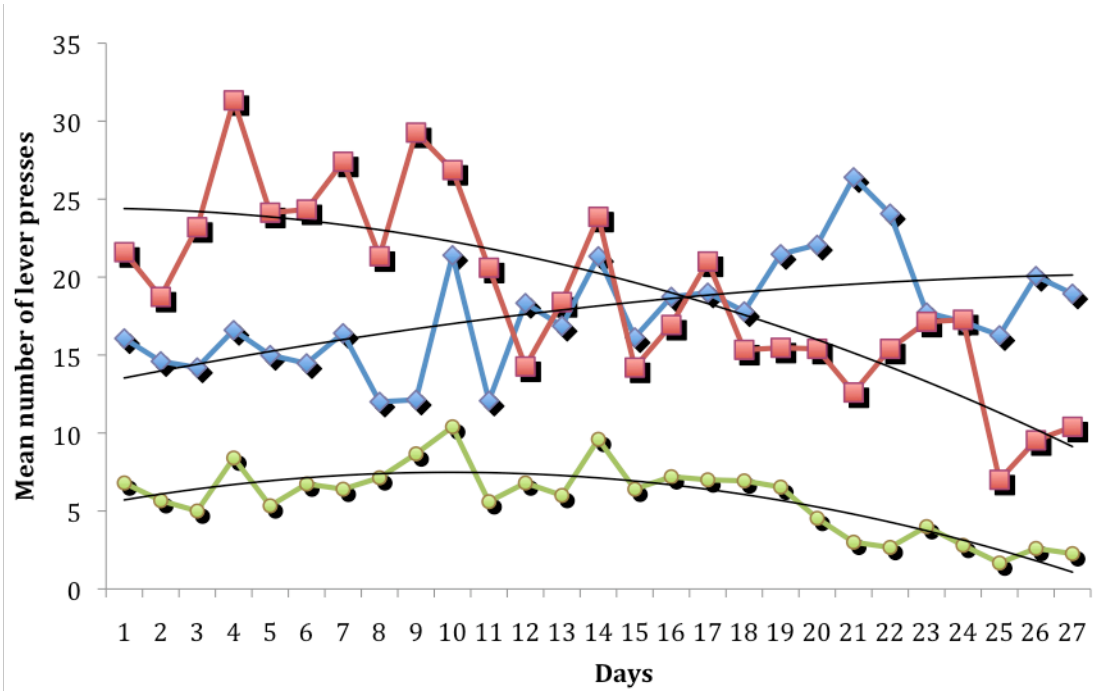

Figure 22 – Mean number of lever presses in experimental (▲), yoked (■) and control (●) animals for sessions 0 – 27 in the operant cage with best-fit trendlines

Operant conditioning (28 – 55 days)

There was a significant difference in lever pressing across the three groups on the number of lever presses for the light stimulus made in the second half of the operant

conditioning phase (repeated measures ANOVA) [F (1,2) = 11.17, p =.00]. The LSD post-hoc test showed a significant decrease in lever pressing between the control group and both the experimental and yoked groups for the light stimulus (Figure 23). Figure 24 displays the average number of lever presses that the experimental animals produced over days. Only five lever presses are necessary in order to obtain all illumination levels. As shown the subjects pressed the lever more than this minimum amount. These results suggested that animals are chose to control the stimulus rather than have a preference over a particular level of illumination.

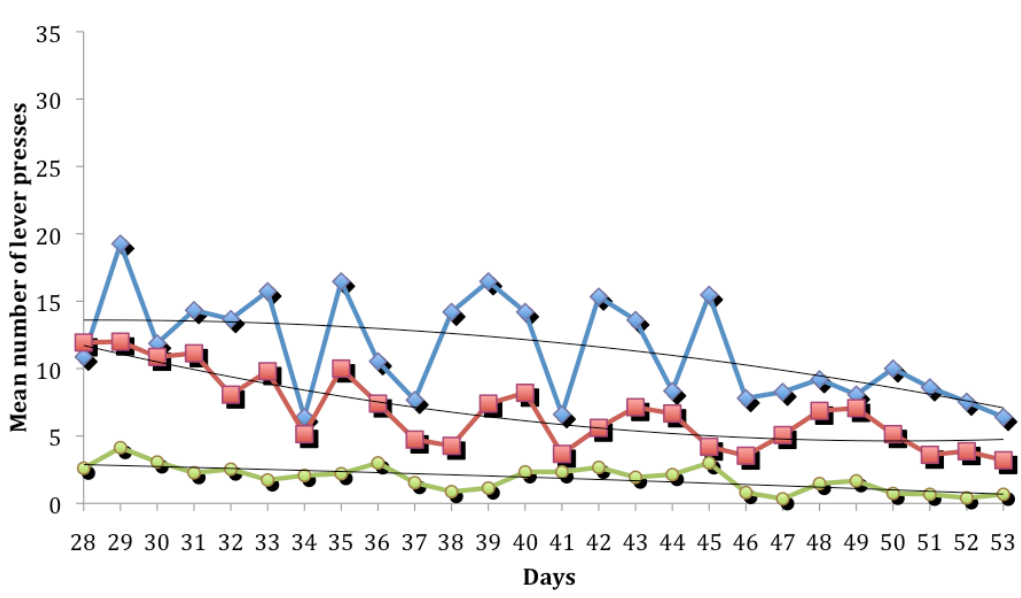

Figure 23 – Mean number of lever presses in experimental (▲), yoked (■) and control (●) animals for sessions 28 – 55 in the operant cage with best-fit trendlines

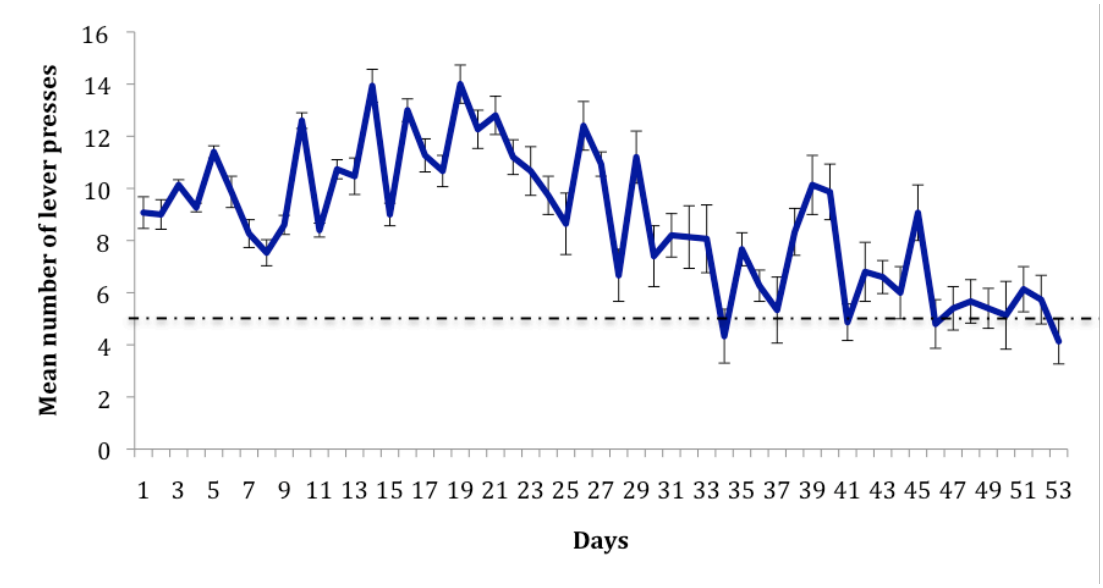

Figure 24 – Mean number of lever presses changing the light stimulus by experimental group. Dotted line represents five lever presses

Discrimination task

The total number of correct choices were for the experimental animals (n = 8): M = 74.5, SEM = 3.19; for the yoked group (n = 8): M = 71.25, SEM = 3.27; and for the control group (n = 8): M = 68.0, SEM = 1.95. There was no significant difference in the total number of correct choices performed on the discrimination task across groups (one-way ANOVA) [F (2,14) = 2.85, p >.05]. Linear regression functions were performed to test if the experimental group increased the number of correct choices made over time (Figures 25, 26 and 27). The experimental subjects significantly increased the number of correct choices over days in the discrimination task as compared to yoked and control subjects (one-way ANOVA [F (2, 21) = 4.22, p >.05] on the slopes of the functions (Fig. 28).

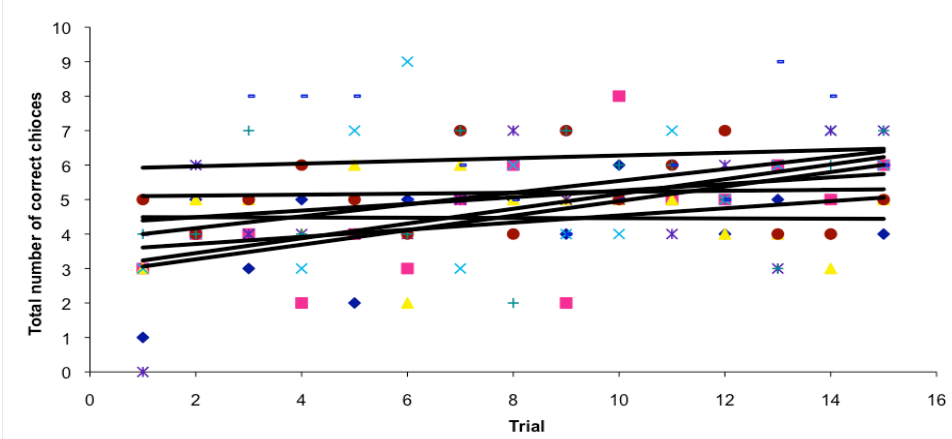

Figure 25 – Mean number of correct choices on the discrimination task for the experimental group; linear trendlines for each subject are added

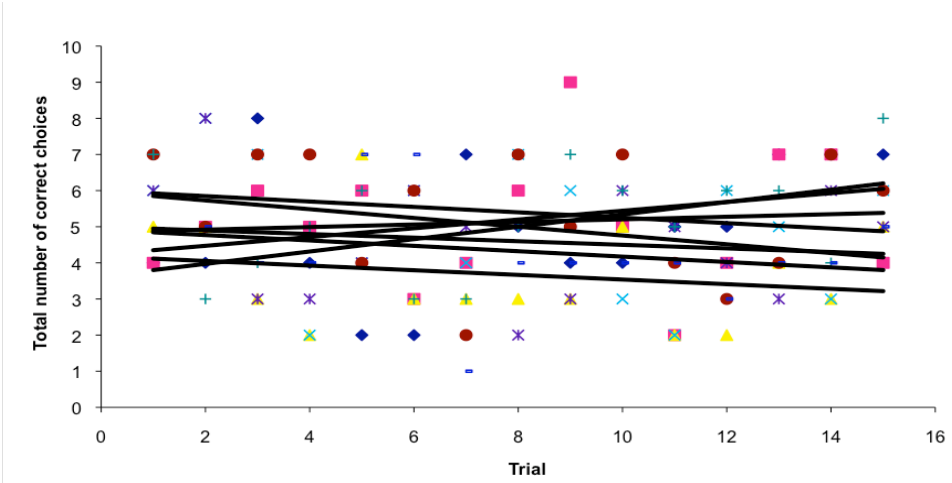

Figure 26 – Mean number of correct choices on the discrimination task for the yoked group; linear trendlines for each subject are added

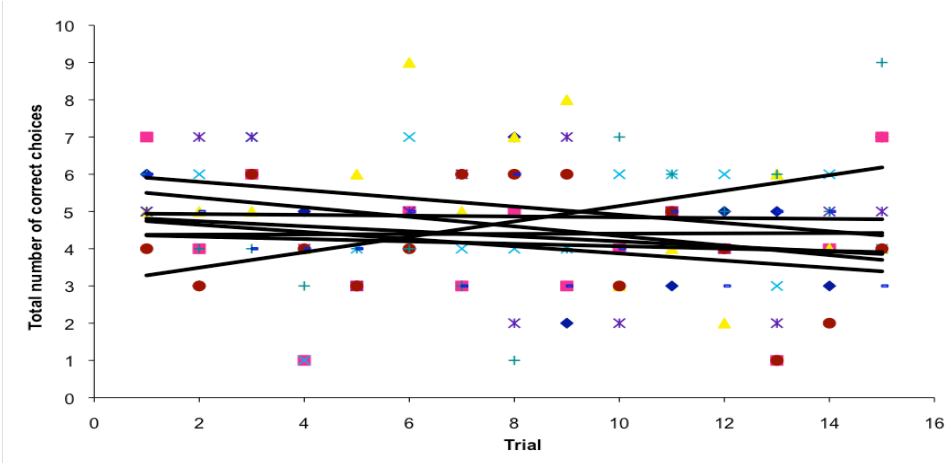

Figure 27 – Mean number of correct choices on the discrimination task for the control group; linear trendlines are added for each subject

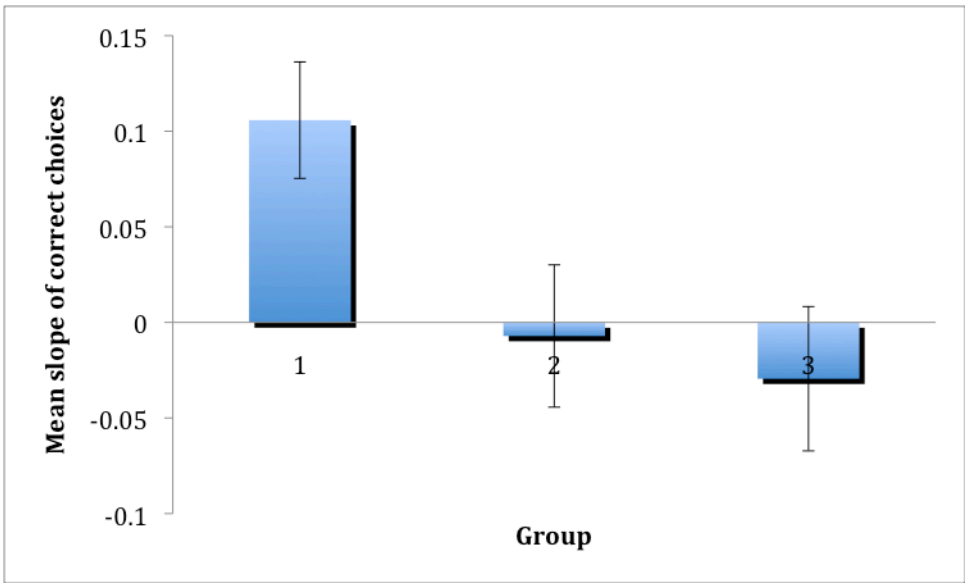

Figure 28 – Mean slope for the number of correct choices on the discrimination task for the experimental (1), yoked (2) and control (3) group with +/- 1 SEM

Hormonal Assay

Levels of corticosterone were not significantly different across the three groups (repeated measures ANOVA) [F (1,2) = 1.23, p <.05]. Still there was a noticeable trend indicating lower levels of corticosterone in the experimental subjects as compared with either yoked or control animals. Yoked animals displayed the highest levels of corticosterone throughout the operant phase (Figure 29). Means and SEMs are reported in Table 10.

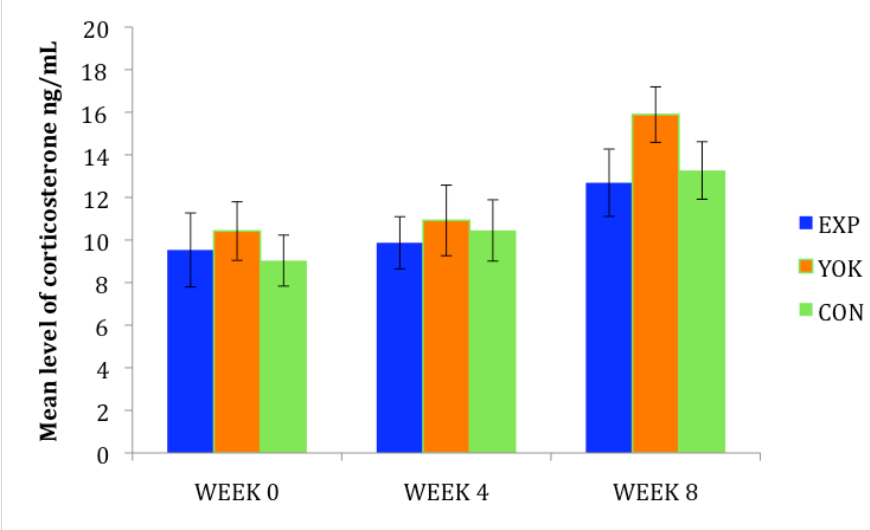

Figure 29 – Mean and ± 1 SEM levels of corticosterone in ng/mL for the three groups at three time points during the operant phase of the experiment with +/- 1 SEM.

Table 10 - Means and ± 1 SEM of corticosterone levels (ng/mL) for experimental yoked and control groups at three time points.

Group	Experimental		Yoked		Control	
Week	Mean	SEM	Mean	SEM	Mean	SEM
0	9.53	1.74	10.42	1.38	9.03	1.20
4	9.87	1.23	10.92	1.66	10.45	1.44
8	12.69	1.58	15.89	1.30	13.27	1.35

Correlations between tasks

In order to compare performances across tasks, animals were rank-ordered by their lever pressing activity in the operant cage (Table 11 & Figures 30, 31 & 32). Subsequently, experimental, yoked and control groups were divided into two subgroups each by selecting the five most active (high performance group, HPG) and the five least active animals (low performance group, LPG). Using Pearson's product-moment correlations, the performance of HPG and LPG animals was compared with their behavior on the object approach test, activity levels during the operant phase, performance on the discrimination task, and associated corticosterone levels (Table 12).

Table 11-Rank order for total number of bar presses for the light stimuli in the experimental, yoked and control groups.

Group	**Experimental**	**Yoke**		**Control**	
Animal ID	presses	Animal ID	presses	Animal ID	presses
4	193	42	129	8	104
11	193	13	240	33	109
10	270	38	276	26	128
39	379	43	283	18	133
28	478	9	327	7	147
35	483	32	332	23	156
2	564	37	430	30	160
25	617	34	512	1	181
44	646	22	516	17	185
40	732	20	740	14	185
16	1128	29	1030	27	196
12	1151	3	1114	41	210
36	1188	24	1155	6	339
31	1398	5	1584	45	405
19	2177	15	1661	21	428

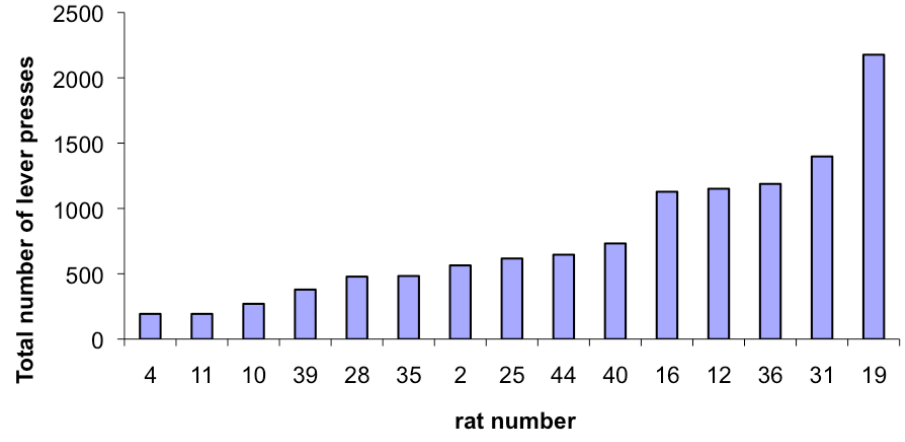

Figure 30 –Total number of lever presses in the experimental animals for light from 0 – 55 days

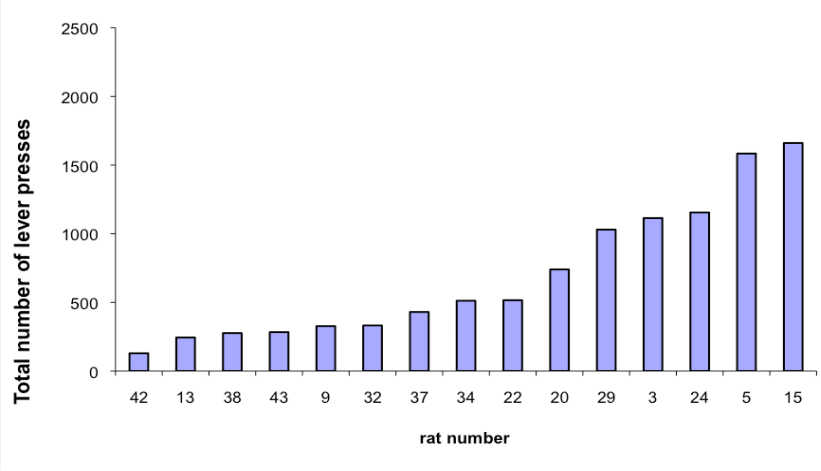

Figure 31 – Total number of lever presses in the yoked animals for light from 0 – 55 days

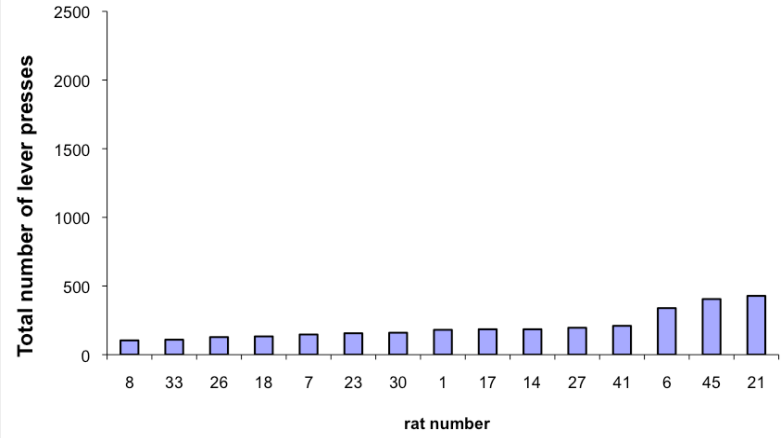

Figure 32 – Total number of lever presses in the control animals for light from 0 – 55 days

Table 12 – Pearson product-moment correlations between all experimental variables (* denotes significance between variables)

Tasks correlated	Experimental	Yoked	Control
Activity/Approach time			
Total group (n=15)	.10	.19	.03
HPG (n=5)	.92 *	.46	-.60
LPG (n=5)	-.98 *	.72	-.20
Activity/Discrimination (n=8)	-.21	.55	-.47
Approach time/ Discrimination (n=8)	.02	-.07	.33
Discrimination/Hormone (n=8)	-.28	.40	.09
Approach time/Hormone (n=15)	-.30	-.19	-.55 *
Activity/Hormone (n=15)	.30	.24	-.16

The following correlations were significant at alpha level p=.05: comparing the operant activity and object approach, there was a positive correlation in HPG animals and a negative correlation in LPG animals. Control animals that spent more time with the object showed lower corticosterone levels during the operant phase.

Discussion

The present study showed that experimental animals would control access to a light stimulus, but that the act of control over a variable stimulus in any direction appeared to be more important than its consequence, i.e., a particular level of illumination. Lever pressing with control of illumination levels did improve cognitive performance on the discrimination task. Further, corticosterone levels in experimental, yoke and control subjects were not affected by the animals' performance during the operant phase.

The first aim of this study was to test if experimental animals would press levers to control a light stimulus in an operant paradigm. Experimental subjects pressed the lever in the operant cage to change the illumination more often than the control animals, confirming the prediction that subjects would control their environment. Robust results have been reported in several studies where subjects controlled the ambient light level in an operant cage (McCall, 1965; Lockard, 1962; Kish, 1955; Kavanau, 1975).

Experiment 2 showed that control rather than preference was more important to experimental subjects. Rats needed only five lever presses to be exposed to every possible light level in the operant cage, but experimental subjects were found to press the lever more than five times (Figure 24), at least initially. The rate of lever pressing, however, did decrease to an average of five lever presses per day in the last few days of the operant phase, a phenomenon also described in other studies (Lockard, 1962; Lockard

1964; Kavanau & Havenhill, 1975). It is possible that the experimental subjects did acquire a preferred illumination level or that they habituated to the continuous changes in their environment.

Lockard (1966) found the opposite results to the ones in the current experiment that in one his experiments, subjects had control of light and from the outset and seemed to prefer a particular stimulus status. This might be due to the fact that in some cages animals had a choice between two extremely different (and possibly noxious) light conditions, whereas in the current experiment light levels differed only in small amounts and were not noxious. Illumination levels used in this study were similar in that levels did not exceed 140 lx so that animals were not motivated to adjust a preferred level but rather to control changes.

Yoked animals that only experienced ambient changes without actual control of the illumination also pressed the lever significantly more often than control subjects. This may have been due to superstitious behavior, i.e., yoked animals may have perceived that they were controlling the changing illumination levels even though their presses had no functional consequences. Davis and Hubbard (1972) observed that superstitious behavior is formed when an animal relates a particular action with a specific outcome in the absence of an actual reinforcer. Yoked animals may have pressed the levers at similar times as experimental subjects and associated changes in illumination with their action. Such 'superstitious behavior' decreased over sessions as the yoked group most likely learned the absence of any consequence of their activity in the operant cage (Figure 23).

The operant phase of the experiment was monitored during three different time periods (0-55, 0-27, and 28-55 days) to test for temporal differences. Although the findings did not differ from each other (experimental and yoked groups pressed the lever

significantly more often than control animals), there were some interesting trends. In the 0-55 day period, experimental animals appeared to press the lever controlling illumination less often than yoked animals, which changed half way through the operant phase with the experimental subjects pressing more than the yoked subjects (Figure 20). This gradual switch of activity between groups may have been due to animals learning the consequences of their lever pressing.

Though the experimental animals did not outperform yoked or control subjects in the total number of correct choices in the discrimination task, the experimental animals made more correct choices over time than both the yoked and control animals. Krech, Rosenzweig, and Bennett, (1962) found that rats raised with access to environmental enrichment were able to discriminate between doors that would lead to goal boxes with reward and goal boxes without reward. Also, Rothblat and Hayes (1987) were able to successfully train rats to discriminate between three-dimensional cues. The results in the present study might have been different and/or significant had subjects been presented with a spatial discrimination task as in the Krech et al. (1962) study, or with three-dimensional objects as in the Rothblat and Hayes (1987) study. The current research used a two-dimensional card with a graphic, which might not have been easily discernible. Using a three-dimensional object may have represented a naturalistic stimulus with ethological validity (Gerlai & Clayton, 1999). However, in support of the use of two-dimensional graphics, Bussey, Saksida, and Rothblat (2001) found that mice could successfully discriminate between graphic images on a computer screen. The difference between their study and the current one may be due to the type of images, but more importantly, the increased efficacy of the computer presentation of both the stimulus and reward given to the subjects as compared to the possible interference by the researcher.

Prior to dividing subjects into groups at the start of the operant phase, an object approach test was performed to examine whether this task could be used as a predictor of performance on other tasks. Two significant correlations were of interest: one was found in HPG and the other in LPG animals during the operant phase. Experimental subjects that approached the object more often also displayed more frequent lever presses. The opposite also held for animals that approached the object less often and subsequently displayed fewer lever presses. These findings support the concept of animal personality traits (behavioral syndromes) when animals sustain their characteristic behavior regardless of the environmental circumstances (Sih, Bell, & Johnson, 2004). An object approach test may thus be helpful in selecting experimental subjects that will in all likelihood perform at chosen levels of activity on operant and other locomotor tasks.

General Discussion

Experimental subjects that were allowed control of light made use of this opportunity as established in both experiments. Stimulus control (pressing a lever to affect any change) took precedence over adjusting a particular stimulus condition, i.e., lights on-off or illumination levels. Similar behavior was reported in earlier studies that tested laboratory rats that were in control of their environment and allowed to select various levels of light (Marx, Henderson, & Roberts, 1955; Lockard, 1963; McCall, 1965). One aim of the current research was to explore how the animals' performance in an operant conditioning task would affect their cognitive abilities. Experimental subjects did not show an increased performance on the Barnes maze in experiment 1, but did show improved discrimination performance over time in experiment 2. These results, where control was used as a tool of enrichment to improve laboratory subjects' performance on cognitive tasks, should encourage further research, as control of environmental features

has a significant influence on the behavior of animals (Kavanau, 1962). Also, studies of control allow insight into an animal's decision making as manifest by its overt preferences (Fraser, 1993).

Past research has established that light, sound, and access to a running wheel, and moreover control of these stimuli, are important to the animals' well-being (Kavanau, 1963). The current results on the Barnes maze may have been significant if all of the stimuli offered (light, sound, and use of the wheel) had resulted in reinforcing consequences. As there was no preference, it stands to reason that all light levels used in the operant phase of the present experiment served as positive reinforcers. It might therefore be of interest to perform the experiment using negative reinforcers. The 'Executive Monkey' experiment provides an excellent example (Brady, 1958). Within this experimental protocol, an experimental animal placed in a restraining chair could press a lever to prevent it from receiving an electric shock. A yoked monkey, equally restrained, had no control over the lever, but received the same shock. Following a 23-day regimen of 6 hours on, 6 hours off exposure to electric shocks, the executive monkey, psychologically stressed from having to push the lever in time, died of stomach ulcers, whereas the yoked animal remained relatively healthy and survived. Research has shown that during and immediately following such emotionally arousing learning tasks the adrenals release epinephrine (medulla) and corticosterone/cortisol (adrenal cortex) (de Kloet et al., 1999). Brady found that the executive's stomach acidity was greatest during the rest period. The severity and the type of stressor, i.e., the degree of emotional arousal, critically affect these hormonal systems (de Kloet et al., 1999; Korte, 2001; McIntyre & Roozendaal, 2007).

Although levels of corticosterone did not differ significantly among groups in the current study, they were lowest in experimental subjects and highest in the yoked animals while performing in the operant cage. This trend is noteworthy assuming that the animals with some aspect of control of their environment, and having learned the positive consequences of their actions (in contrast to the 'executive' monkey (Brady, 1958)), were less stressed than animals in a yoked situation, which experienced uncontrolled changes all the time. Thus allowing subjects to control aspects of their environment may result in decreased corticosterone production, therefore aiding in creating healthier subjects, as was found in a study performed by Davis and Levine (1982).

Animals kept for longer periods of time in the operant learning conditions will habituate to positive reinforcers associated with low arousal levels. (Data not reported here show that within a 60-min session individuals did in fact decrease their lever pressing activity). On the other hand, animals will not habituate to negative reinforcers and continue pressing the lever to avoid the aversive stimulus associated with increased arousal level as demonstrated by Brady's (1958) executive monkeys.

Memory consolidation is generally characterized by an inverted-U shaped stress hormone dose-response effect. This has been shown by administration of moderate doses of epinephrine or glucocorticoids leading to improved memory consolidation, whereas lower or higher doses were either less effective or impaired it (Gold & van Buskirk, 1975; Roozendaal, Williams, & McGaugh, 1999; McIntyre & Roozendaal, 2007). Experimentally 'optimizing' a subject's arousal level by identifying and applying the appropriate contingency of reinforcement schedule, should result in optimal corticosterone release and subsequent memory consolidation. This in turn could become

evident in the animals' improved performance on cognitive tasks as predicted in this study.

Several studies have addressed both positive and negative reinforcing qualities of light in rats; unfortunately, there is no congruence among these findings. One predictor of what a laboratory subject perceives as reinforcing is the intensity of a light stimulus, as extremely bright lights are turned off (by animals given control over this stimulus) more often than dimmer ones. The perception of how adverse a stimulus is may also rely on the individual animal. In order to obtain valid data for what laboratory subjects find reinforcing, further research on environmental control should be more sensitive to the choice of stimuli so as to promote natural, species-typical behaviors (Newberry, 1995) that focus on positive rather than negative reinforcers.

When using devices that animals can manipulate in an operant chamber, it is important to realize that the intended experimental reinforcers for an animal's lever pressing (e.g., changing light levels in the context of this research) might not the only reinforcing stimuli within the animal's environment. The sound or motion of pressing the lever might be sufficiently reinforcing even when control of the stimulus is not (Kish, 1955; Kish & Antonitis, 1956). The control animals in the current experiments provide clear evidence that animals pressed a lever even though there was no reinforcing consequence.

The results of the object approach task conducted prior to the second experiment indicated its usefulness as a predictor of 'animal personalities' (behavioral syndromes) (Sih, Bell, & Johnson, (2004). Such a tool would be helpful in selecting populations of animals with desired locomotor activity levels, which might result in time saving strategies and increased validity of experimental data. Rundquist & Hebon (1935) noted

that the more active an animal is the better it usually performs on cognitive tasks. Although the correlation between HPG animals and their performance on the discrimination task in the current study was not significant, nevertheless the predicted trend was present.

Renner & Rosenzweig (1987) noted that when behavioral changes such as higher-level cognitive abilities are induced by enrichment, they are likely to be found in higher level functioning areas of the brain such as the hippocampus and/or the cerebral cortex. A common method of trying to understand the effects of pharmaceuticals within clinical trials is testing animals in behavioral tasks, which often have little relevance to human conditions. It has been suggested that the tasks used in human cognitive research, should be adapted to animal subjects, which might result in higher test validity (Garner, Thogerson, Würbel, Murray, & Mench, 2006). It was hypothesized in the current study that the discrimination task would produce analogous results to those found in tasks performed by humans (Garner, Thogerson, Würbel, Murray, & Mench, 2006), and thus was more appropriate than the Barnes maze. Based on the findings in experiment 1, the Barnes maze was therefore replaced with a discrimination task in the second experiment.

When choosing research subjects for cognitive tasks, it is important to consider their developmental stage and sex as both affect physiology and behavior (Renner & Rosenzweig, 1987). Animals in this experiment were young 27-day old males. The predicted relationship between activity in the operant cage and cognitive performance may differ depending on the animals' sex and developmental age. Future studies should investigate applying intended treatments at different developmental stages of laboratory subjects, as differences in test results may be affected by the animals' age.

Further investigation along the lines of the current research should be pursued. Studies using control as a form of enrichment might include having the subjects control aspects of their environment over days or weeks at a time rather than running short sessions of a half an hour to an hour long. Having animals control a stimulus (such as light) for a longer time may lead to more significant physiological differences, which might result in clearer distinctions in the performance on a cognitive task between experimental and control animals than what was established in the current study.

The sound stimulus should still be considered as a possible stimulus suitable for studies focusing on control over the environment even though the lever pressing for the sound stimulus was not different between control and experimental groups. It was concluded that although the stimulus was well within the hearing range of the subjects, the intensity between the two sounds was not substantial enough to motivate the experimental animals to press the lever more than the control animals. A more effective design would have the sound stimulus mimic the regimen of the light stimulus, where the two possible settings for the subjects to control were on or off, rather than two different intensities. Control of multiple stimuli as investigated in experiment 1, should be further considered, starting with two stimuli and subsequently adding more stimuli over the course of a long-term study, which might aid in the understanding of the relative importance and interaction of preferred or controlled stimulus conditions on the animals' cognitive abilities. Although a significant difference was found in animals performing on the discrimination task, previous research suggests that the discrimination task, with the addition of an attention set shifting task should be included to aid in testing cognitive differences between experimental and control subjects. In order to complete the attention set shifting task the subject first learns the discrimination paradigm and then is asked to

remember the distinct feature from the discrimination task but with an added component. Previous research has found robust results when animals were subjected to this task than just using the discrimination task alone (Bussey et al., 2001; Garner, Thogerson, Würbel, Murray, & Mench, 2006).

The procedure for the discrimination task can be improved by taking human presence out of the procedure. Within the current research, the stimulus was manually manipulated, and the investigator reinforced the subjects. The apparatus of the task needs to be computer driven to eliminate any possible confounds due to human influence.

Though animals were subjected to the open field box a few times during the course of the experiment where the discrimination task was performed, more time to habituate to the apparatus may have decreased the animals' anxiety at the start of this phase, and results with increased validity may have been obtained. Also, animals were food deprived to 95% of their body weight, and although this is less than current standards used in research designs, it may once again benefit the results by finding another method in which to motivate the subjects to work. The actual stimulus used for the subjects to discriminate might be changed from a two dimensional stimulus to a three dimensional object. Rats may be more motivated to work using their natural tendency to be inquisitive about objects in their environment, where a two dimensional stimulus may not elicit as much of a response.

It may be beneficial to collect fecal samples from more time points during the course of the operant phase to more precisely follow the effects of the operant task in all groups. Also, since there was a trend in the experimental animals having lower levels of the hormone than the yoked and control groups, the collection of samples for a longer

time period should be considered, which might strengthen the differences between the three groups.

Guided by this plan, results of future experiments will not only be important for the welfare of animals in captive environments, but will also aid in designing better scientific methods, yielding animal models with increased research validity and reliability. Animals raised and maintained in improved conditions will provide better substitutes for human conditions.

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