THE EFFECTS OF AN ACUTE NOISE EXPOSURE ON RODENT SPATIAL WORKING MEMORY AND THE RODENT STRESS RESPONSE

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Abstract

Acute stress can sensitize or desensitize responses to later stress, but it is not known if this has any lasting effect on spatial working memory. In this study, male Long-Evans rats were stressed with exposure to 60 or 90 dBA of white noise. Spatial working memory was assessed by monitoring rats in a cross-maze during an exposure or 1, 4, or 24 hr after an exposure. When rats were exposed and assessed simultaneously, noise reduced spontaneous alternation without increasing corticosterone levels or extracellular signal-regulated kinase 1 and 2 (ERK1/2) phosphorylation. However, transportation and exposure to the testing area introduced confounding stress that may have masked significant changes in physiology. Rats also displayed enhanced corticosterone and amygdalar ERK1/2 responses to the testing area 24 hr after noise exposure, which suggests that ERK1/2 activity in the basolateral amygdala may play a role in neuroendocrine sensitization.

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List of Abbreviations

ACTH ..... Adrenocorticotropic hormone

ANOVA ..... Analysis of variance

AVP ..... Arginine vasopressin

CA ..... *Cornu ammonis*

CRH ..... Corticotrophin-releasing hormone

ERK ..... Extracellular signal-regulated kinase

HPA ..... Hypothalamic-pituitary-adrenal

HSD ..... Honestly Significant Difference test

PBS ..... Phosphate-buffered saline

pERK ..... Phosphorylated extracellular signal-regulated kinase

TX ..... Triton X-100

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The Effects of an Acute Noise Exposure on Rodent Spatial Working Memory and the Rodent Stress Response

Stress is a ubiquitous fact of life, and many occupations demand performance and efficiency under varying forms and levels of stress. This is especially true for military and emergency service personnel (Driskell & Johnston, 1998; Driskell, Salas, & Johnston, 2006; Driskell, Salas, Johnston, & Wollert, 2008; Keinan & Friedland, 1996). Emergency situations can expose response teams to physical stressors, including extreme environments, heavy workloads, poor sanitation, inadequate sleep, dehydration, and malnutrition. Psychological stressors might include fear, anxiety, uncertainty, isolation, loud noises, and information overload. Responders may also witness death or gruesome injuries and may risk being killed or injured themselves (Beaton, Murphy, Johnson, Pike, & Corneil, 1998; Driskell et al., 2006; Harris, Hancock, & Harris, 2005; Helmus & Glenn, 2005; Krueger, 2008; Lieberman et al., 2005; Kavanagh, 2008).

Stress is a potent modulator of learning and memory processes, and some researchers have demonstrated that stress can detrimentally affect the cognitive performance of emergency responders. For example, LeBlanc, MacDonald, McArthur, King, and Lepine (2005) found that paramedics calculated drug dosages with significantly reduced accuracy after exposure to a stressful clinical simulation. Oudejans (2008) noted that while performance on standard shooting tests is generally good, the accuracy of a police officer firing a handgun in a stressful, real-life incident varies between about 15 and 60%, with 60% being the exception rather than the rule. The effects of stress on cognition in military personnel have also been studied by a number of researchers (e.g. Driskell et al., 2008; Harris, Hancock, & Harris, 2005; Harris, Hancock,

& Morgan, 2005; Harris, Ross, & Hancock, 2008; Helmus & Glenn, 2005; Lieberman et al., 2005; Vaisman-Tzachor, 1997). In one example, Lieberman and his colleagues studied the cognitive performance of US Army officers who were exposed to realistic combat stressors, including sleep loss, dehydration, malnutrition, and simulated explosions and gunfire. They observed severe decrements on standard psychological tests of attention, pattern recognition, reasoning, and memory.

The Mammalian Stress Response

Neurological research has attempted to describe and explain the effects of stress on cognition. A stressor is usually defined as a situation or event that an organism recognizes as threatening or challenging. It elicits a series of physiological and behavioural reactions that mobilize stored energy and facilitate an organism's ability to cope (Anisman & Merali, 1999). The two most commonly studied physiological systems that respond to stress are the sympathetic nervous system and the neuroendocrine system (McEwen, 2000; McEwen & Sapolsky, 1995). The sympathetic nervous system is a series of interconnected neurons that connects the brain and spinal cord to almost every peripheral organ system in the body. Activation of these organ systems primes the body for physical activity. For example, sympathetic innervations that reach the respiratory system can dilate air passageways, increasing the oxygen that is available every time an animal takes a breath. Innervations in the cardiovascular system can accelerate an animal's heart rate, increasing the circulation of energy and nutrients to vital systems. Some innervations also reach the adrenal glands, located on top of the kidneys. The adrenal glands respond to sympathetic activation by secreting catecholamine hormones into the bloodstream. These hormones, called epinephrine and norepinephrine, are

associated with the rapid increases in energy, alertness, and arousal that characterize the fight-or-flight response (Cannon, 1953, 1963; Meany, 2000).

The sympathetic nervous system acts very quickly. Innervations from the central nervous system activate peripheral organs almost instantaneously (Cannon, 1953, 1963). Catecholamines are released rapidly and typically return to resting levels about 10 min after the onset of stress (de Boer, Koopmans, Slangen, & van der Gugten, 1990; de Quervain, Roozendaal, & McGaugh, 1998). The neuroendocrine system, by contrast, is significantly slower. It exerts its effects through a cascade of hormone secretions that activate the brain structures in the hypothalamic-pituitary-adrenal (HPA) axis. In this system, stress first activates the hypothalamus, which is a structure near the base of the brain that controls basic metabolic processes, such as hunger, thirst, or body temperature. The hypothalamus secretes corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into a small system of blood vessels that connect the hypothalamus to the anterior lobe of the pituitary gland. The pituitary gland is a pea-sized structure that protrudes off the bottom of the hypothalamus. It is sometimes called the master gland because it secretes hormones that can activate or deactivate all other endocrine glands in the body. When CRH and AVP bind to the anterior pituitary, they promote its secretion of adrenocorticotropic hormone (ACTH). This hormone travels through the bloodstream to the adrenal glands, which respond by releasing glucocorticoid hormones into general circulation. Glucocorticoids bind to receptors on cells throughout the body, triggering a number of metabolic reactions that help direct oxygen and nutrients to the stressed body site (Anisman & Merali, 1999; Harbuz & Lightman, 1992; Matteri, Carroll, & Dyer, 2000; Selye, 1976). Glucocorticoid binding in the brain modulates a variety of processes,

including arousal, attention, mood, and memory (Anisman & Merali, 1999; McEwen & Sapolsky, 1995).

Because a stressful event usually elicits a glucocorticoid secretion, researchers often use glucocorticoid levels as a relative index of HPA axis activity (Anisman & Merali, 1999; Harbuz & Lightman, 1992). In rodents, the primary glucocorticoid secretion is corticosterone (Matteri et al., 2000). To characterize the stressful effects of loud noise, Burow, Day, and Campeau (2005) exposed groups of rats to different levels of white noise and measured blood concentrations of corticosterone. They observed a gradual increase in its blood concentration that occurred as the noise intensity increased. Corticosterone elevations began at 85 dBA, and a peak was reached at 110 dBA, the maximum noise intensity used in their study.

The implication in Burow et al.'s (2005) study and in much of the stress research is that stressor intensity and glucocorticoid levels maintain a strict dose-response relationship where increasing the stressor intensity increases the glucocorticoid release. However, it should be noted that a sudden glucocorticoid release does not necessarily indicate a stress response. For example, Merali, McIntosh, Kent, Michaud, and Anisman (1998) observed that rats offered a familiar but especially palatable food demonstrated a corticosterone release that was approximately comparable to being grasped and manually restrained by an experimenter for 20 min. Woodson, Macintosh, Fleshner, and Diamond (2003) also observed corticosterone elevations in male rats exposed to a sexually-receptive female rat, and those elevations were equivalent to those seen in males exposed to a predatory cat. Thus, it could be argued that glucocorticoid activity simply represents a response to any strong stimulus (Anisman & Merali, 1999; Merali et al., 1998). Also,

like those of many other mammalian hormones, glucocorticoid levels follow a normal cyclic variation, reaching their highest point shortly after awakening. Blood concentrations then decline gradually over the course of a day, showing transient increases throughout the waking hours. The peaks occur as part a normal circadian variation and do not necessarily indicate a stress response (Kräuchi, Wirz-Justice, Willener, Campbell, & Feer, 1983; Seale et al, 2004; Windle et al., 2001).

Stress and Memory Processes

The relationship between stressor intensity and cognitive performance is generally thought to follow an inverted U-shaped curve (Hancock & Szalma, 2008; Mendl, 1999; Sandi & Pinelo-Nava, 2007). That is, a moderate level of stress improves cognitive performance while very high or very low levels of stress are detrimental. This generalization is rooted in the seminal work of Yerkes and Dodson (1908). In their original paper, Yerkes and Dodson reported on a set of experiments using mice and a visual discrimination task. Each mouse was placed in the start-box of a maze and had to proceed through a white or black passageway to reach the end-box. If a mouse proceeded through the white passageway, it completed the trial, but if it proceeded through the black passageway, it received a non-injurious electric shock. Yerkes and Dodson started by giving very weak shocks, and they found that it took the mice a relatively long time to learn to complete the task. When a moderate-intensity shock was used, the mice learned much more quickly. However, their learning slowed again when the strength of the shock was increased to a very high level. After a series of follow-up experiments, Yerkes and Dodson concluded that stimulation can facilitate learning, provided that it is not too weak or too strong. Hebb (1955) argued that stimulation gives arousal and with his writing,

pushed Yerkes and Dodson's concepts into the mainstream. Behavioural science now recognizes a general relationship between arousal and performance, which is commonly referred to as the Yerkes-Dodson Law (Teigen, 1994).

Contemporary research typically uses a stressor to induce arousal, and the Yerkes-Dodson Law persists in most studies of cognition (Mendl, 1999). However, research has demonstrated that the pattern does not necessarily hold for all cognitive processes, and experimental results are especially heterogeneous when specific memory operations are considered. For example, moderate levels of stress facilitate the retrieval of stored memories, whereas very high levels of stress are usually detrimental to the process (de Quervain et al., 1998; Diamond et al., 2006; Diamond, Ingersoll, Fleshner, & Rose, 1996; Manikandan, Padma, Srikumar, Jeya Parthasarathy, Muthuvel, & Sheela Devi, 2006; Pakdel & Rashidy-Pour, 2006; Roozendaal, 2002; Sajadi, Samaei, & Rashidy-Pour, 2007; Sandi & Pinelo-Nava, 2007). This is a biphasic effect that the Law predicts. In contrast, high stress usually enhances memory consolidation, the process by which information is transferred from active consciousness to long-term memory for storage (de Quervain et al., 1998; Diamond et al., 2006; McGaugh, 2000; Pakdel & Rashidy-Pour, 2006; Sajadi et al., 2007; Sandi & Pinelo-Nava, 2007). The effect does not follow the Law's inverted U-shaped curve. In fact, stress appears to enhance consolidation according to a linear-asymptotic function, where increasing a stressor's intensity proportionately increases the effectiveness of consolidation until effectiveness approaches a limiting maximum value (Sandi & Pinelo-Nava, 2007). Clearly, it is essential that memory researchers explicitly define the specific processes that they intend to study.

Some studies have observed impaired memory consolidation when the intensity of a stressor is enhanced with post-training glucocorticoid injections (e.g. Pugh, Tremblay, Fleshner & Rudy, 1997; Sandi, Loscertales, & Guaza, 1997; Sandi & Rose, 1997). For example, Sandi and Rose (1997) injected low or high doses of corticosterone into the brains of day-old chicks immediately after the chicks tasted low or high doses of a disgusting and stress-inducing bird repellent. A low dose of the bird repellent failed to produce avoidant behaviour, but avoidance was observed when the stressor's intensity was augmented with a low dose of corticosterone. A high dose of the repellent by itself produced avoidance, but this effect disappeared when the exposure was followed by a high dose of corticosterone. It is possible that biphasic effects on memory consolidation are only observable when high stress is induced shortly after performance on a cognitive task. Few (if any) studies have observed impaired consolidation when stress occurs simultaneously with the task. Thus, the distinction between cognitive performance *after* stress and cognitive performance *under* stress is important (Sandi & Pinelo-Nava, 2007). Such a distinction may help to clarify the differential effects that stress and glucocorticoids can have on various memory processes.

In rodents, high stress and elevated glucocorticoids levels consistently disrupt spatial working memory, the ability to hold visual information about an environment in short-term storage or active consciousness (Bats et al., 2001; Birnbaum et al., 2004; Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007; Diamond et al., 1996; Diamond, Park, Heman, & Rose, 1999; Lieberman et al., 2005; Manikandan et al., 2006; Roozendaal, 2002; Stillman, Shukitt-Hale, Levy, & Lieberman, 1998; Trofimiuk & Braszko, 2008; Woodson et al., 2003). Some researchers have demonstrated the effects of stress on

spatial working memory by using a spontaneous alternation task. In this paradigm, a rodent is usually placed in the stem of a T- or Y-maze and allowed to enter one of the two arms. The animal is then returned to its starting location for a second trial, where it has the choice of either repeating the same response or alternating (Lalonde, 2002). Rats and mice normally alternate at levels significantly greater than chance, demonstrating a natural tendency to explore a novel environment (Dember & Fowler, 1958, 1959). A high rate of alternation indicates spatial working memory because it demonstrates that the rodent can remember the arm that was entered last and can avoid re-entering it.

Bats et al. (2001) used a spontaneous alternation task to demonstrate the negative effects of stress on spatial working memory. They forced mice to explore a brightly lit open field shortly before their second trial in a T-maze. The bright light stressor reduced alternation to chance levels and induced concordant increases in blood corticosterone. A similar spatial memory impairment was observed when Mitchell, Koleszar, and Scopatz (1984) tested mice in a T-maze after exposure to an electric shock stressor.

A cross-maze (with four arms intersecting at a central platform) or a radial arm maze (with greater than four arms arranged in the same design) can also elicit spontaneous alternation (Isaacson, Karoly, & Caldwell, 1957; Olton & Samuelson, 1976). In these paradigms, the increased number of arms increases the probability of alternation (Lalonde, 2002). Rodents can also be trained in baited mazes (with a reward at the end of some arms) to compare working memory with other spatial memory processes. For example, Diamond et al. (1996) trained rats to locate food pellets in a 14-arm radial arm maze. Throughout the study, the same seven arms were always baited, and with training, the rats learned to ignore the arms that never had food. This avoidance indicated good

reference memory, a type of long-term spatial memory that involves reference to external cues (Neves et al., 2008). To compare this process with working memory, Diamond and his colleagues removed each rat from the maze after it had eaten food in only four of the seven arms. After a short delay period, each rat was returned to the maze and challenged to locate the three remaining food pellets. A rat demonstrated good spatial working memory if it could remember and avoid the areas that it had already searched before the delay.

To test the effects of stress on working and reference memory, Diamond et al. (1996) transferred the rats to an unfamiliar and stress-provoking environment for the duration of the delay period. When the stressed rats were returned to the maze, they frequently revisited arms, apparently forgetting where they had searched just a few hours ago. However, the stressed rats still tended to avoid the arms that had never been baited. Woodson et al. (2003) and Diamond et al. (1999) replicated these findings using another version of the radial arm maze. Together, Diamond and his teams concluded that stress selectively inhibits hippocampus-dependent memory processes—including spatial working memory—but has comparatively less impact on hippocampus-independent processes—including reference memory.

The Hippocampus, the Amygdala, and Spatial Memory Impairment

The hippocampus is a brain structure that is heavily involved in most learning and memory processes. It is located inside the medial temporal lobe of the cerebral cortex, and its distinctive curved and convoluted figure is given by two interlocking U-shaped substructures called the dentate gyrus and the hippocampus proper. The dentate gyrus is composed of densely packed granule cells that encircle the inferior end of the

hippocampus proper. This marks the beginning of the hippocampal circuit. The hippocampus proper can be further divided into four *cornu ammonis* (CA) regions based on differences in cell morphology and fibre projections. The first region, CA4, designates a group of scattered mossy cells that receive inputs from nearby granule cells in the dentate gyrus. The circuit continues with CA3 and CA2, respectively. Both regions are composed of distinctly large pyramidal neurons, but only the CA3 region receives mossy fibre projections from the dentate gyrus. The circuit ends with CA1, the largest and most superior region (O'Keefe & Nadel, 1978). The smaller pyramidal cells in CA1 cast efferent projections into neighbouring temporal lobe cortices and the amygdala (Ishikawa & Nakamura, 2006; O'Keefe & Nadel, 1978).

The amygdala is a small, almond-shaped group of cell clusters typically associated with emotion and memory. It can be divided structurally into three major regions, including the cortical region, the centromedial region, and the basolateral region. The cortical region of the amygdala is a section of the olfactory cortex. It receives inputs from the main and accessory olfactory bulbs and may play a role in processing pheromonal information. The centromedial region is a specialized expanse of the striatum that projects to autonomic centres in the brainstem (Swanson & Petrovich, 1998). This region is associated with some of the passive motor behaviours (e.g. freezing in rats) that occur in response to stress (Roozendaal, Koolhaas, & Bohus, 1991; Swanson & Petrovich, 1998). The basolateral amygdala is an extension of the claustrum that has bidirectional projections connecting it to the frontal and temporal lobes of the cerebral cortex (Swanson & Petrovich, 1998). This region is thought to play a significant role in conditioned fear responses and memory for emotionally arousing events (McGaugh &

Roozendaal, 2002; McIntyre, Power, Roozendaal, & McGaugh, 2003; Paré, 2003; Phelps, 2004). The basolateral region is particularly notable for at least two reasons. First, receptors in this area are thought to modulate the effects of glucocorticoids and catecholamines on some memory processes (McIntyre et al., 2003; McGaugh & Roozendaal, 2002; Roozendaal, 2000; Roozendaal, 2002). Second, the basolateral amygdala projects heavily back to the hippocampus and the associated temporal lobe cortices. In fact, the most widespread projections from the amygdala to the hippocampus originate in the basal nucleus, which projects substantially to the CA3 and CA1 fields of the hippocampus proper (Pikkarainen, Rönkkö, Savander, Insausti, & Pitkänen, 1999).

Major insights into the function of the hippocampus have come from lesion studies and observations of amnesia in human patients with brain damage (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999). Early studies demonstrated that removing medial temporal lobe structures significantly impaired long-term retention for new information while leaving preoperative memories intact (Scoville & Milner, 1957). Later research observed a similar condition in patients where the brain damage was strictly limited to the hippocampus or to particular hippocampal CA regions (Varghan-Khadam et al, 1997; Zola-Morgan et al., 1986). For example, one patient exhibited marked anterograde amnesia after bilateral lesions to the CA1 field (Zola-Morgan et al., 1986). In animals, researchers can lesion specific CA fields to demonstrate their importance in particular memory processes (Martin & Clark, 2007). In one study, Gilbert and Kesner (2006) injected an acid into the brains of anesthetised rats to destroy the CA3 region of the dorsal hippocampus. When the rats recovered, they were unable to complete a spatial working memory task. Selective lesioning has also demonstrated the importance of CA1

in spatial working memory (Dillon, Qu, Marcus, & Dodart, 2008; Lee & Kesner, 2003), but other research has shown that CA3 and CA1 contribute differentially to other short-term spatial memory processes, such as pattern separation and sequence learning (Glibert & Kesner, 2006; Gilbert, Kesner, & Lee, 2001; Lee, Jerman, & Kesner, 2005).

It has been consistently demonstrated that performance on a vast number of spatial memory tasks is dependent on the integrity of the hippocampus (Martin & Clark, 2007; Liu & Bilkey, 2001; for a seminal review, see O'Keefe & Nadel, 1978). The hippocampus codes spatial information using specialized neurons that dramatically increase their firing rate when an animal explores a familiar environment. Individual clusters of cells in CA1 and CA3 only fire when the animal faces a particular direction or moves through a particular location (Eichenbaum et al., 1999; Kim et al., 2007; Nakazawa, McHugh, Wilson, & Tonegawa, 2004; Neves, Cooke, & Bliss, 2008; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978; Quirk, Muller, Kubie, & Ranck, 1992). This pattern of activity is established within a few minutes of entering a novel environment (Kim et al., 2007; Nakazawa et al., 2004; Wilson & McNaughton, 1993), and it remains stable across repeated exposures to the same area, which allows the animal to determine its relative position (Knierim, Kudrimoti, & McNaughton, 1995; Nakazawa et al., 2004; Neves et al., 2008; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978; Wilson & McNaughton, 1993).

The molecular mechanism underlying this activity is still largely undetermined, but some results suggest that extracellular signal-regulated kinases (ERKs) may play a crucial role (Blum, Moore, Adams, & Dash, 1999; McGauran et al., 2008; Nagai et al., 2006; Thiels & Klann, 2001). A kinase is an enzyme that transfers phosphate groups from

donor molecules to specific target proteins. The addition of a phosphate group activates or deactivates the target by changing its conformation. In neurons, ERK1 and ERK2 activate transcription factors, initiating protein synthesis. Some memory processes are thought to require the production of new proteins (Blum et al., 1999; McGauran et al., 2008; Nagai et al., 2006), and a number of findings have suggested that the ERK1/2 system regulates memory-induced protein synthesis in the hippocampus. For example, it has been demonstrated that ERKs are part of a complex multistage signalling pathway that is regulated by certain memory-associated kinases, including protein kinase A and protein kinase C. ERKs may also contribute to the regulation of cAMP response-element binding protein, a particular transcription factor known to be involved in long-term memory (Impey et al., 1998; Thiels & Klann, 2001; Roberson et al., 1999).

Recently, Nagai et al (2006) reported that levels of hippocampal ERK1/2 were significantly increased in rats immediately after they completed a spatial working memory task in a radial arm maze. This increase persisted for at least 60 min but faded naturally within 2 hr. Nagai and his colleagues also observed a decrement in performance with bilateral microinjections of an ERK1/2 inhibitor into the hippocampus. The inhibitor significantly increased the number of arms that rats erroneously revisited, implying memory impairment. Taken together, these results suggest that neurons in the rat hippocampus require normal ERK1/2 activation to completely process spatial information in the short term.

ERK1/2 activation also seems to be involved in physiological responses to stress. For example, Yang, Huang, and Hsu (2004, 2008) reported that rats restrained and subjected to unpredictable and inescapable tailshocks expressed significantly higher

ERK1/2 levels in the basolateral amygdala and the CA1 region of the hippocampus when compared to a control group. Pardon et al. (2005) detected a similar hippocampal ERK1/2 increase in mice that were exposed to a novel environment or placed near a threatening and aggressive rival mouse. These results are interesting because stress disrupts rodent spatial working memory, as discussed previously. Yang et al. (2004) hypothesize that the stress response and certain cognitive tasks require use of the same molecular machinery. They suggest that the ERK1/2 pathway has a maximum operating capacity and that cognitive performance is impaired by stress because multiple processes are demanding significant or sustained activation from the same limited resources.

Research has suggested that stress-induced cognitive impairments result from activity in several brain structures, including the hippocampus and the basolateral amygdala. For example, Roozendaal, Griffith, Buranday, de Quervain, and McGaugh (2003) showed that rats failed to retrieve spatial memories when a glucocorticoid receptor agonist was injected directly into the dorsal hippocampus shortly before retention testing. This effect was dose-dependent: Higher doses of the agonist gave proportionately greater impairment. Injections of the same glucocorticoid agonist into the basolateral amygdala had no effect on rodent memory, but the retrieval impairment was blocked when the basolateral region was chemically lesioned. Woodson et al. (2003) also demonstrated that corticosterone elevations could only impair spatial working memory when rats were stressed with exposure to a frightening stimulus. This is interesting because fear and emotional arousal strongly modulate activity in the basolateral amygdala (Paré, 2003; Pelletier, Likhtik, Filail, & Paré, 2005).

Preemptive Exposure to Stress

Military and emergency service personnel are frequently exposed to stressful and arousing stimuli that detrimentally affect their cognitive performance (Driskell et al., 2008; Harris et al., 2005; Harris et al., 2005; Harris et al., 2008; Helmus & Glenn, 2005; LeBlanc et al., 2005; Lieberman et al., 2005; Oudejans, 2008; Vaisman-Tzachor, 1997). Anecdotal evidence suggests that their performance under stress improves with field experience, and some studies suggest that emergency responders who have become accustomed to stressful work may be better equipped to deal with stress in the future (Helmus & Glenn, 2005; Meichenbaum & Novaco, 1985; Solomon, Mikulincer, & Jakob, 1987; Vaisman-Tzachor, 1997). A number of researchers have described stress training programs that protect task performance by preemptively exposing recruits to stressors (e.g. Driskell & Johnston, 1998; Driskell, Johnston, & Salas, 2001; Driskell et al., 2008; Friedland & Keinan, 1986; Friedland & Keinan, 1992; Inzana, Driskell, Salas, & Johnston, 1996; Johnston & Cannon-Bowers, 1996; Keinan & Friedland, 1996; Keinan, Friedland, & Sarig-Naor, 1990; Meichenbaum & Novaco, 1985; Oudejans, 2008).

In a simple laboratory demonstration such a program, Keinan et al. (1990) trained students to complete a visual search task with one hand submerged in ice-water. Students who experienced the cold water stress as part of their training outperformed others who simply practiced the task. Oudejans (2008) described a very realistic training program that was intended to prevent stress-induced degradation of handgun-shooting performance by exposing police officers to the pressure of a threatening gunfight situation. Firearms training for ordinary police officers usually takes place in a range where an instructor teaches participants to shoot at stationary cardboard targets. In

Oudejans's study, participants in the experimental group practiced shooting at an opponent (an instructor) who could fire back with coloured soap cartridges. Being hit with one of these cartridges caused intense pain, and the threat of being hit caused elevations in heart rate and self-reported anxiety. When compared to a control group, officers who had completed this high-pressure training program demonstrated significantly better shooting accuracy on a test that featured an armed and threatening opponent.

The mechanism underlying the effectiveness of these training programs remains largely undetermined. Most proponents hypothesize that a preemptive exposure to stress improves performance because it helps build familiarity and predictability (e.g. Driskell & Johnston, 1998; Driskell et al., 2001; Driskell et al., 2008; Friedland & Keinan, 1986; Friedland & Keinan, 1992; Inzana et al., 1996; Johnston & Cannon-Bowers, 1996; Keinan et al., 1990; Keinan & Friedland, 1996). A stressor elicits a variety of physiological, emotional, and cognitive reactions, and some researchers believe that these reactions can impair performance by distracting an individual from the task. Familiarity and predictability might decrease distractions and reduce the attentional demands of having to monitor and interpret novel events (Cohen, 1978; Driskell et al., 2008). Stress can also detract from performance by directly affecting the activation of relevant brain structures, such as the hippocampus. Familiarity might help to counter this effect by reducing the extent to which a stressor can elicit a physiological response. Animal research demonstrates the biological plausibility of this effect: In some studies, it has been shown that repeatedly exposing a rodent to an expected and predictable stressor can

attenuate normal sympathetic and neuroendocrine responses (de Boer, van der Gugten, & Slangen, 1989; Weiss, 1970; Bassett, Cairncross, & King, 1973).

Animal research has also demonstrated that a single preemptive stressor exposure can desensitize neuroendocrine responding to later stress on a long-term basis. For example, Martí, García, Vellès, Harbuz, & Armario (2001) restrained rats for 60 min by taping their limbs to metal mounts on a wooden board. The researchers measured the concentration of hormones released from the HPA axis throughout this first immobilization to observe baseline responding to stress. Nine days later, the rats were subjected to the same procedure a second time. Compared to the first exposure, rats expressed significantly lower levels of corticosterone and ACTH shortly after the stressor had been withdrawn, suggesting a more rapid recovery after their second experience. This effect became progressively accentuated with time and persisted for at least four weeks. Single-trial desensitization has also been observed when rats are stressed with electric footshocks (Belda, Márquez, & Armario, 2004) or different restraint procedures (Dal-Zotto, Martí, Delgado, & Armario, 2004; Dal-Zotto, Martí, & Armario, 2003; Martí et al., 2001).

However, some animal research indicates that a preemptive stressor exposure can also sensitize an organism's normal stress response, inducing long-lasting physiological and behavioural changes. For example, van Dijken et al. (1993) reported that subjecting rats to a single 15-min session of inescapable footshocks resulted in persistent and exaggerated HPA responses when rats were later exposed to novelty. The responses included significant elevations in AVP and ACTH, which occurred with anxiety-like behaviours that intensified progressively over a week and remained present for at least a

month (van Dijken, van der Heyden, Mos, & Tilders, 1992; van Dijken et al., 1993). Shocked rats displayed less activity and more defecation in an open field when compared to control rats, and they reacted with more immobility and freezing in response to a sudden drop in background noise. In rodents, freezing is a species-specific defensive response to threatening stimuli (Blanchard & Blanchard, 1988; Bolles, 1970), and defecation is thought to indicate anxiety and stress (File & Vellucci, 1979; Gentsch, Lichtsteiner, & Feer, 1981; Levine, Madden, Conner, Moskal & Anderson, 1972).

Rodents have exhibited similar progressive and/or lasting sensitization after only one trial of physical restraint (Belda, Fuentes, Nadal, & Armario, 2008; Gagliano, Fuentes, Nadal, Armario, 2008; Klenerová, Krejčí, Šída, Hliňák, & Hynie, 2006; Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005), forced underwater submersion (Richter-Levin, 1998), defeat by a more aggressive rodent (Koolhaas et al., 1990), exposure to a predator (Adamec, Kent, Anisman, Shallow, & Merali, 1998; Adamec & Shallow, 1993), or exposure to a predator's odour (Cohen, Benjamin, Kaplan, & Kotler, 2000; Cohen, Kaplan, & Kotler, 1999). Because these behaviours can be relieved with anxiolytic (van Dijken, Tilders, Oliver, & Mos, 1992) or antidepressant medications (Koolhaas et al., 1990), some authors have suggested that sensitized rodents may be displaying a persistent state of stress sensitivity or vulnerability that is thought to occur in a number of psychiatric disorders (Tilders & Smchidt, 1999; Tilders, Schmidt, Hoogendijk, & Swaab, 1999). Note that posttraumatic stress disorder often occurs with sensitized HPA responses (Yehuda, 1997).

Some authors have hypothesized that a stressor exposure will desensitize the neuroendocrine response only to a later encounter with the same stressor. The response to

a novel stressor, they predict, will be sensitized and enhanced for days (Armario, Escorihuela, Nadel, 2008; Armario, Vallès, Dal-Zotta, Márquez, & Belda, 2004; Martí & Armario, 1998; Martí et al., 2001). This follows Groves and Thompson's (1970) dual-process theory of habituation, which generally holds in chronic stress paradigms when animals are exposed to the same stressors repeatedly or daily (de Boer, Slangen, & van der Gugten, 1988; de Boer et al., 1989; Konarska, Stewart, & McCarty, 1990; Natelson et al., 1988; Pitman, Ottenweller, & Natelson, 1990). Findings from Martí et al. (2001) support this conjecture. Martí and his colleagues stressed rats using immobilization and observed neuroendocrine desensitization to a second experience with the same stressor. In a later experiment, the researchers stressed rats using immobilization and observed sensitized responding to a novel experience with a forced swim stressor. However, it should be noted that sensitization has been observed with repeated exposures to the same stressor (Konarska et al., 1990; Pitman et al., 1990) or when the stressor is a direct physiological insult, such as a drug (Vanderschuren et al., 1999) or an immune system challenge (Anisman, Merali, & Hayley, 2003; Haley, Brebner, Lacosta, Merali, & Anisman, 1999). As well, under certain conditions, some forms of desensitization or tolerance can apparently generalize across different stressors in humans (Driskell et al., 2001) and fish (Todgham, Schulte, & Iwama, 2005).

The extent to which previous experience with a stressor results in sensitization or desensitization probably depends on a number of factors, including the intensity of the stressor (Armario et al., 2008; Armario et al., 2004; Adamec et al., 1998). Most researchers consider the stress response to be an adaptive process that mobilizes physiological resources and helps to confront a threat or challenge. Negative effects are

expected only when the process is overly taxed (Anisman & Merali, 1999). For example, a diagnosis of posttraumatic stress disorder requires that a patient has been exposed to an extremely intense stressor that "threatened death or serious injury" and fostered feelings of "intense fear, helplessness, or horror" (American Psychological Association, DSM-IV-TR, pp. 467-468). To test the effects of stressor intensity, Martí et al. (2001) induced stressor desensitization in separate groups of rats using two restraint paradigms of significantly different intensity. Their results suggest that neuroendocrine desensitization is *enhanced* with increasing stressor intensity.

This result is surprising because it is at odds with an established rule of habituation. Groves and Thompson (1970) predicted that decrements in responding would occur with *decreasing* stimulus intensity. This rule has been consistently demonstrated in chronic stress paradigms, where low-intensity stressors are more likely to produce habituation and high-intensity stressors are more likely to produce sensitization (Konarska et al., 1990; McCarty et al. 1994; Natelson et al., 1987; Pitman et al., 1990). Groves and Thompson also wrote that adaptation to a stimulus is negatively related to the time lapse between repeated exposures. However, the sensitization and desensitization that occur with a single exposure to stress progressively *intensify* over time. These apparent contradictions have led some authors to conclude that the long-term desensitization induced with a single stressor exposure is distinctly different from the habituation process described by Groves and Thompson (Armario et al., 2004; Armario et al., 2008).

The Present Study

Most studies that have investigated the long-term effects of a single stressor exposure have examined spontaneous behaviour in rodents. Stress is a potent modulator of learning and memory processes, and very few studies have investigated the effects of stress-induced sensitization or desensitization on cognitive performance. Some human research has demonstrated that a preemptive stressor exposure can protect performance under stress, (e.g. Keinan et al., 1990; Driskell et al., 2001; Oudejans, 2008) but the conclusions drawn from these results are limited for several reasons. First, many of these studies involve tasks with a complex or poorly-defined cognitive component. In cognitive studies, definition is critically important because stress affects many learning and memory processes differentially. Second, very few of these studies have gathered useful physiological data, and for this reason, they cannot speak to the neurological processes that underlie their results. Finally, these studies have not investigated long-term sensitization or the effects of high-intensity stress. This area of human research is probably limited by practical or ethical considerations, and animal studies will be necessary to thoroughly investigate these phenomena.

The present study sought to investigate the cognitive effects of a single exposure to stress. In particular, the effects of acute noise exposure on rodent spatial working memory were examined. Loud noise was selected as the stressor in this research for a number of reasons. First, loud noise reliably provokes behavioural and physiological indicators of stress, including secretions of glucocorticoids, catecholamines, ACTH, and CRH (Burow et al., 2005; Dayas, Buller, Crane, Xu, & Day, 2001; de Boer et al., 1988; de Boer et al., 1989; Driskell et al., 2001; Inzana et al., 1996; Kim et al., 2007;

Manikandan et al., 2006; Masini, Day, & Campeau, 2008; Michaud et al., 2003; Prior, 2006; Samson, Sheeladevi, Ravindran, & Senthilvelan, 2007; Seale et al., 2004; Windle et al., 2001). Second, the decibel scale can be used to describe magnitude differences in the intensity of the stressor. Stressor intensity appears to be an important variable in single-trial sensitization and desensitization, and the intensity of most other stressors (e.g. restraint, odour, predator, or novelty) cannot be measured with the same level of precision. Third, noise can be activated by remote, which allows a researcher to avoid contact with animal subjects. This is important because handling can evoke a confounding stress response (Balcombe, Barnard, & Sandusky, 2004; Gärtner et al., 1980; Keith & Michaud, 2008) and the presence of a human observer can influence animal behaviour in testing (Lay, Buchanan, & Haussmann, 1999). Finally, noise can be delivered *while* an animal completes a task, which allows for a simultaneous behavioural assessment. Most other stressors can be delivered only in advance of a task, and for this reason, other stress and behaviour paradigms may lack some ecological validity. In the present study, this characteristic of noise exposure allowed for a novel distinction between performance under stress and performance after stress.

Spatial working memory was assessed with a modified spontaneous alternation task. In this task, rats freely explored a cross-maze, which allowed them to demonstrate a variety of spontaneous behaviours (e.g. alternation, exploration, freezing, and defecation). These behaviours could then be compared with those observed by other researchers. Blood and neural tissue samples were harvested at the end of each experiment to quantify plasma glucocorticoid secretions and to characterize ERK1/2 activity in the hippocampus and the amygdala.

Experiment 1

In the first experiment, locomotor activity and spontaneous alternation were measured in the cross-maze while rats were exposed to 60 or 90 dBA of white noise. This experiment characterized the extent to which loud noise elicited stress and altered behaviour. It was hypothesized that noise-exposed rats would exhibit physiological signs of stress, including increased plasma corticosterone levels, increased ERK1/2 activity in the basolateral amygdala and the CA1 region of the hippocampus, and decreased ERK1/2 activity in the dentate gyrus. Noise-exposed rats were also expected to demonstrate signs of a spatial working memory deficit, including a reduced level of spontaneous alternation, and it was expected that increasing the noise intensity would proportionately increase indicators of stress and behavioural disruption.

Method

Subjects

The first experiment included 24 male Long-Evans rats (Charles River, QC). The rats were approximately 45 days old and weighed 176-200 g when they arrived from the supplier. They were housed in pairs in clear, polycarbonate cages (48 x 26 x 20 cm^3) in a dedicated cage room (300 x 390 x 280 cm^3) inside the Life Sciences Research Centre vivarium at Carleton University. The cages contained wood shavings for bedding and were covered with wire lids that provided the rats with *ad libitum* access to standard Purina Rat Chow and tap water. The temperature and humidity of the vivarium ranged from 19-22°C and 25-62%, respectively. Rats were housed under a normal 12:12 h light-dark cycle with lights on at 8:00 a.m. and off at 8:00 p.m. Lighting in the cage room was provided by two fixtures that each contained two 32 W linear T8 fluorescent tube lamps

(Sylvania, ON). Each lamp was covered by an acrylic diamond-shaped prismatic diffuser and had a colour temperature rating of 3500 K. The background noise in the cage room was determined by an expert from the Acoustics Division at Health Canada (Consumer and Clinical Radiation Protection Bureau, Healthy Environments and Consumer Safety Branch). A single measurement from the centre of the cage room suggested a varying pressure level, ranging 47-52 dBA (depending on the activity and vocalizations of the rats). Rats were left to habituate to these conditions for at least 5 days before any handling or experimental manipulation occurred.

At the start of the experiment, rats were randomly assigned to three independent groups. Each group (n = 8) completed a test of visuospatial working memory while being simultaneously exposed to a stressor. The first group was exposed to 60 dBA of continuous white noise in a designated testing area. The second group was exposed to 90 dBA of the same stressor, and the third group served as a no-noise control. All experimental manipulations were conducted according to guidelines provided by the Canadian Council on Animal Care and the experimental protocol (P08-4; see Appendix A) approved by the Carleton University Animal Care Committee.

Procedure

Handling. Handling began at least 5 days after the rats arrived from the supplier. Each rat was transferred from its home cage to a transport cage and then carried up two flights of stairs to a behaviour room where testing occurred. The transport cage was identical to the home cage except that it contained no bedding and had no wire lid. This cage was also cleaned with a 70% ethanol solution just before a new rat was placed inside. In the behaviour room, each rat was held in a gloved hand for 5 min before being

carried back down the stairs to its home cage. This procedure was continued for 5 consecutive days to habituate the animals to any effect of transportation, handling, or ethanol exposure. Handling always occurred between 12:00 p.m. and 3:00 p.m. to further habituate the rats to a consistent interruption in their sleep cycles. Stressing and testing began at least 24 hr after the fifth day of handling.

Behavioural assessment. Visuospatial working memory was assessed by monitoring rats in a cross-maze (see Appendix B). The cross-maze was a Plexiglas structure with four fenced arms radiating at 90° from a 14 x 14 cm^2 central platform. This platform and the floor of each arm were glossy black, but the walls of the maze were transparent and colourless. Each arm measured 46 x 14 x 25 cm^3 and was labelled A, B, C, or D to indicate southeast, northeast, northwest, or southwest, respectively. The cross-maze sat inside an enclosed testing chamber (220 x 220 x 215 cm^3) in the behaviour room on a circular platform (123 cm in diameter) that elevated the maze 30 cm above the ground. The maze was also deliberately positioned so that each arm faced an ordinal direction inside the chamber. This ensured that the noise equipment suspended directly above the maze could project an equivalent signal into each arm.

Scoring. An arm entry was counted only when all four paws left the centre platform of the cross-maze. The number of arm entries was recorded to obtain an index of spontaneous exploration and general locomotion. The order in which the entries occurred was also recorded to provide an index of spontaneous alternation. One successful alternation was defined as any non-repetitive sequence of four arm entries. For example, if a rat followed the entry sequence ABCDABBCDA, an experimenter would count 2 complete alternations (i.e. ABCD and BCDA). Overlapping alternations were not

counted. To control for locomotor behaviour, the number of alternations was converted to a percent score by dividing the ratio of alternations-to-entries by 1 / 4. (One-fourth is the ratio of alternations-to-entries given by perfect performance in the cross-maze.) For example, if a rat made 3 alternations over 15 entries, the ratio would be 3 / 15. This value would be divided by 1 / 4 to give a final score of 80%. A rat's alternation score was recalculated after every minute of testing. Each recalculation considered all the entries and alternations that had occurred up to that point; thus, alternation score is a cumulative value. A high alternation score indicates a consistent and logical search pattern, which in turn indicates good spatial working memory. In this experiment, scoring excluded the first 10 sec of video to accommodate any delay before noise onset.

Noise stress. Immediately (~5 sec) after a rat was placed into the cross-maze for testing, a 10-min burst of continuous white noise was delivered through two JBL4892 Array Series loudspeakers (JBL Professional, CA). The loudspeakers were suspended from a metal chassis approximately 120 cm above the floor cross-maze (see Appendices B and C). The chassis consisted of two rectangular frames joined together by two crossbars and was constructed using 10 pieces of Wheatland ASTM A-53 Type F Schedule 40 Extra Heavy 1.25-in (3.18-cm) steel piping (Wheatland Tube Company, PA) and 12 aluminum slip-on pipe fittings (The Wagner Companies, WI). Each rectangular frame (200 x 130 cm^2) was constructed by cutting down four pieces of piping and connecting them together with four 90-degree elbow fittings. Each crossbar was constructed from a single 220-cm piece of piping and was attached to the top pipe of each frame using a crossover fitting. Four Omega-95 MD12S3 screw-lock carabiners (Omega Pacific, WA) joined the loudspeakers to the crossbars. The loudspeakers and an attached

Logitech QuickCam Messenger webcam (Logitech International S.A., ON) were connected to a laptop and signal processing equipment located outside of the testing chamber. This allowed an experimenter to activate the noise equipment and monitor a test subject's response without being present in the testing chamber to influence the animal's behaviour.

The signal processing equipment used in this experiment included a Sony XA20ES current pulse digital-to-analog converter (Sony Corporation, ON), which played a white noise CD produced specifically for this study by the Acoustics Division at Health Canada. This CD played a repeating 64-sec white noise signal, band-limited from 100 Hz to 22 kHz. The signal was modulated by a TOA DP-0204 digital signal processor (TOA Corporation, ON) and amplified by a 120 W Bryston 8B-ST multichannel amplifier (Bryston Limited, ON) before being fed to the loudspeakers. The signal processor was configured to ensure that only 60 or 90 dBA were delivered during stressing. However, because their drivers activated sequentially instead of simultaneously, the loudspeakers projected 57 and 95 dBA of white noise for approximately 1.5 sec during the 60 and 90 dBA exposures, respectively. Sound level measurements were conducted by an expert from Health Canada to ensure that sound pressure levels varied consistently and negligibly throughout each arm of the maze. After the last exposure, a final sound level measurement confirmed that the equipment had functioned properly throughout the experiment.

Blood and tissue processing. Stressing, testing, and euthanizing always occurred between 12:00 p.m. and 2:00 p.m. to minimize the regular circadian variability in blood corticosterone concentrations. Immediately (<60 sec) after testing, each rat was removed

from the cross-maze and rapidly decapitated using a guillotine and a DecapiCone™ restrainer (*Braintree* Scientific Incorporated, MA). Brains were removed and stored temporarily in 14-ml vials containing a solution of 4% (wt/vol) paraformaldehyde and 0.1 M phosphate buffer. Trunk blood was collected in 1.5 ml microcentrifuge tubes and vortexed at 3000 rpm in 10 µl EDTA. Blood samples were then centrifuged at 4300 rpm for 15 min at 4°C or less. The supernatant plasma was decanted and stored at -80°C until corticosterone levels could be determined using a commercially available radioimmunoassay kit (MP Biomedicals, OH). When ready for measurement, stored samples were thawed, gently vortexed, and added to the assay according to the manufacturer's specifications. Samples were assayed twice. The first assay provided results for a preliminary analysis that is not featured in this report. The second assay included samples obtained from rats in this and all subsequent experiments. This ensured that the results were comparable throughout the study. The final concentration of corticosterone was recorded in micrograms per decilitre of blood.

Brains were transferred to a solution of 30% (wt/vol) sucrose and 0.1 M phosphate buffer approximately 48 hr after removal. After at least 72 hr in sucrose, the brains were hemisected. The right hemisphere was sectioned on a cryostat at 25 µm and collected in a 0.1 M phosphate-buffered 0.1% (wt/vol) sodium azide solution. Two brain sections from each animal were then processed for immunohistochemistry that assessed activation (phosphorylation) of ERK1/2. Each step of this process took place at room temperature. First, sections were washed three times for 5 min each in a solution of phosphate-buffered saline (PBS) and 0.2% (vol/vol) Triton X-100 (TX; Union Carbide Corporation, ON). The PBS solution included 0.15 M sodium chloride and 2.68 mM

potassium chloride in 0.01 M phosphate buffer. Next, sections were blocked in 0.3% (vol/vol) hydrogen peroxide for 15 min and then washed three more times for 5 min each in the PBS-TX solution. A second blocking in 3% (vol/vol) normal goat serum (Vector Laboratories Incorporated, CA) and PBS-TX followed and continued for 30 min. Sections were then incubated in the primary antibody (ab50011; Abcam Incorporated, MA) overnight. This monoclonal antibody was specific for the active forms of ERK1/2. It was raised in mouse but known to react with rat tissue (Abcam Incorporated, 2008). The antibody was tested in four different dilutions with PBS-TX before tissue processing began, and it was found that dilutions greater than 1:5000 lacked the staining intensity necessary for easy quantification (Figures 1-3). For this reason, a 1:5000 dilution in PBS-TX was used.

The following day, sections were washed three times for 10 minutes each in PBS-TX and incubated in the secondary antibody (goat antimouse diluted 1:500 in PBS-TX; Vector Laboratories Incorporated, CA) for 2 hr. During a second set of three 10-min washes in PBS-TX, a solution of 15% (vol/vol) Avidin Biotin Complex (Vector Laboratories Incorporated, CA) in PBS-TX was prepared and allowed to conjugate for 30 min. After a 1-hr incubation in this solution, sections were washed three times for 5 min each in PBS and developed for approximately 5 min in a 2.33 mM 3,3`-diaminobenzidine and PBS solution. The diaminobenzidine solution included 0.02 M cobalt chloride and 1.05 mM nickel ammonium sulphate as development enhancers. The sections were float mounted on Fisherbrand Superfrost microscope slides (Thermo Fisher Scientific Incorporated, ON) and left to air-dry overnight. The next day, the slides were washed in distilled water and dehydrated in graded alcohol (75, 95, and 100% for 1, 5, and 10 min,

respectively) before being submerged for 20 min in a laboratory solvent (Clearene™; Surgipath Canada Incorporated, MB) and coverslipped. Cells expressing the phosphorylated form of ERK1/2 (pERK1/2) appeared black or dark grey after staining.

pERK1/2 quantification. For each stained section, bright field images of the CA1 region, the dentate gyrus, and the basolateral amygdala were captured at 10x magnification using a Retiga-2000R camera (QImaging, BC) and an Olympus BX61 research microscope (Olympus Canada, ON). Regions of interest were selected and traced (freehand) with reference to Paxinos and Watson (1998). When the entire CA1 region or dentate gyrus could not be captured in a single image, a sample of the area was acquired instead. The CA1 sample region was identified by the string of pyramidal neurons that could be viewed through the camera lens when the most superior point of the hippocampus was centred horizontally and vertically (Figure 1). Dentate gyrus counts were carried out on the two blades of neurons that were viewable when the end of the superior blade reached the lateral edge of the camera window (Figure 2). Images were acquired using InVitro version 3.2.2 (Media Cybernetics, MD), and pERK1/2-stained cell counts were obtained using Image-Pro Analyzer version 6.2.1.491 (Media Cybernetics, MD).

Cells expressing pERK1/2 within each region of interest were identified on the basis of staining intensity (average grey value). To ensure that the image intensity values were unaffected by any digital adjustments, the camera's brightness, contrast, and gamma corrections remained at their default linear settings. Gain and binning were also set to their default values (1.0 and 1, respectively), and the exposure value was held constant at

40 ms. For each section, the microscope lighting was adjusted until the widest range of intensity values could be detected by the camera.

Stained cells were segmented out by manually adjusting the greyscale threshold for each section. Threshold values ranged from 1500-3450 ($M = 2405$, $SD = 345.60$), depending on the intensity of the staining. The mean diameter of each segmented object was calculated by averaging the length of all diameters that could be measured at two-degree intervals around each object's perimeter. Objects with a mean diameter of less than 10 μm were filtered out to increase the likelihood that only pyramidal neurons would be included in the cell count. Each of the remaining segmented objects was then inspected by an experimenter who excluded objects that were obviously composed of stained background material. The final count was divided by the area of the region of interest to obtain a measure of cell density, which was recorded in number of pERK1/2-labelled cells per millimetre squared. Two sections from each animal were quantified, and the mean of every two corresponding density values was entered into the analysis as a single data point.

Results

The statistical analyses considered the concentration of plasma corticosterone; the density of pERK1/2-labelled cells in the CA1 region, the dentate gyrus, and the basolateral amygdala; the latency of the first alternation; the number of arm entries; and the alternation score. Mean corticosterone levels and mean pERK1/2 cell densities for each region of interest were analyzed in separate one-way analyses of variance (ANOVAs) that included three independent groups (Control, 60 dBA, and 90 dBA). Four

identical analyses were conducted, and in each case, pairwise planned comparisons analyzed all non-redundant differences between the groups.

Each of the behavioural variables was analyzed in a 3 x 2 (noise exposure x phase) mixed ANOVA that examined the three groups across the two phases (Habituation and Testing) of the experiment. In this design, only the simple effects of the experimental phases were considered: The simple effect of noise exposure in the habituation phase was analyzed to confirm that there were no differences in baseline performance, and simple pairwise planned comparisons were conducted to investigate group differences due to noise exposure in the testing phase. Stressed rats demonstrated immediate disorientation at the start of testing followed by a gradual recovery that occurred within the first few minutes of the experiment (Figure 4; see also Appendix D). For this reason, the mean alternation scores and arm entries were calculated for each minute of the experiment, and a series of analyses examined each minute sequentially, starting with the first minute, until significant differences were no longer detectable in the testing phase.

The modified Bonferroni correction was applied when the number of planned comparisons conducted in each analysis exceeded the available degrees of freedom (Keppel, 1982; Schaffer, 1995). This procedure reduced the acceptable significance level to .03 for the pairwise comparisons; however, in all other analyses, a significance level of .05 was used. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 16.0.

Corticosterone Concentration and pERK1/2 Cell Density

Descriptive statistics for corticosterone concentration and pERK1/2 cell density are displayed in Tables 1 and 2, respectively. Interestingly, the control group did not

differ significantly from either noise-exposed group. The difference between the 60- and 90-dBA groups was also nonsignificant.

Latency of the First Alternation

The mean latencies and standard errors are displayed in Figure 5A. No significant differences were detected among the groups in the habituation phase, $F(2, 21) = 0.22$, $p = .81$, power =.08. In the testing phase, simple planned comparisons were unable to detect significant differences between any of the groups.

Number of Entries

The mean number of entries and standard errors for the first minute of each experimental phase are depicted in Figure 5B. There was no simple effect of noise exposure in the first minute of the habituation phase, $F(2, 21) = 1.29$, $p = .30$, power = .25. In the first minute of the testing phase, the control group made significantly more arm entries than the 60- and 90-dBA groups, $F(1, 21) = 13.19$, $p = .002$, $\eta_p^2 = .39$ and $F(1, 21) = 8.95$, $p = .007$, $\eta_p^2 = .30$, respectively. However, there was no significant difference between the two noise-exposed groups.

Descriptive statistics for the number of entries completed over the second minutes of habituation and testing are displayed in Figure 5C. In the second minute of the habituation phase, there was no simple effect of noise exposure, $F(2, 21) = 1.61$, $p = .22$, power = .30, and in the second minute of the testing phase, simple planned comparisons could not detect significant differences between any of groups. No further analyses were conducted using this dependent variable.

Alternation Score

Mean alternation scores and standard errors for the first minute of each experimental phase are depicted in Figure 5D. In the first minute of the habituation phase, no simple effect of noise exposure was indicated, $F(2, 21) = 1.12, p = .35.$, power = .22. In the first minute of the testing phase, the control group scored significantly higher than the 60- and 90-dBA groups, $F(1, 21) = 9.64, p = .005, \eta_p^2 = .31$ and $F(1, 21) = 6.02, p = .02, \eta_p^2 = .22$, respectively. No significant difference was detected when the 60- and 90-dBA groups were compared.

Alternation scores and standard errors up to the second minute of each phase are displayed in Figure 5E. In the second minute of the habituation phase, there was no simple effect of noise exposure, $F(2, 21) = 1.61, p = .22$, power =.30, and in the second minute of the testing phase, simple planned comparisons could not detect significant differences between the any of the groups. No further analyses were conducted on this dependent variable.

Experiment 2

The results of Experiment 1 suggest that exposure to loud noise disrupts behaviour but does not elicit physiological changes indicative of stress. The second experiment tested rodent performance after noise exposure to assess the time course of these effects. Groups of rats were exposed to 90 dBA of white noise and were allowed to rest in their home cage for 1, 4, or 24 hr before testing. The 60 dBA noise category was dropped because no significant differences were detected between 60 and 90 dBA exposures in the previous experiment. Corticosterone levels, ERK1/2 phosphorylation, and indicators of behavioural disruption were expected to regress to control or baseline levels gradually as the rest period increased.

Method

Subjects

The second experiment included 48 male Long-Evans rats (Charles River, QC). The rats were approximately 45 days old and weighed 176-200 g when they arrived from the supplier. They were housed as described in the previous experiment and were similarly left to habituate to those conditions for at least 5 days before any handling or experimental manipulation occurred. At the start of the experiment, rats were randomly assigned to six independent groups (n = 8). The first three groups were stressed with exposure to 90 dBA of white noise and were left to rest for 1, 4, or 24 hr before testing. The next three groups served as no-noise controls. These rats underwent the same procedure without noise exposure and were similarly allowed to rest for 1, 4, or 24 hr before testing.

Procedure

Rats were handled in the behaviour room and tested in the cross-maze according to the procedures described in the previous experiment. Testing and euthanizing continued to occur between 12:00 p.m. and 2:00 p.m. to minimize circadian effects. For this reason, the 1-hr rest groups were stressed between 11:00 a.m. and 12:00 p.m., the 4-hr rest groups were stressed between 8:00 a.m. and 9:00 a.m., and the 24-hr rest groups were stressed one day in advance between 12:00 p.m. and 2:00 p.m. Twenty-four hours after a 5-min habituation trial, rats were placed in a holding cage inside the testing chamber. The holding cage was identical to a rat's home cage except that it contained no bedding and had no wire lid. It sat on top of the cross-maze approximately 55 cm above the ground and was cleaned with a 70% ethanol solution just before a new rat was placed inside. Rats in the control groups were placed in the holding cage and allowed to explore freely for 10 min, whereas rats that experienced noise stress were placed in the cage and exposed to 90 dBA of continuous white noise for 10 min. The noise was delivered by the apparatus described previously, but in this experiment, noise was faded in over 5 sec to mask the sequential activation of the loudspeakers' drivers.

After stressing, each rat was returned to its home cage in the vivarium via the transport cage used in handling. After a 1-, 4-, or 24-hr rest period, each rat was brought back to the testing chamber and tested for 10 min in the cross-maze. Immediately after testing, each rat was removed from the cross-maze and rapidly decapitated. Neural tissue and blood samples were obtained and processed according the procedures described in the previous experiment.

Results

Mean corticosterone levels and mean pERK1/2 cell densities were analyzed in 2 x 3 independent groups ANOVAs that included two noise exposure groups (Control and 90 dBA) and three rest period groups (1, 4, or 24 hr). In each case, only simple effects were considered: First, the simple effect of noise exposure at each rest period was examined to determine extent to which noise elicited stress after a rest period. Next, the simple effect of rest period on the control groups was analyzed to confirm that different periods of rest had no effect in the absence of noise. Finally, simple pairwise planned comparisons examined all non-redundant differences between the rest periods for the 90-dBA groups to determine the extent to which responses to stress developed or persisted over time.

Each of the behavioural variables was analyzed in a 2 x 3 x 2 (noise exposure x rest period x phase) mixed ANOVA that examined the same noise and rest groups across different phases (Habituation and Testing) of the experiment. Again, only simple effects were considered. In the habituation phase, the simple main effects and simple interaction of noise exposure and rest period were analyzed. Nonsignificance in these tests confirmed the absence of differences in baseline performance. In the testing phase, the analysis considered the same set of comparisons described for the corticosterone and pERK1/2 data, including the simple simple effect of noise exposure at each rest period, the simple simple effect of rest period on the control groups, and simple simple pairwise planned comparisons that analyzed differences between the rest periods for those rats exposed to 90 dBA of noise.

The mean alternation scores and arm entries were calculated and analyzed per minute, as described in the previous experiment. A significance level of .05 was used throughout, but the modified Bonferroni correction was applied to accommodate the

planned comparisons (Keppel, 1982). This reduced the acceptable significance level for the comparisons to .03. All analyses were conducted using SPSS version 16.0.

Corticosterone Concentration

Mean concentrations of blood corticosterone and standard errors are displayed in Table 3. Rats that were exposed to 90 dBA of white noise exhibited a mean corticosterone level after 1 hr of rest that was statistically equivalent to that of a control group never exposed to noise. However, noise-exposed rats that were allowed to rest for longer periods exhibited corticosterone levels that were higher than their corresponding control groups. After 4 hr of rest, the elevation fell just short of statistical significance, $F(1, 42) = 3.83, p = .06, \eta_p^2 = .08$, but after 24 hr of rest, the difference met the specified criteria, $F(1, 42) = 4.68, p = .04, \eta_p^2 = .10$. Interestingly, the simple effect of rest period on the control rats was significant, $F(2, 42) = 3.75, p = .03, \eta_p^2 = .15$, while simple pairwise planned comparisons were unable to detect any differences between the rest periods when rats were exposed to noise. Tukey's Honestly Significant Difference test (HSD) was used to compare the control group corticosterone levels after each rest period. In this analysis, the mean level observed after 1 hr of rest was significantly higher than the level observed after 4 hr of rest, $F(1, 42) = 5.90, p < .05$. When the 4 and 24 hr groups were compared, the difference was not significant, $F(1, 42) = 5.35, .05 < p < .10$, but Tukey's test statistic was very similar to the critical value (HSD = 3.27 vs. $q = 3.43$).

pERK1/2 Cell Density

Mean cell densities and standard errors for all three regions of interest are displayed in Table 4. One animal's data were deleted from the analyses of the CA1 region and the dentate gyrus because its hippocampus was destroyed during processing.

In the CA1 region, significantly more cells were active in noise-exposed rats after 4 hr of rest when compared to control rats, $F(1, 41) = 6.25$, $p = .02$, $\eta_p^2 = .13$. However, no significant differences were observed when the same comparison was made after 1 and 24 hr of rest. There was no simple effect of rest period on the control groups, $F(2, 41) = 0.74$, $p = .49$, power = .17, and simple pairwise planned comparisons were unable to detect any significant differences between the rest periods when the 90-dBA groups were considered alone. The main effect of noise exposure was considered post hoc, and it was found that on average, noise-exposed rats expressed significantly higher pERK1/2 cell densities in the CA1 region than controls, $F(1, 41) = 6.29$, $p = .02$, $\eta_p^2 = .13$.

When cell densities from the dentate gyrus were compared, no significant differences were observed between the control and 90-dBA groups after any rest period. There was also no simple effect of rest period on the control groups, $F(2, 41) = 1.91$, $p = .16$, power = .37, and when the 90-dBA groups were considered in planned comparisons, no significant differences between the rest periods were detectable.

pERK1/2 cell densities in the basolateral amygdala were reminiscent of the corticosterone results. Rats that were exposed to 90 dBA of noise exhibited mean cell densities after 1- and 4-hr rest periods that were statistically equivalent to the corresponding control groups. However, noise-exposed rats that were allowed to rest for 24 hr exhibited a significantly higher mean cell density when compared to controls, $F(1, 41) = 10.19$, $p = .003$, $\eta_p^2 = .20$. In addition, the simple of effect of rest period on the control groups was significant, $F(2, 42) = 3.49$, $p = .04$, $\eta_p^2 = .14$, while differences between the rest periods for noise-exposed rats were nonsignificant. Tukey's HSD

indicated that the mean pERK1/2 cell density observed after 1 hr of rest was significantly higher than the density observed after 24 hr of rest, $F(1, 42) = 6.08, p < .05$.

Latency of the First Alternation

The mean latencies and standard errors are displayed in Figure 6A. In the habituation phase, the simple main effects of noise exposure and rest period were nonsignificant, $F(1, 42) = 0.26, p = .61$, power = .05 and $F(2, 42) = 2.05, p = .14$, power = .40, respectively. The simple interaction of the two variables was also nonsignificant, $F(2, 42) = 0.46, p = .64$, power = .12. In the testing phase, latency scores were significantly skewed and kurtotic, $z = 5.74, p < .0001$ (two-tailed) and $z = 5.97, p < .0001$ (two-tailed), respectively. No significant differences were observed between the control and 90-dBA groups after any rest period. The rest periods had no significant effect on the control groups, $F(2, 42) = 0.11, p = .89$, power = .07, and simple simple pairwise planned comparisons were unable to detect any differences between the rest periods when the three 90-dBA groups were considered.

Number of Entries

The mean number of entries and standard errors for the first minute of each experimental phase are depicted in Figure 6B. In the habituation phase, group differences were detectable at the outset: The simple main effects of noise exposure and rest period were significant, $F(1, 42) = 5.58, p = .02, \eta_p^2 = .12$ and $F(2, 42) = 5.75, p = .006, \eta_p^2 = .21$ respectively. However, the simple interaction of the two variables was not significant, $F(2, 42) = 0.25, p = .78$, power = .08. Tukey's HSD was used to analyze the differences between the rest periods. On average, rats assigned to the 1 hr rest group completed fewer arm entries than rats assigned to the 4 or 24 hr rest groups, $F(1, 42) = 4.54, p < .05$ and

$F(1, 42) = 11.22, p < .01$, respectively. However, the difference between the 4 and 24 hr groups was not significant.

Data from the first minute of the testing phase demonstrated a significant negative skew, $z = 2.42, p = .02$ (two-tailed). In this phase, no significant differences were observed between the control and 90-dBA groups after any rest period. The rest periods had no significant effect on the control groups, $F(2, 42) = 1.56, p = .22$, power = .31, and simple simple pairwise planned comparisons did not indicate differences between the rest periods when the 90-dBA groups were considered. No further analyses were conducted on this dependent variable.

Alternation Score

Mean alternation scores and standard errors for the first minute of each experimental phase are depicted in Figure 6C. In the habituation phase, the data were highly variable, and the simple main effects of noise exposure and rest period were not significant, $F(1, 42) = 0.56, p = .46$, power = .11 and $F(2, 42) = 2.74, p = .08$, power = .51, respectively. The simple interaction of the two variables was also nonsignificant, $F(2, 42) = 0.37, p = .69$, power = .14. In the first minute of the testing phase, alternation scores were significantly kurtotic, $z = 2.83, p = .005$ (two-tailed). No significant differences were observed between the control and 90-dBA groups after any rest period. The rest periods had no significant effect on the control groups, $F(2, 42) = 0.09, p = .91$, power = .07, and simple simple pairwise planned comparisons were unable to detect any differences between the rest periods when the three 90-dBA groups were considered. No further analyses were conducted on this dependent variable.

Experiment 3

In the third experiment, different handling and habituation procedures were compared to indentify and characterize sources of extraneous stress. Confounding stress might have been produced by human activity in the vivarium or transport to the testing chamber. Entry into the testing chamber was also accompanied by significant changes in lighting and sound, which might have been another source of extraneous stress. In this experiment, four main hypotheses were tested. First, it was hypothesized that rats that never underwent any handling procedure and only experienced routine human contact in the vivarium would express lower levels of plasma corticosterone when compared to control rats from Experiment 1. Second, it was hypothesized that rats transported to the testing chamber would express levels of corticosterone that were significantly higher than levels expressed by rats that were only exposed to routine vivarium activity. Third, it was hypothesized that rats would express a corticosterone level after transport to the testing chamber that was significantly higher than the level expressed by rats before transport. Finally, it was hypothesized that rats provided with extended habituation to the testing chamber would demonstrate improved behavioural performance and exhibit reduced physiological signs of stress, including changes in corticosterone level and ERK1/2 phosphorylation.

Method

Subjects

The third experiment included 24 male Long-Evans rats (Charles River, QC). The rats were approximately 45 days old and weighed 176-200 g when they arrived from the supplier. They were housed as described in Experiment 1 and were similarly left to

habituate to these conditions for at least 5 days before any handling or experimental manipulation occurred. Rats were randomly assigned to four different groups. The first two groups underwent a novel handling procedure that included habituation to the environmental conditions inside the testing chamber. One of these groups ($n = 4$) was sacrificed before the testing phase while the other group ($n = 8$) was sacrificed afterwards. These groups were used to determine the extent to which stress was induced by transport to the testing area. The next two groups did not complete any particular habituation or testing procedure and were instead removed directly from the vivarium for blood sampling. One of these groups ($n = 8$) was sacrificed on a day when vivarium activity was limited to routine maintenance (e.g. changing water bottles, refilling food baskets). This group served as an absolute no-stress condition. The other group ($n = 4$) was sacrificed on a busy cage-changing day when maintenance and cleaning activities in the vivarium were very high. It should be noted that rats in these last two groups were subjected to another experiment before participating in the present study. This experiment included injection of a non-competitive NMDA receptor antagonist (MK-801) and reinforced bar-pressing in an operant chamber with concomitant food restriction (Clarke, 2009). Rats were dissociated from these procedures for at least a week before their participation in this experiment.

Procedure

Rats in the routine and high vivarium activity groups were removed directly from the cage room on the appropriate day and decapitated within approximately 60 sec of transport. Trunk blood was collected and assayed according to the procedures described in Experiment 1.

Rats that underwent testing chamber habituation were exposed to the chamber immediately after each handling trial for 5 consecutive days. The testing chamber that was used in all experiments was a shutdown and defrosted walk-in freezer (Can-Trol Environmental Systems Limited, ON; see Appendix C). This area was noticeably darker and quieter than the room immediately outside it. The chamber was lit with a single 60 W 120 V A19 soft white incandescent lamp (Sylvania, ON) that had a colour temperature rating of 2700 K and was covered with a shatterproof grille. This lamp sat at the approximate centre of the chamber's ceiling but was partially occluded by the loudspeakers suspended below it. In contrast, the room where rats were handled was lit with seven fixtures that each contained two 32 W linear T8 fluorescent tube lamps (Sylvania, ON). Each lamp was covered by an acrylic diamond-shaped prismatic diffuser and had a colour temperature rating of 3500 K. The ambient noise levels in the testing chamber and the behaviour room were determined by personnel from the Acoustics Division at Health Canada. A single measurement from inside the testing chamber revealed a background noise level of approximately 40 dBA, compared to a noise level of approximately 46 dBA in the room immediately outside.

Handling for rats in testing chamber habituation groups followed the same procedure described in Experiment 1, except for one modification. Before being returned to the vivarium, each rat was placed in a holding cage inside the testing chamber where it sat for approximately 10 min. This cage was identical to the holding cage described in Experiment 2 and was similarly cleaned between subjects. After 5 days of habituation, rats were provided with one habituation trial. Twenty-four hours later, one group of rats was taken directly from the cage room in the vivarium and decapitated within

approximately 60 sec of transport. The second group underwent testing. The behavioural assessment and blood and tissue processing were performed as described in Experiment 1.

Results

The physiological and behavioural data obtained from these animals were compared to the data obtained from the Experiment 1 control group, which completed the testing phase after a handling procedure that did not include exposure to the testing chamber. Significance in all tests was defined at the .05 level, and all analyses were conducted using SPSS version 16.0.

Corticosterone Concentration

Mean corticosterone concentrations and standard errors are displayed in Table 5. A one-way ANOVA with planned comparisons considered a limited number of contrasts between five independent groups. The modified Bonferroni correction was applied to accommodate these planned comparisons (Keppel, 1982), reducing the significance criteria to $\alpha = .03$. The Experiment 1 control group exhibited a corticosterone concentration that was significantly higher than the no-stress baseline provided by the routine vivarium activity group, $F(1, 27) = 13.35$, $p = .001$, $\eta_p^2 = .33$. High activity in the vivarium appeared to have no effect on corticosterone level relative to this baseline, $F(1, 27) = 030$, $p = .59$, power = .06. When handling included habituation to the testing chamber, corticosterone levels before and after testing were statistically equivalent, $F(1, 27) = 2.44$, $p = .13$, power = .33, and neither group exhibited a level that was significantly different from the vivarium baseline, $F(1, 27) = 0.06$, $p = .80$, power = .05 and $F(1, 27) = 2.57$, $p = .12$, power = .34 for the before- and after-testing groups, respectively. However,

it should be noted that the corticosterone level expressed after testing was almost twice that which was expressed before testing. Also, rats that were habituated in the testing chamber exhibited a mean corticosterone level after testing that was almost significantly lower than the level exhibited by the Experiment 1 control group, $F(1, 27) = 4.21$, $p = .05$, $\eta_p^2 = .13$.

pERK1/2 Cell Density and Behavioural Measures

pERK1/2 cell densities and behavioural data were obtained only for rats that underwent habituation in the testing chamber because only these rats progressed to the testing phase. Mean pERK1/2 cell densities and standard errors for this group are displayed with descriptive statistics from the Experiment 1 control group in Table 6. Three one-way ANOVAs compared the two groups and found no significant differences in activated cell densities in the CA1 region, the dentate gyrus, or the basolateral amygdala.

Descriptive statistics for latency of the first alternation, number of arm entries, and the alternation score are displayed with the equivalent Experiment 1 control group data in Figure 7. The mean alternation scores and arm entries were calculated and analyzed per minute, as described in Experiment 1. Three 2 x 2 (group x phase) mixed ANOVAs compared the behavioural data obtained from these two groups across both phases (Habituation and Testing) of the experiment. Only the simple effects of group were considered. No significant effects were detected during any phase of the experiment for any behavioural measure. Further analyses did not consider data beyond the first minute.

Discussion

These results provide very little support for the hypothesis that an acute noise exposure has a lasting effect on rodent spatial working memory or the rodent stress response. When rats were exposed to white noise in a cross-maze, their ability to search and navigate was impaired for the first minute of testing. However, noise-exposed rats expressed levels of circulating corticosterone that were similar to those expressed by rats that had never been exposed to the white noise. Noise-exposed rats also exhibited levels of ERK1/2 activity in brain regions associated with stress and memory that were equivalent to levels expressed by unexposed control rats. These results suggest that while acute noise exposure can disrupt spatial working memory for about a minute, it does not elicit an elevation in corticosterone or pERK1/2 that would suggest a stress response. When rats were exposed to the white noise and then allowed to rest for 1, 4, or 24 hr before testing, their behavioural performance was identical to that of control rats and was unaffected by the length of the rest period. However, noise-exposed rats expressed elevated levels of plasma corticosterone and hippocampal pERK1/2 that were equivalent across all three rest periods. These results suggest that an acute noise exposure has no delayed or lasting effect on spatial working memory, but it may inhibit the normal decline of corticosterone levels and ERK1/2 activity that occurs after a stressor exposure has been terminated.

It should be noted that these results are confounded by the influence of extraneous stressors. When groups of rats were exposed to a quiet testing chamber and allowed a period of rest, they often expressed elevated levels of corticosterone and ERK1/2 activity at testing, even though they were never exposed to the white noise. These increments

persisted when the rest period was short and faded when the rest period was long, following a time course that one might expect from a mild stress exposure. Furthermore, rats that underwent testing but were never exposed to noise exhibited corticosterone levels that were significantly higher than those exhibited by a group of rats completely dissociated from the noise exposure and the behavioural assessment, which strongly suggests that some aspect of the testing procedure was inherently stressful.

When another group of no-noise rats was repeatedly exposed to the testing environment during handling, they expressed an average corticosterone level at testing that was significantly lower than the average level expressed by a group of no-noise rats without this habituation. However, there were no differences in behavioural performance or ERK1/2 activity between the two groups. These results suggest that the testing environment itself may have been a considerable source of stress, but it had no affect on spatial working memory or neurological activity in the hippocampus or the amygdala. It should also be noted that these specially habituated rats still expressed levels of corticosterone that were nearly double that of rats that had not undergone testing. At testing, rats were transferred from their home cage to a transport cage and then carried up two flights of stairs. The resultant corticosterone elevation was not statistically significant, but it suggests that the transport process that occurred immediately before testing might have been another important source of stress.

In the literature, loud noise reliably elicits behavioural and physiological indicators of stress (Burow et al., 2005; Dayas et al., 2001; de Boer et al., 1988; de Boer et al., 1989; Driskell et al., 2001; Inzana et al., 1996; Kim et al., 2007; Manikandan et al., 2006; Masini et al., 2008; Michaud et al., 2003; Prior, 2006; Samson et al., 2007; Seale et

al., 2004; Windle et al., 2001). For this reason, it seems unlikely that the noise exposure used in the present study was completely unable to induce stress. However, while noise is an established and reliable stressor, a number of caveats can complicate its use. For example, Keith and Michaud (2008) noted that rats can hear noises that range from about 100 Hz to 80 kHz, which is very different from the upper limit of the typical human auditory system. This means that rats are able to perceive auditory signals that humans cannot, and it is possible that they may as a result experience a significant difference in the subjective loudness of an exposure. Keith and Michaud also recommended that researchers consider the subject's position within the exposure area and the impact that objects in that environment (e.g. shielding or reflecting walls, bedding in a cage) might have on the noise administration. Prior (2006) advised that background sound levels should also be considered and measured to facilitate replicability across trials and in different settings. This measurement quantifies the extent to which the noise stressor is different from the noise already present in the testing environment, which is important because subjects may have already habituated to a significant level of background noise.

In the present study, thorough sound level assessments were conducted by personnel from the Acoustics Division at Health Canada. This ensured that the noise exposure occurred within the frequency limits of rodent hearing and at a pressure level significantly greater than the background noise. A set of measurements taken from inside the cross-maze also confirmed that the noise levels varied consistently and negligibly throughout each arm, and after the final exposure, the sound level assessments were repeated to confirm that there had been no changes in the noise throughout the experiments. Thus, it seems highly unlikely that variance in a subject's physiological

response to stress would be due to improper use of the noise exposure equipment featured in this study.

It is possible that differences in physiology were not significant when the noise and testing occurred simultaneously because the 10-min exposure/testing period before decapitation was too short. Sampling time is a particularly important consideration when measuring HPA activation, because maximum corticosterone levels are not usually reached before 15-20 min (Gagliano et al., 2008; Harbuz & Lightman, 1992; Le Mevel, Abitbol, Beraud, & Maniey, 1979). For this reason, some authors consider ACTH to be a better index of neuroendocrine activity after a short-term (less than 15-min) exposure to stress (Gagliano et al., 2008). With a longer testing period, the corticosterone level of our noise-exposed rats might have continued to increase, reaching a maximum point where differences would become observable. However, corticosterone levels can persist at their maximum concentration for at least 60 min after single experience with stress (Gagliano et al., 2008; Harbuz & Lightman, 1992; Le Mevel et al., 1979; Martí et al., 2001; Pasketti et al., 2000; Qin & Smith, 2008), and when our noise exposure was separated from testing and blood sampling by 1 hr, the corticosterone difference between the control and noise-exposed rats was still nonsignificant. This suggests that increasing the length of the sampling period used in the present study would have no effect on the corticosterone results that were obtained.

Sampling time may have had an effect on the amount of ERK1/2 activity that was observed. Increased ERK1/2 phosphorylation is usually observed in the hippocampus (Pardon et al., 2005; Yang et al., 2004, 2008) and the amygdala (Akirav, Sandi, & Richter-Levin, 2001; Yang et al., 2008) after exposure to acute stress. However, in the

present study, there was no effect of noise exposure on cellular activity in the CA1 region, the dentate gyrus, or the basolateral amygdala when stressing and testing occurred simultaneously over a 10-min period. Most studies seem to find significant ERK1/2 elevations after much longer (60-min) exposures to stress (Akirav et al., 2001; Pardon et al., 2005; Yang et al., 2004, 2008), but to our knowledge, a specific time-course for the immediate effects of stress on ERK1/2 activity has not been firmly established.

In our study, significantly more cells were active in the CA1 region of noise-exposed rats after 4 hr of rest when compared to unexposed control rats. However, no significant differences were observed when the same exposed and unexposed rats were compared after 1 and 24 hr of rest. This could suggest that noise-induced pERK1/2 elevations in this area of the hippocampus require up to 4 hr of incubation to become significant, which is much longer than the 60 min typically reported in the literature. Some brain areas take up to 2 hr for ERK1/2 effects to develop (Yang et al., 2008), and it is possible that the 4-hr delay observed in the present study was a unique response to noise or very short-term stress. It should be noted, however, that this particular difference may have resulted from declining pERK1/2 levels in the control group rather than increasing pERK1/2 levels in the noise-exposed group. In this case, the elevation could simply point to an intrinsic group difference or to the selective influence of extraneous factors. Note that rats assigned to different rest periods and different noise exposure conditions completed a significantly different number of arm entries in the preliminary habituation phase before any experimental manipulations occurred.

Extraneous factors could have also artificially elevated corticosterone and pERK1/2 levels shortly before blood and tissue sampling occurred. This might have

resulted in a ceiling effect that masked significant differences when the noise stress was applied. For example, corticosterone levels follow a circadian variation that begins with a peak shortly after awakening. Rats in the present study were always tested and decapitated in the middle of their light cycle (between 12:00 p.m. and 2:00 p.m.). If the rats had been woken up just before testing, their corticosterone levels may have already been at or near a maximum when the noise stress or blood sampling occurred. A similar ceiling for ERK1/2 activity in the CA1 region of hippocampus might have been reached simply because the rats completed a spatial working memory task. Nagai et al. (2006) showed that ERK1/2 activity in the hippocampus increases immediately with spatial memory processing, and it is possible that the task used in the present study increased pERK1/2 levels in the CA1 region to a point where the addition of noise stress had no observable effect. Extraneous stressors probably also inflated corticosterone levels, pERK1/2 levels in CA1 region, and pERK1/2 levels in the basolateral amygdala. High amounts of human activity in the vivarium may have had no effect on physiology, but other routine procedures—especially transport and exposure to the testing environment—probably induced stress. This could have pushed corticosterone or pERK1/2 levels to a ceiling, masking any additional effect of noise.

Extraneous stress might also explain why control rats that were exposed to only a quiet testing chamber exhibited higher corticosterone levels after a 1-hr rest period than they did after 4- or 24-hr rest periods. When rats are exposed to acute stress, corticosterone levels generally reach a peak after 15-20 min and can persist at a high concentration for at least 60 min. Levels then generally return to baseline within 2-3 hr (Le Mevel et al., 1979; Martí et al., 2001; Pasketti, McCreary, & Herman, 2000; Qin &

Smith, 2008). This was the approximate time course displayed by control rats in the present study, and it suggests that they were exposed to a stressor in the absence of noise. The same rats exhibited a very similar pattern of ERK1/2 activity in the basolateral amygdala, where the number of activated cells was higher after 1- and 4-hr rest periods than it was after a 24-hr rest period. Acute stress can increase amygdalar ERK1/2 phosphorylation, and high levels can persist for at least 2 hr (Yang et al., 2008). In some brain regions, pERK1/2 levels can persist for a surprisingly long time. For example, in the CA1 region of the hippocampus, ERK1/2 phosphorylation can be observed up to 12 hr after a stressor has been delivered and withdrawn (Yang et al., 2004).

It is interesting that rats exposed to loud noise did not display a similar pattern of declining corticosterone or pERK1/2 levels. They instead expressed consistently higher levels that were equivalent across all three rest periods. The elevations were not always statistically significant, but the corticosterone levels were significantly greater than control levels after 4- and 24-hr rest periods. The elevated pERK1/2 levels in the basolateral amygdala were also significant after a 24-hr rest period, and the pERK1/2 levels in the CA1 region of the hippocampus were significantly higher than the control levels on average. These elevations are consistent with the application of a stressor and could suggest that an acute noise exposure inhibits the normal decline and recovery of corticosterone levels and ERK1/2 activity after stress. However, it seems unlikely that corticosterone and pERK1/2 elevations would persist for 24 hr.

The elevated levels in noise-exposed rats after each rest period were probably the result of a new stress response elicited at some point during the testing procedure. This might point to the importance of contextual factors in eliciting stress. Richter-Levin

(1998) stressed rats using forced underwater submersion and observed behavioural deficits that were context-specific. In his study, one group of rats was taken to the testing area and submerged in a water maze, while another group was taken to a different room and submerged in a small tank. Both groups were tested in the water maze, but only the group that was submerged in it beforehand had difficulty completing the task. In our study, some groups of rats were exposed to loud noise, and some were simply brought into the exposure area. When the rats were later returned to that area for testing, the noise-exposed rats exhibited higher corticosterone levels and increased ERK1/2 phosphorylation. This could suggest that rats had learned to associate contextual cues in the exposure area with the stress-inducing noise. However, it should be noted that these elevations occurred without coincident behavioural deficits.

A new, heightened stress response could also be evidence of single-trial stressor sensitization. Armario et al. (2008) theorized that a single exposure to a psychogenic stressor could sensitize responding to heterotypic stressors for days or weeks, and a number of researchers have observed sensitized HPA responding to combinations of stressors (e.g. Martí et al., 2001; Belda et al., 2008; Gagliano et al., 2008; Klenerová et al., 2006; Koolhaas et al., 1990). For example, van Dijken et al. (1993) observed AVP and ACTH sensitization in rats that were exposed to a short session of inescapable footshocks and then later exposed to environmental novelty. In the present study, rats were exposed to loud noise (a psychogenic stressor) and then stressed again with transport and exposure to the testing area (heterotypic stressors). The persistent corticosterone elevations that were observed after the testing phase could indicate HPA sensitization.

To our knowledge, the same kind of progressive and lasting sensitization has not been demonstrated in the ERK1/2 pathway. However, Yang et al. (2008) observed that rats stressed with unpredictable and inescapable tailshocks expressed a small but significant pERK1/2 elevation in the centromedial amygdala that increased dramatically when the rats were later exposed to a novel environment. This could be evidence of heterotypic stressor sensitization, but it should be noted that Yang and his colleagues also noticed a similar pERK1/2 increase in the basolateral amygdala that was suddenly suppressed when the rats were transferred to the novel environment. In the present study, this was one of the brain areas that appeared to demonstrate sensitized ERK1/2 responding.

Stressor sensitization is usually accompanied by anxious behaviour that develops progressively and persists for hours, days, or even weeks after the initial stressor exposure (Adamec & Shallow, 1993; Adamec et al., 1998; Belda et al., 2008; Cohen et al., 1999; Cohen et al., 2000; Gagliano et al., 2008; Haley et al., 1999; Klenerová et al., 2006; Koolhaas et al., 1990; Mitra et al., 2005; Richter-Levin, 1998; van Dijken, Tilders, et al., 1992; van Dijken, van der Heyden, et al., 1992). In the present study, no significant behavioural changes were observed as the period after stress lengthened. However, behavioural deficits were evident when rats were tested and exposed to noise simultaneously. Physiological signs of stress were also apparent throughout the study, regardless of when the behavioural deficits were observable. This suggests that the mechanism through which noise affected spatial working memory was not related to stress-induced corticosterone elevations or ERK1/2 activity.

Cohen (1978) suggested that the presence of a stressor can create demands on attentional capacity because an organism is compelled to monitor and interpret the stimulus or event. These demands increase with the intensity of the stressor, and the result is a decrement in task performance. Thus, the simultaneous presentation of noise may have simply distracted our rats from the task. Driskell et al. (2008) suggested that familiarity and predictability can reduce the attentional demands imposed by a stressor, which could explain why rodent performance was only impaired for the first few minutes of testing. Presumably, attentional demands are also reduced when the stressor is temporally separated from the task. However, a number of studies have shown that performance on a spatial working memory task declines when a stressor immediately precedes testing (Bats et al., 2001; Cerqueira et al., 2007; Diamond et al., 1996; Diamond et al., 1999; Woodson et al., 2003).

The noise stressor used in the present study might have had a significant behavioural effect that was simply masked by our scoring procedures. When rats had finished exploring the cross-maze, an experimenter reviewed the video data and noted the number and sequence of arm entries. The total number of arm entries per minute was taken as an index of locomotor activity, while the sequence of arm entries was used to determine an alternation score. The alternation score served as an index of spatial working memory and expressed a relationship between the number of alternations that were performed and the number of alternations that were possible. However, the number of alternations that were possible heavily depended on the number of arm entries that were performed, and the mathematical relationship between score and entries probably introduced a significant amount of extraneous variability into the statistical analyses. For

example, significant effects on behaviour were observed during the first minute of testing when most rats had completed only 3-5 entries. Because one alternation required a non-repetitive sequence of *four* arm entries, many rats received extreme alternation scores (i.e. 0 or 100%) in this first minute. This variability could have masked significant differences in performance.

It is also possible that behavioural effects existed but were not measurable using this particular spatial memory task. Maze tasks differ on many dimensions, and the spatial abilities measured by one procedure may not resemble those engaged by another (Hodges, 1996). Some evidence suggests that the capacity for stress to impair spatial working memory is influenced by the difficulty of the task used in the study. For example, Diamond et al. (1999) trained rats to locate a submerged platform in a radial arm water maze. On each testing day, the platform was pseudorandomly placed in a different arm of the maze, and each rat was given five trials to learn its location. After the fourth trial, each rat was exposed to a cat for 30 min and then returned to the maze. The rat had to remember the platform's location after the stressful experience with the cat. Stress had no effect on spatial working memory in the easiest version of the task, when rats were allowed to stand on a platform in the centre of the maze and examine the apparatus before each trial began. When this observation period was eliminated, stress impaired working memory, but the rats' performance recovered after only two days. When the experiment was repeated with a significantly more difficult, six-arm version of the maze, cat exposure impaired rodent spatial working memory for at least six days.

The present study measured spatial working memory using a simple spontaneous alternation task. Spontaneous alternation is a natural tendency, and most (if not all) rats

will alternate without any prior training or extrinsic reinforcement (Dember & Fowler, 1958; Lalonde, 2002; Olton & Samuelson, 1976). In our version of this task, rats freely explored a cross-maze and were never challenged to remember any specific location for more than a few seconds. Most spatial working memory paradigms are significantly more difficult, often challenging rats to remember their movements for much longer periods of time (Bats et al., 2001; Cerqueira et al., 2007; Diamond et al., 1996; Diamond et al., 1999; Woodson et al., 2003). For example, Bats et al. (2001) tested mice in a traditional spontaneous alternation task and found that exposure to a bright light stressor severely degraded performance. However, in their study, the retention interval between alternations was 10-24 min.

To draw firm conclusions about noise stress and spatial working memory, it may be necessary to replicate this study using a more demanding task. A number of researchers have trained animals to find food in a cross- or radial arm maze to examine learned behaviour instead of spontaneous behaviour. This procedure measures spatial working memory when the rodents are interrupted partway through the task and challenged to continue after a short delay (Diamond et al., 1996; Nagai et al., 2006). A T- or Y-maze could also be used in a traditional spontaneous alternation task, where rodents enter one arm of the maze and then, after a delay, restart and must choose to enter the other. In both paradigms, an experimenter generally records the number of incorrect or repetitive arm entries, and the difficulty of the task can be easily manipulated by lengthening the delay periods (Lalonde, 2002). Stress could be applied simultaneously with the task or at predetermined setback time intervals to replicate the rest period manipulations featured in the present study.

A replication should carefully consider sources of experimental and extraneous stress. We found evidence to suggest that 90 dBA of white noise failed to elicit a stress response, which is inconsistent with data from the literature. For this reason, a replication should compare the effects of noise with those of another established and reliable stressor. Researchers might also consider using other neuroendocrine measures of stress, including CRH, AVP, or ACTH. Corticosterone may only be useful when examining the effects of low- or moderate-intensity stressors because its levels can reach a maximum even when only low levels of ACTH have been released (Gagliano et al., 2007).

We also found strong evidence to suggest that transport and exposure to the testing area induced confounding stress. In the present study, 5 days of repeated exposure may have reduced the amount of stress elicited by the testing area; however, it should be noted that rats with this extra habituation still expressed elevated corticosterone levels, which suggests that a single week of habituation was unable to completely eliminate the extraneous effect. A similar 5-day habituation routine was unable to eliminate a corticosterone elevation that was probably due to transportation. A replication might consider housing animals in the testing area to prevent any effect of transport and novelty. Once these stressors are controlled, their influence can be properly manipulated and examined. This may be important because in the present study, transport and exposure to the testing chamber after an earlier experience with noise stress may have provoked heightened corticosterone and amygdalar ERK1/2 responses.

In summary, the white noise used in the present study detrimentally affected spatial working memory only when rats were tested and exposed to noise simultaneously. This deficit seemed to occur independent of corticosterone elevations or ERK1/2 activity,

and for this reason, we suspect that noise affected behaviour primarily as a distraction that consumed attentional resources. However, this conclusion is tenuous because our testing procedure included confounding stress and may have been a weak spatial memory challenge. Acute noise exposure may have also enhanced normal corticosterone and amygdalar ERK1/2 responses to transportation and novelty, but we observed none of the anxious behaviour that usually accompanies neuroendocrine sensitization. To our knowledge, enhanced ERK1/2 responding in the basolateral amygdala due to a single, short experience with stress has never been documented, and a replication or reversal of this effect could clarify the role of ERK1/2 activity in stressor sensitization. Sensitization in rats resembles posttraumatic stress disorder in humans (Armario et al., 2008; Martí et al., 2001; Richter-Levin, 1998; van Dijken et al., 1993; van Dijken, Tilders, et al., 1992; van Dijken, van der Heyden, et al., 1992), and a better understanding of the molecular mechanism behind the disorder could eventually lead to improved clinical treatments.

Acute stress can also desensitize neuroendocrine responding to later experiences with stress, but it is not known if ERK1/2 activity and cognitive performance are affected by this process. Desensitization is of interest because military and emergency service personnel are frequently exposed to stressors, and some training programs rely on preemptive exposure to help reduce or eliminate stress-induced performance degradation. However, the factors that favour the development of desensitization are not entirely clear, and as a result, training programs that include preemptive exposure to stress risk inducing sensitization. We have shown that exposure to white noise may sensitize physiological responses to stress in rats, demonstrating the plausibility of this effect. However, we found no evidence that sensitization affects spatial working memory. Future studies

should consider replicating our work with a more challenging task to determine if sensitization affects performance when the demands on cognition are increased.

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Table 1

Mean Trunk Blood Corticosterone Concentration (μg/dL) Immediately After a 10-min Exposure to Different Levels of White Noise

Noise exposure[a]	*M*	*SE*
Control	25.06	4.44
60 dBA	19.31	1.53
90 dBA	21.28	2.08

[a] $n = 8$ for each group.

Table 2

Mean pERK1/2 Cell Densities (cells/mm^2) Immediately After a 10-min Exposure to Different Levels of White Noise

Noise exposure[a]	*M*	*SE*
	CA1 region	
Control	120.33	5.58
60 dBA	113.59	6.86
90 dBA	113.74	10.38
	Dentate gyrus	
Control	54.47	2.86
60 dBA	50.79	3.20
90 dBA	53.35	3.16
	Basolateral amygdala	
Control	46.86	3.25
60 dBA	43.65	2.38
90 dBA	41.91	3.76

[a] $n = 8$ for each group.

Table 3

Mean Trunk Blood Corticosterone Concentration (μg/dL) 1, 4, or 24 hr After a 10-min Exposure to 90 dBA of White Noise

	M			*SE*		
Noise exposure[a]	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
Control	25.84_x	16.23_y	16.69_a	3.42	3.06	3.57
90 dBA	24.03	23.97	25.25_b	2.01	2.56	1.62

Note. A mean marked with a subscripted "a" differs significantly from a mean marked with a subscripted "b" at $p < .05$ in the simple effects analysis of a mixed ANOVA. A mean marked with a subscripted "x" differs significantly from a mean marked with a subscripted "y" at $p < .05$ in Tukey's HSD.

[a]$n = 8$ for each group.

Table 4

Mean pERK1/2 Cell Densities (cells/mm^2) 1, 4, or 24 hr After a 10-min Exposure to 90 dBA of White Noise

	M			*SE*			
Noise exposure	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr	n[a]
			CA1 region[b]				
Control	96.34	82.46_a	97.43	10.12	8.89	9.45	7
90 dBA	113.00	117.72_b	105.93	7.70	10.96	11.57	7
			Dentate gyrus				
Control	49.20	54.07	40.53	3.85	5.96	5.28	7
90 dBA	50.21	57.51	50.32	4.38	3.34	6.21	7
			Basolateral amygdala				
Control	50.38_x	48.46	38.90_{ya}	3.89	2.56	3.35	8
90 dBA	51.05	45.50	53.75_b	2.81	3.27	3.67	8

Note. A mean marked with a subscripted "a" differs significantly from a mean marked with a subscripted "b" at $p < .05$ in the simple effects analysis of a mixed ANOVA. A mean marked with a subscripted "x" differs significantly from a mean marked with a subscripted "y" at $p < .05$ in Tukey's HSD.

[a]The hippocampus of one animal was destroyed during processing. [b]The main effect of noise exposure is significant for this region of interest.

Table 5

Mean Trunk Blood Corticosterone Concentration (μg/dL) From Different No-Noise Control Groups After Different Handling Procedures

Control group	*M*	*SE*	*n*
	Experiment 1 handling[a]		
After testing phase	25.06_x	4.44	8
	Testing chamber handling		
Before testing phase	8.23	2.42	4
After testing phase	16.35_y	1.94	8
	No handling		
Routine vivarium activity	9.55_y	3.03	8
High vivarium activity	6.72	2.01	4

Note. A mean marked with a subscripted "x" differs significantly from a mean marked with a subscripted "y" at $p < .05$ in a mixed ANOVA when the comparison is planned *a priori*. Only a limited number of comparisons were performed. Note that the acceptable significance level is .03 according to the modified Bonferroni correction (Keppel, 1982).

[a]This control group data is reprinted from Table 1 to facilitate comparison.

Table 6

Mean pERK1/2 Cell Densities (cells/mm^2) in Two Different No-Noise Control Groups Immediately After a 10-min Testing Phase

Control group[a]	*M*	*SE*
	CA1 region	
Experiment 1 handling[b]	120.33	5.58
Testing chamber handling	112.73	9.57
	Dentate gyrus	
Experiment 1 handling[b]	54.47	2.86
Testing chamber handling	47.14	5.27
	Basolateral amygdala	
Experiment 1 handling[b]	46.86	3.25
Testing chamber handling	46.05	1.87

[a]$n = 8$ for each group. ERK1/2 was measured immediately after the testing phase in both cases. [b]This control group data is reprinted from Table 3 to facilitate comparison.

Figure Captions

Figure 1. Dilution series (primary antibody in PBS-TX) demonstrating pERK1/2 staining of a sample of the CA1 region of the hippocampus, including (A) a no-primary control, (B) a 1:50 dilution, (C) a 1:500 dilution, (D) a 1:5000 dilution, (E) a 1:50,000 dilution, and (F) a no-secondary control. Note that staining fades significantly at a 1:50,000 dilution.

Figure 2. Dilution series (primary antibody in PBS-TX) demonstrating pERK1/2 staining of a sample of the dentate gyrus, including (A) a no-primary control, (B) a 1:50 dilution, (C) a 1:500 dilution, (D) a 1:5000 dilution, (E) a 1:50,000 dilution, and (F) a no-secondary control. Note that staining fades significantly at a 1:50,000 dilution.

Figure 3. Dilution series (primary antibody in PBS-TX) demonstrating pERK1/2 staining of the basolateral amygdala, including (A) a no-primary control, (B) a 1:50 dilution, (C) a 1:500 dilution, (D) a 1:5000 dilution, (E) a 1:50,000 dilution, and (F) a no-secondary control. Note that staining fades significantly at a 1:50,000 dilution.

Figure 4. Mean number of arm entries (A) and mean alternation scores (B) over a 5-minute habituation phase and a 10-minute testing phase. Testing occurred with simultaneous exposure to 60 or 90 dBA of white noise. Alternation score for the noise-exposed groups starts very low and appears to recover within the first few minutes of testing. Error bars represent standard errors of the mean.

Figure 5. Mean behavioural measures during the habituation and testing phases, including latency of the first alternation (A), number of arm entries over the first (B) and second (C) minutes, and cumulative alternation score up to the first (C) and second (D) minutes. Testing occurred with simultaneous exposure to 60 or 90 dBA of white noise.

There was no simple effect of noise exposure in the habituation phase for any measure, but the control group outperformed the 60- and 90-dBA groups in the first minute of testing. No significant differences were detected in the second-minute data. Error bars represent standard errors of the mean. *$p < .01$ (compared to the control group in the testing phase).

Figure 6. Mean behavioural measures during the habituation and testing phases, including latency of the first alternation (A), number of arm entries over the first minute (B), and cumulative alternation score up to the first minute (C). Testing occurred 1, 4, and 24 hr after exposure to 90 dBA of white noise. Differences due to noise exposure or rest period were not indicated in the habituation or testing phase for latency or alternation score. However, the noise exposure and rest period had simple effects on the number of entries in the habituation phase. On average, rats assigned to the control groups completed fewer arm entries than rats assigned to the 90 dBA group ($p < .02$), and rats assigned to the 1 hr rest group generally completed fewer arm entries than rats assigned to the 4 or 24 hr rest groups ($p < .05$). Error bars represent standard errors of the mean.

Figure 7. Mean behavioural measures during the habituation and testing phases for rats that underwent different handling and habituation procedures. These measures included latency of the first alternation (A), number of arm entries over the first minute (B), and cumulative alternation score up to the first minute (C). Only rats that completed both the habituation and testing phases are featured. Experiment 1 handling data are reprinted from Figure 2 (control group) to facilitate comparison. The groups were statistically equivalent on all measures. Error bars represent standard errors of the mean.

Figure 1

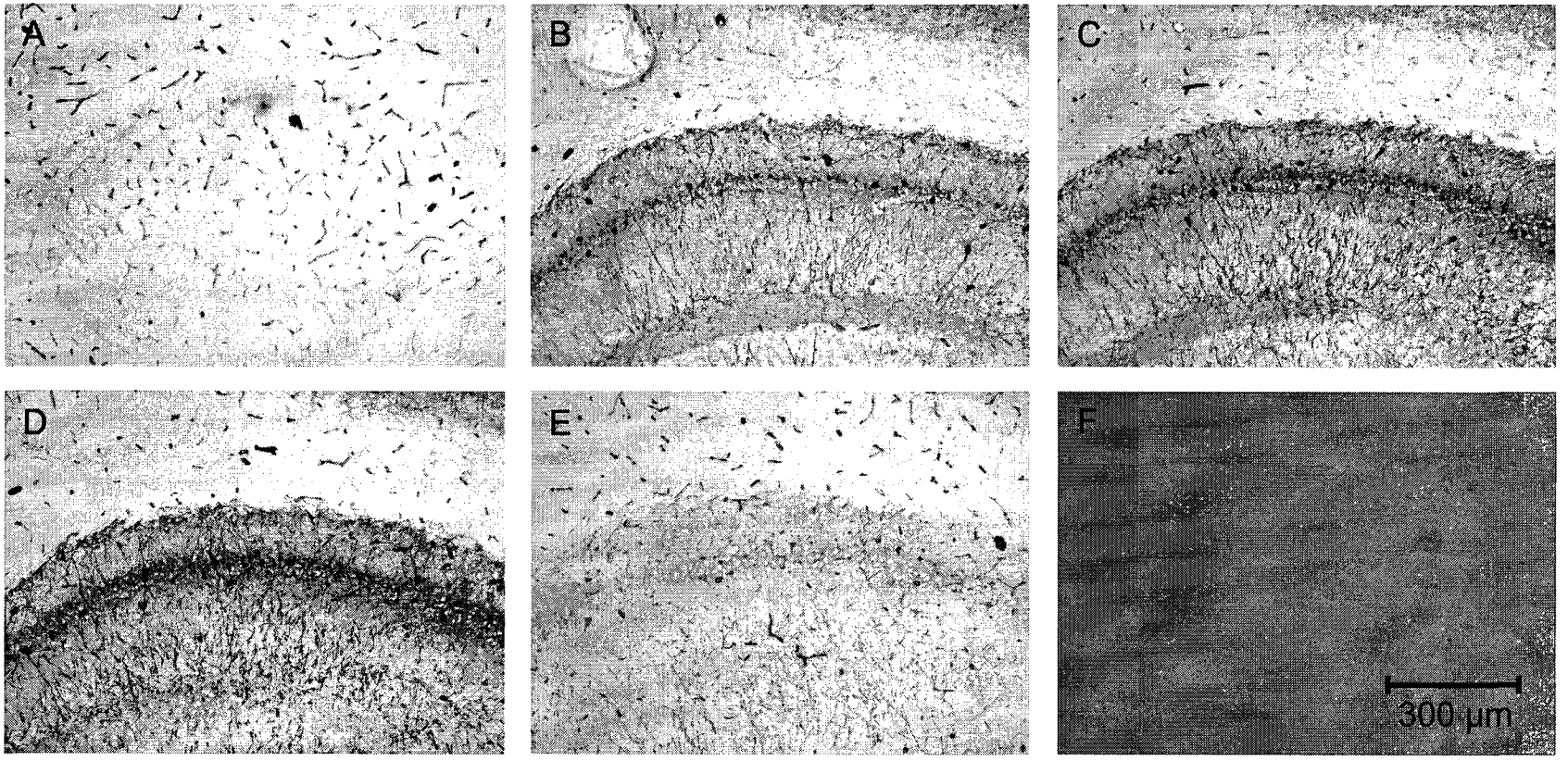


Figure 2

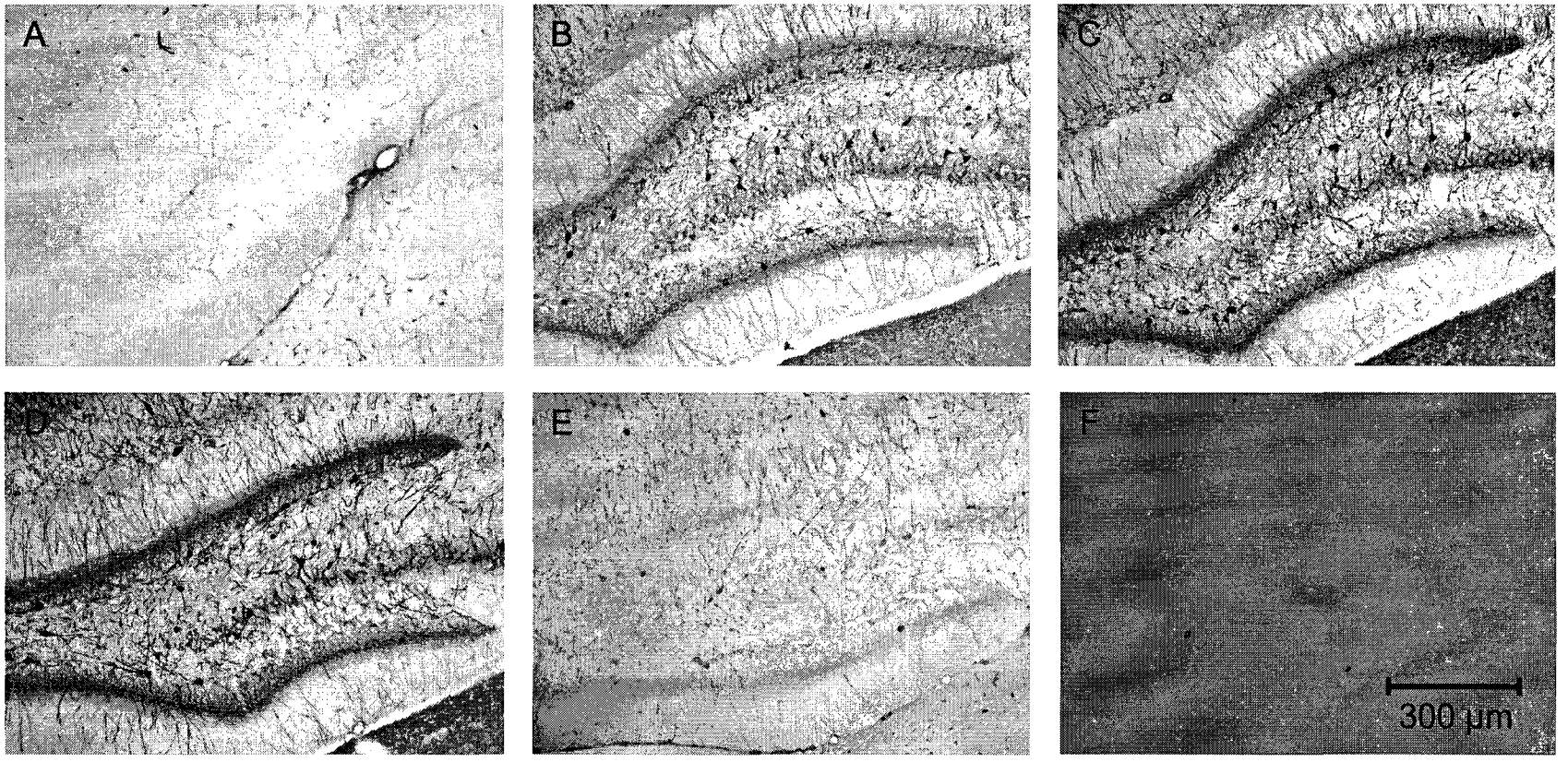


Figure 3

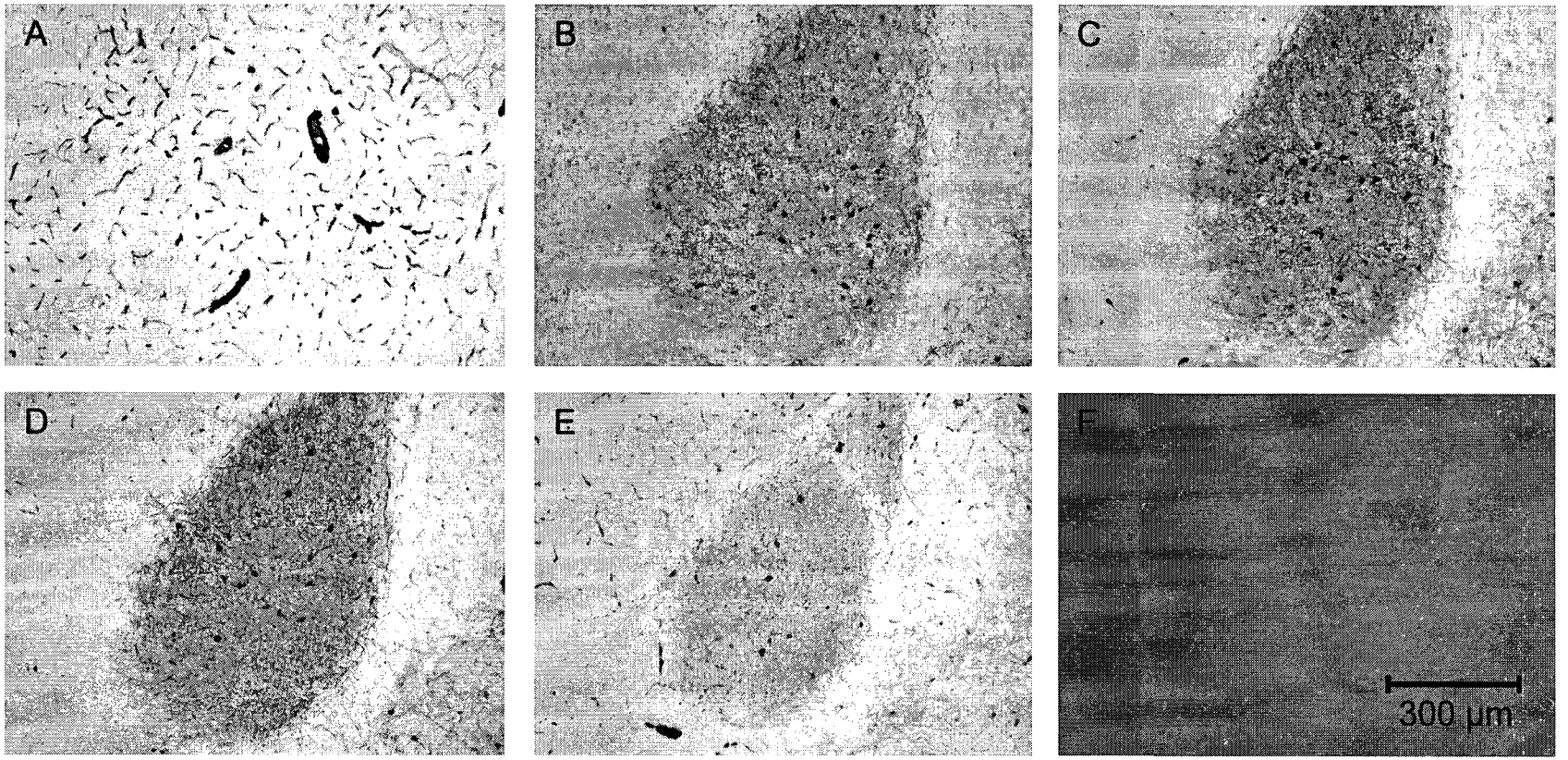


Figure 4

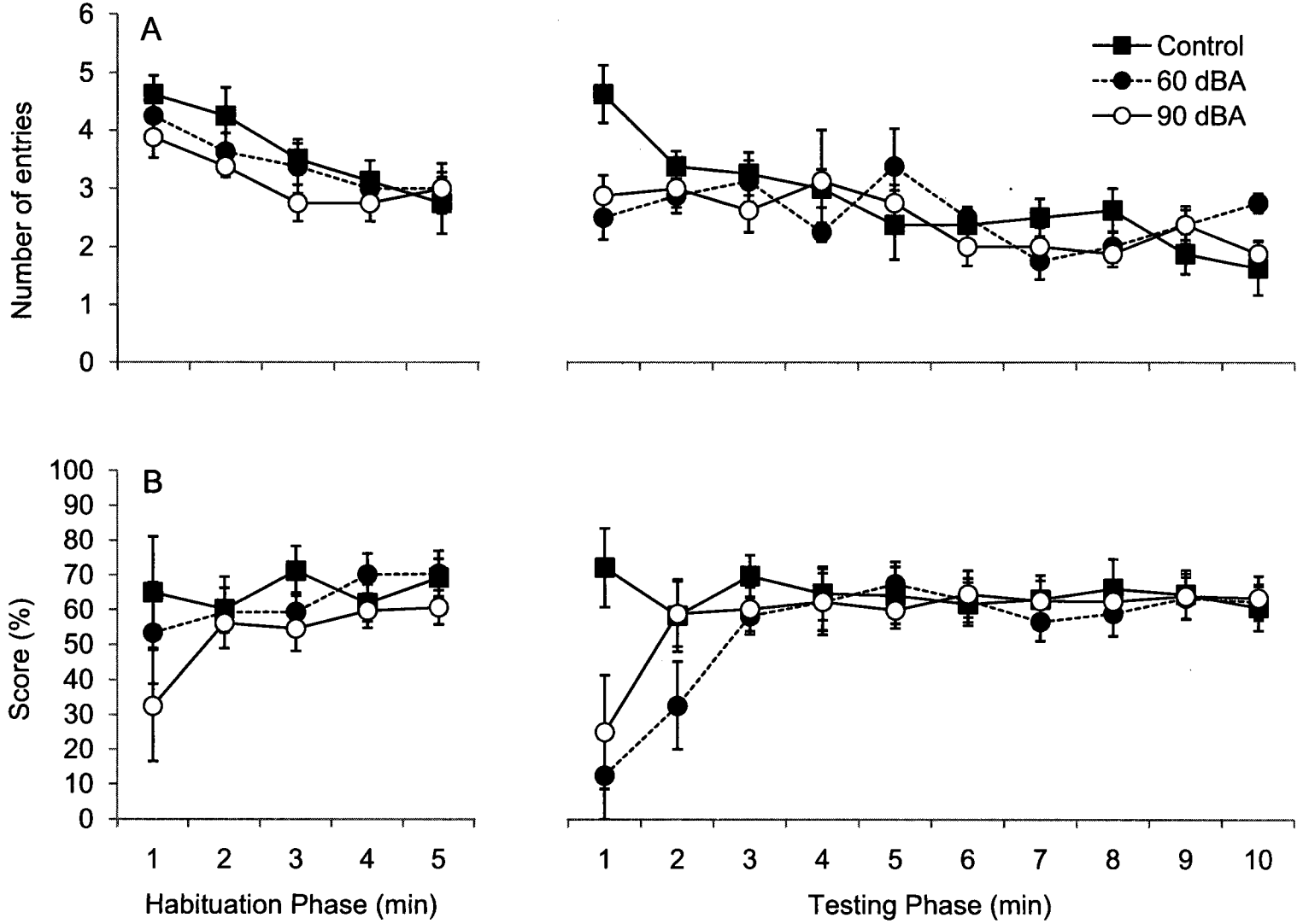

A
B
Number of entries
Score (%)
Control
60 dBA
90 dBA
Habituation Phase (min)
Testing Phase (min)

Figure 5

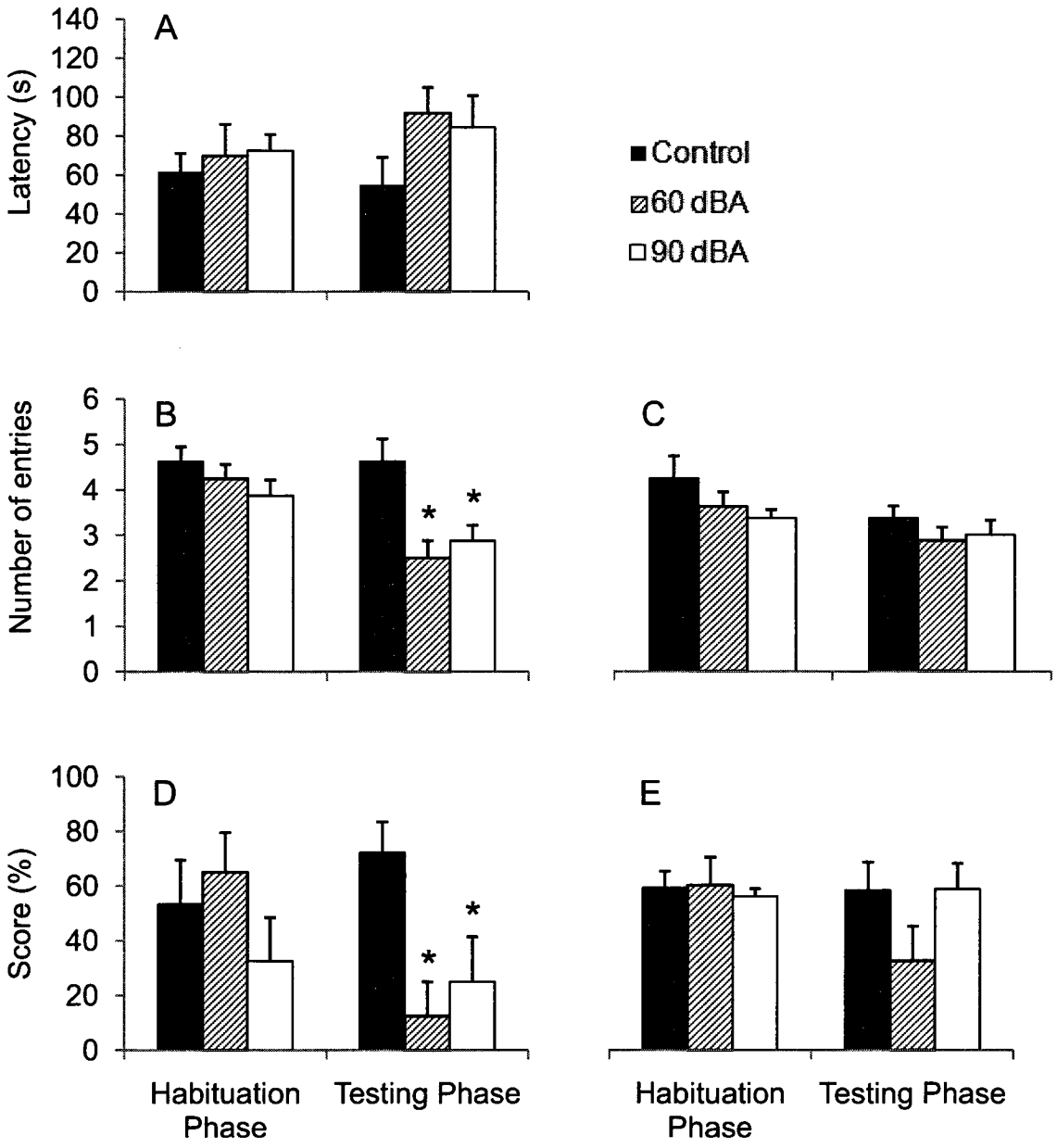

A
Latency (s)
140
120
100
80
60
40
20
0
Control
60 dBA
90 dBA
B
Number of entries
6
5
4
3
2
1
0
*
*
C
D
Score (%)
100
80
60
40
20
0
*
*
E
Habituation Phase
Testing Phase
Habituation Phase
Testing Phase

Figure 6

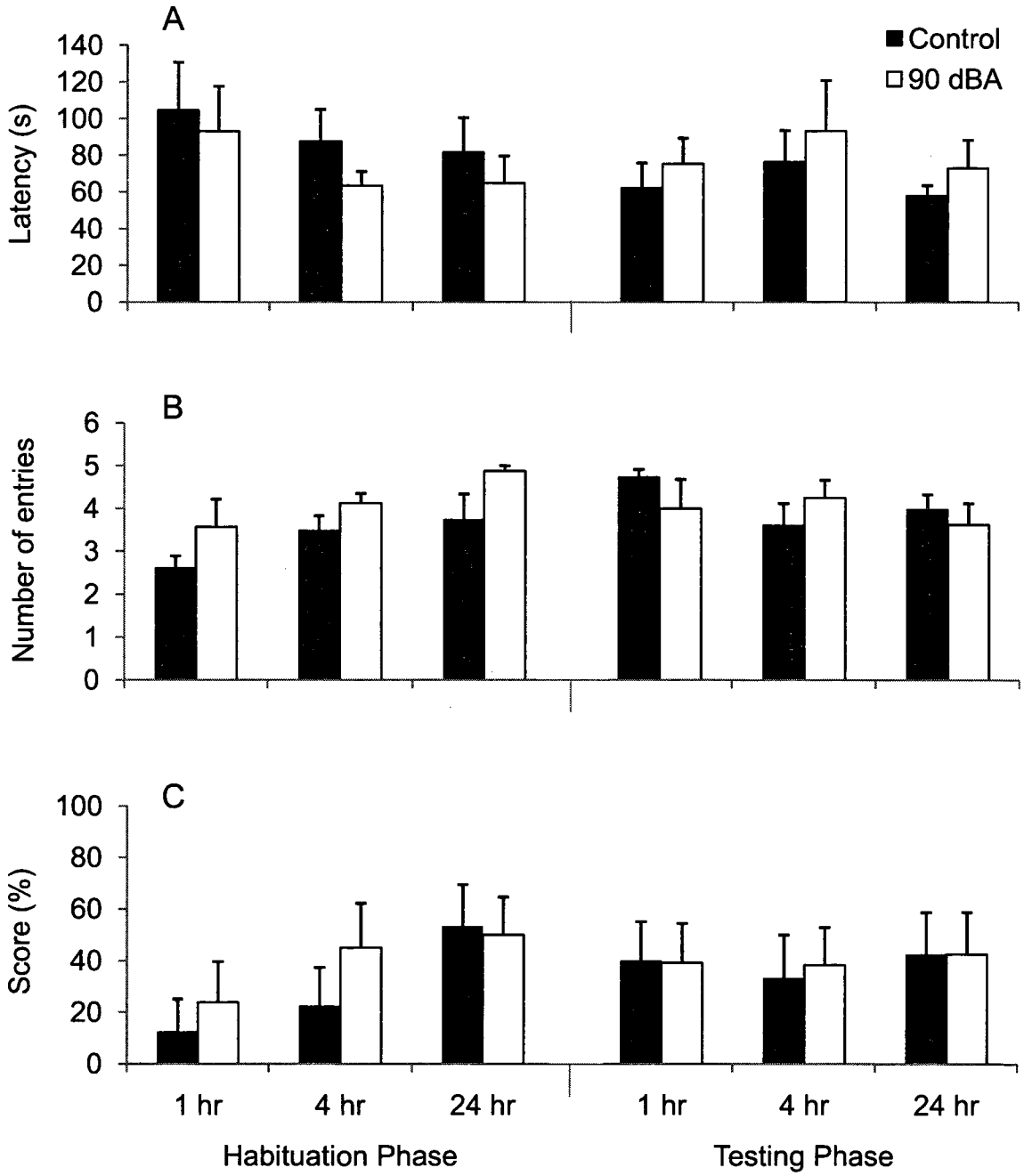
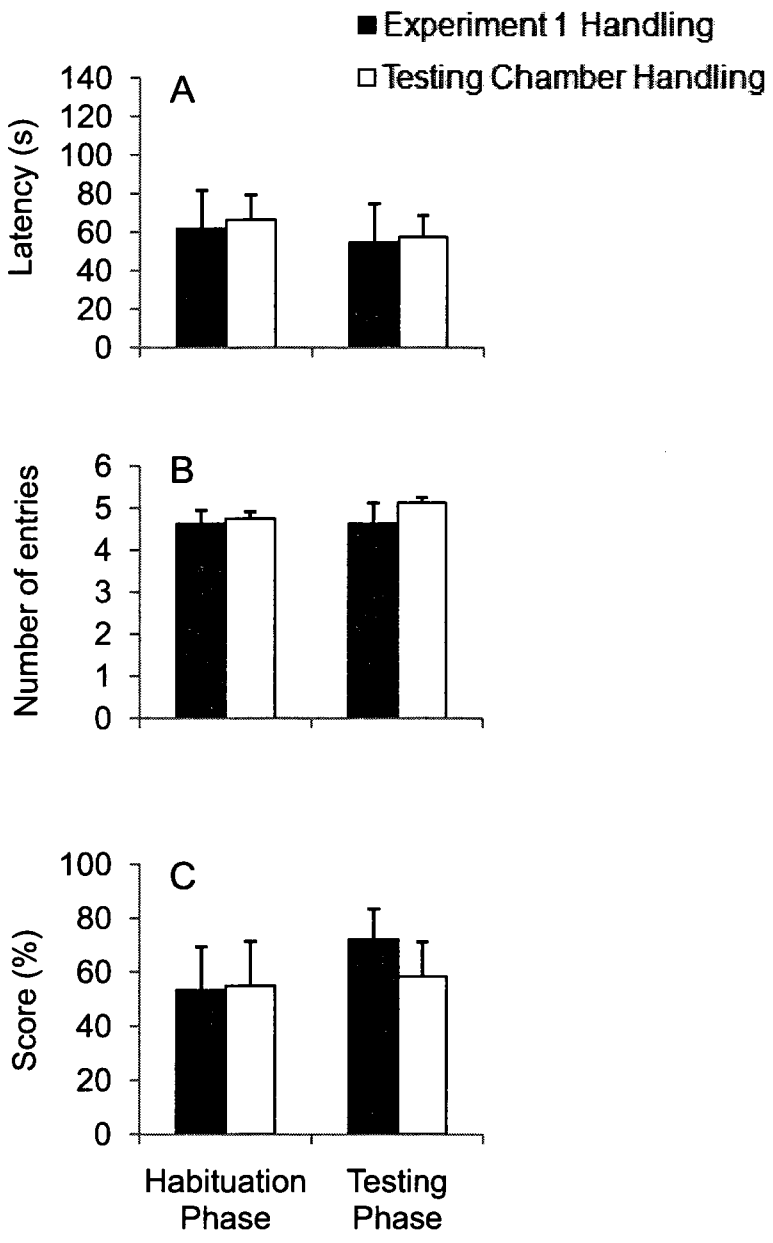

A
Control
90 dBA
Latency (s)
140
120
100
80
60
40
20
0
B
Number of entries
6
5
4
3
2
1
0
C
Score (%)
100
80
60
40
20
0
1 hr
4 hr
24 hr
1 hr
4 hr
24 hr
Habituation Phase
Testing Phase

Figure 7


Experiment 1 Handling
Testing Chamber Handling
A
Latency (s)
140
120
100
80
60
40
20
0
B
Number of entries
6
5
4
3
2
1
0
C
Score (%)
100
80
60
40
20
0
Habituation Phase
Testing Phase

Appendix A

CARLETON UNIVERSITY ANIMAL CARE COMMITTEE

APPLICATION FOR USE OF LIVE ANIMALS IN RESEARCH & TEACHING
- ANIMAL USE PROTOCOL -

PROTOCOL I.D.# P08-4

PART I

1. TITLE OF PROJECT

Phased Training Protects Spatial Working Memory from Noise Stress in Rodents

2. PRINCIPAL INVESTIGATOR

Name: Matthew Holahan
Position: Assistant Professor
Department & Phone #: Psychology / Neuroscience, x1543

3. ANIMALS (anticipated requirements)

Species	Strain	Age/Weight	Sex	Total No. for project
1. Rat (Rattus norvegicus)	Long-Evans	90 days	Male	260
2.			Male	
3.			Male	

4. CLASS OF ANIMAL USE & MAX. CATEGORY OF INVASIVENESS (according to CCAC)

Maximum Duration of Survival:	Max. Invasiveness (A-E)
Chronic ☐ Acute ☒	C

5. PROJECT PERIOD ANTICIPATED

From: May 2008 To: August 2009

6. DECLARATION

All animal manipulations described in this protocol will be carried out by trained and competent personnel. All animals in this project will be cared for and used in accordance with the principles of the Canadian Council on Animal Care stated in the "Guide to the Care and Use of Experimental Animals."

It is understood that the protocol number assigned to this submission will be used when ordering animals, and that these animals will be used only for the project described.

Principal Investigator **Date**

Appendix B

Cross-Maze and Chassis Schematics

Figure B1. Schematic diagram of the cross-maze including side (A) and top-down (B) views.

Figure B2. Schematic diagram of the metal chassis. Loudspeakers were suspended over the maze from the crossbars using screw-lock carabiners.

Figure B1

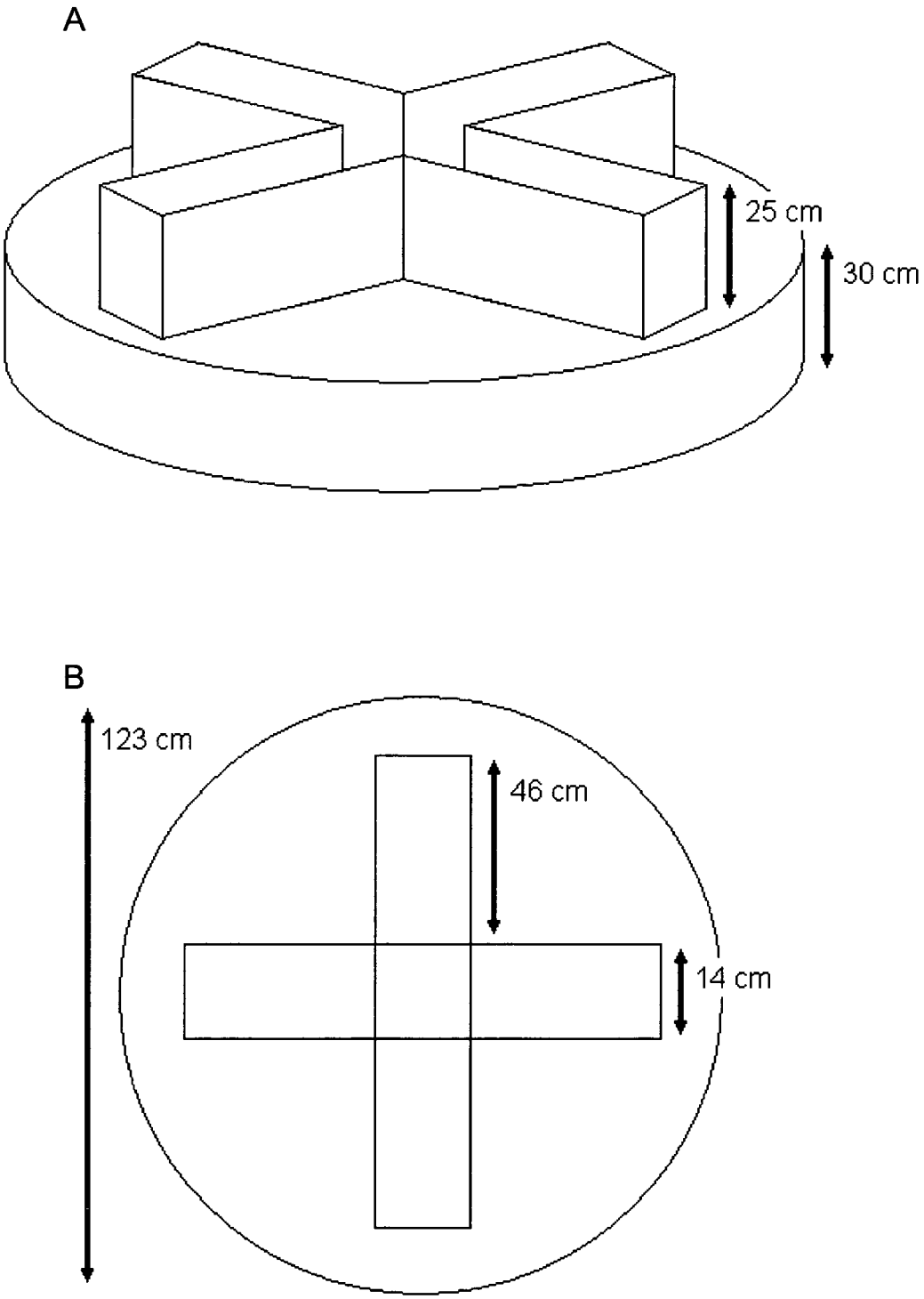
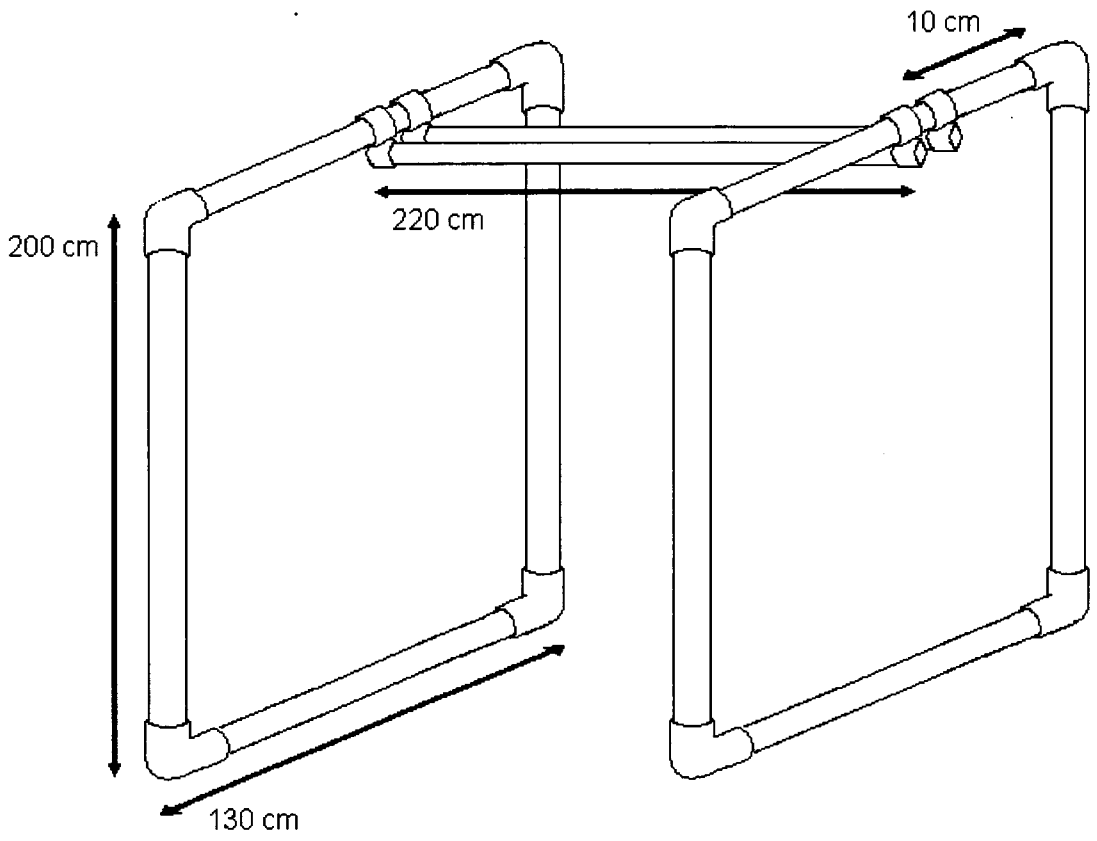


Figure B2



Appendix C

Noise Exposure Apparatus inside the Testing Chamber

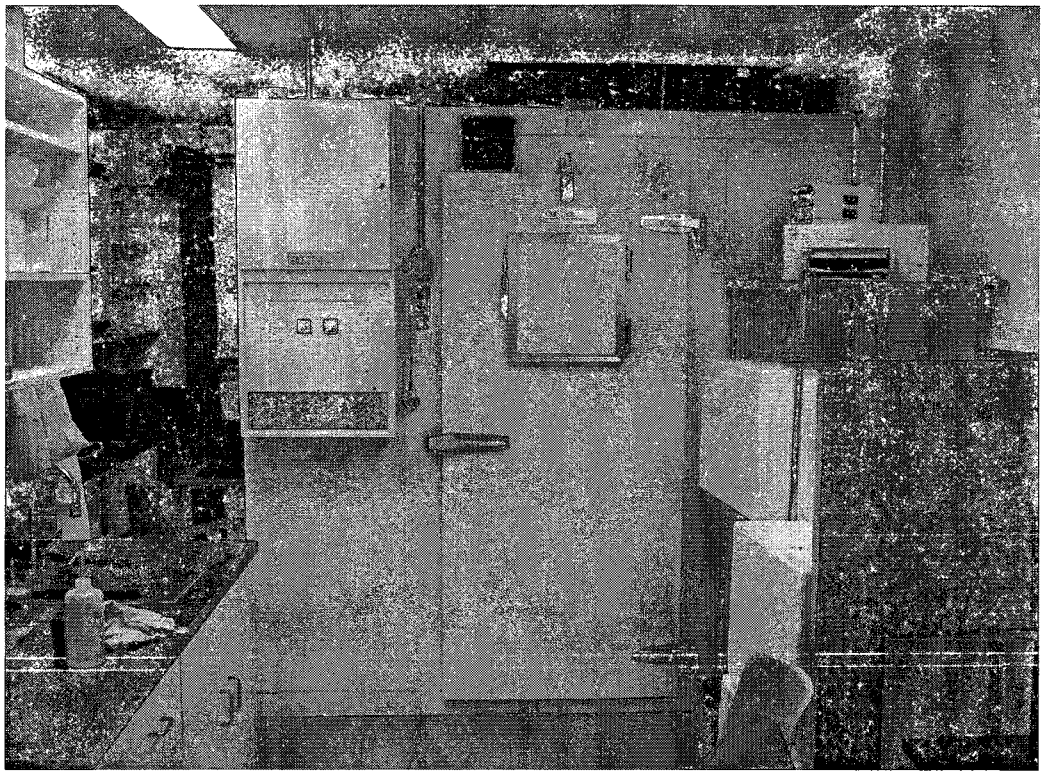
Figure C1. The testing chamber (A) and the data recording equipment (B) outside it. An experimenter activated the noise equipment and monitored each subject's response from a laptop outside the testing chamber. The chamber was a shutdown and defrosted walk-in freezer.

Figure C2. The loudspeakers and webcam suspended from the chassis, including side (A) and bottom-up (B) views. A single incandescent lamp provided light in the testing chamber, but was partially occluded by the loudspeakers.

Figure C3. Screw-lock carabiners (A) joined the loudspeakers to the crossbars of the chassis. The chassis was constructed by joining pieces of steel piping with elbow and crossover fittings (B).

Figure B1

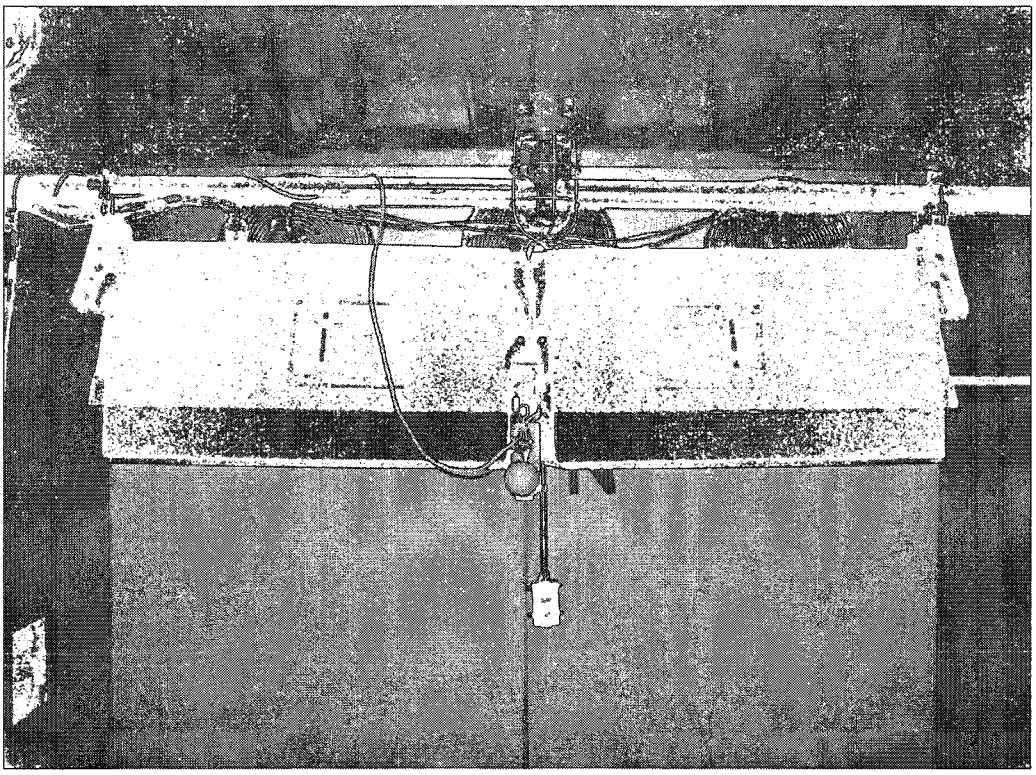
A



B

Figure B2

A



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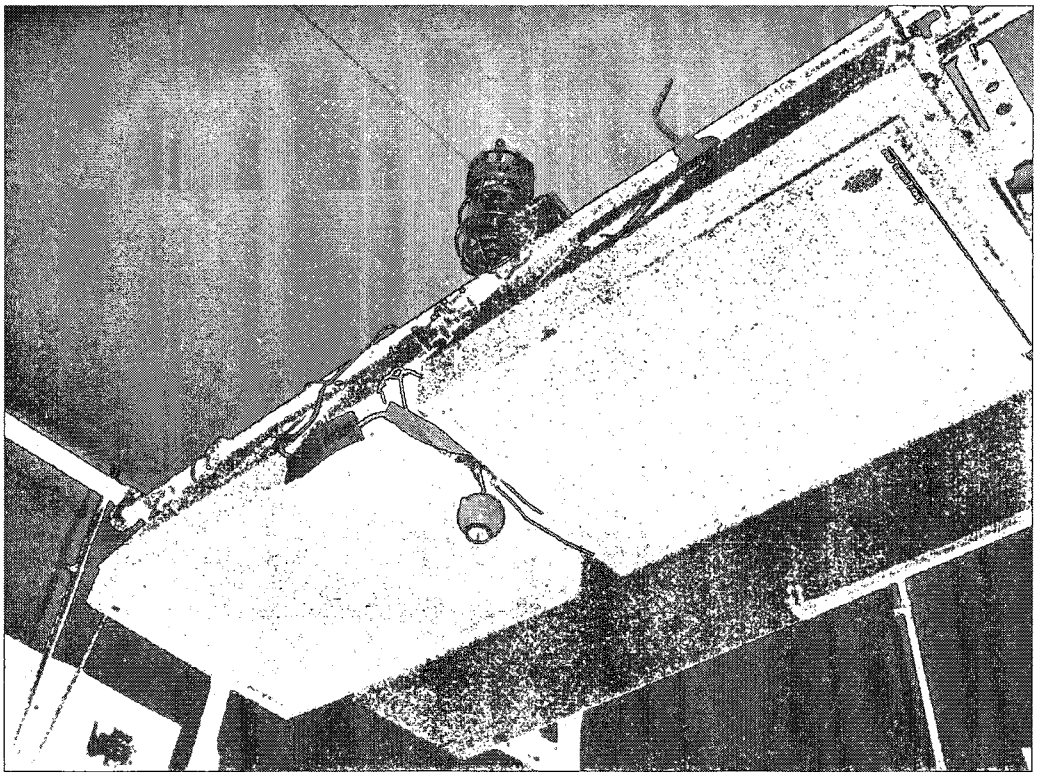
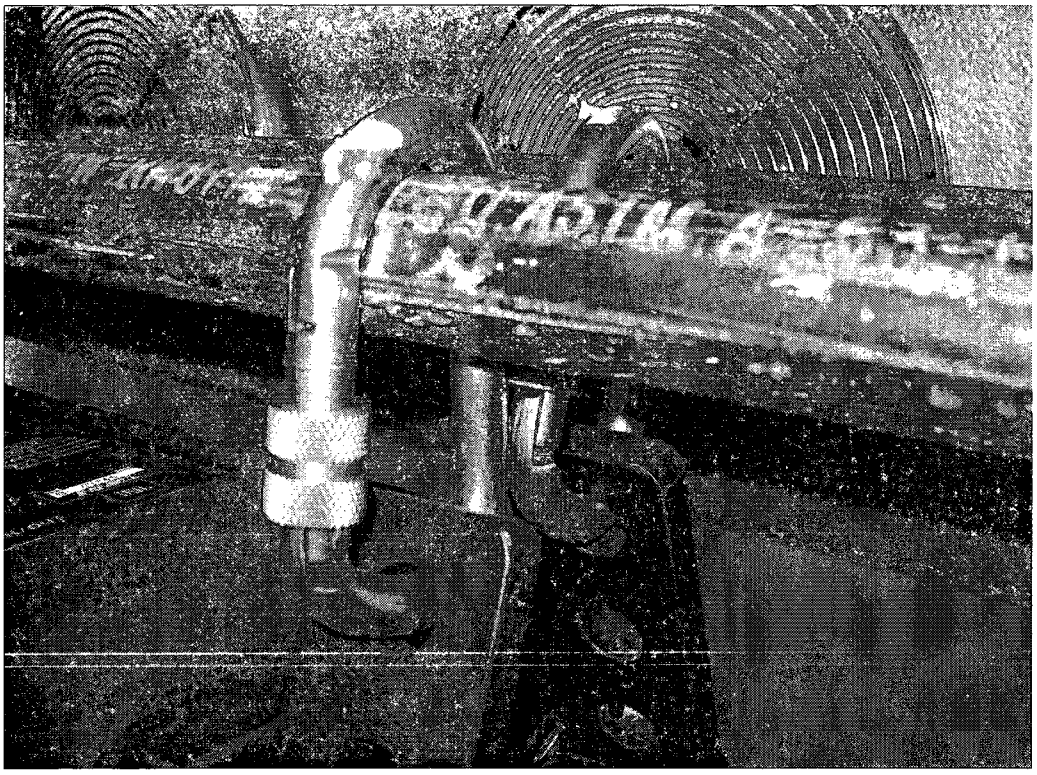
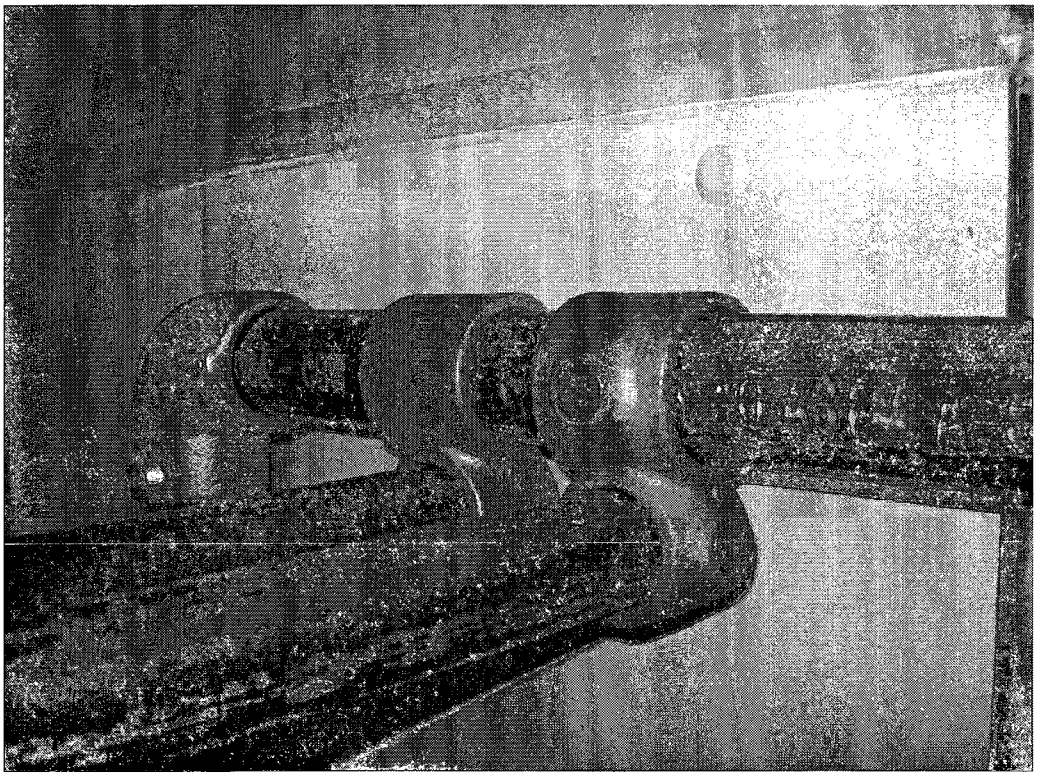


Figure B3

A



B



Appendix D

Supplementary Data

Figure D1. Mean number of arm entries (A) and mean alternation scores (B) over a 5-minute habituation phase and a 10-minute testing phase. Testing occurred 1 hr after exposure to 90 dBA of white noise. Error bars represent standard errors of the mean.

Figure D2. Mean number of arm entries (A) and mean alternation scores (B) over a 5-minute habituation phase and a 10-minute testing phase. Testing occurred 4 hr after exposure to 90 dBA of white noise. Error bars represent standard errors of the mean.

Figure D3. Mean number of arm entries (A) and mean alternation scores (B) over a 5-minute habituation phase and a 10-minute testing phase. Testing occurred 24 hr after exposure to 90 dBA of white noise. Error bars represent standard errors of the mean.

Figure D4. Mean number of arm entries (A) and mean alternation scores (B) over a 5-minute habituation phase and a 10-minute testing phase for rats that underwent different handling and habituation procedures. Error bars represent standard errors of the mean.

Figure D1

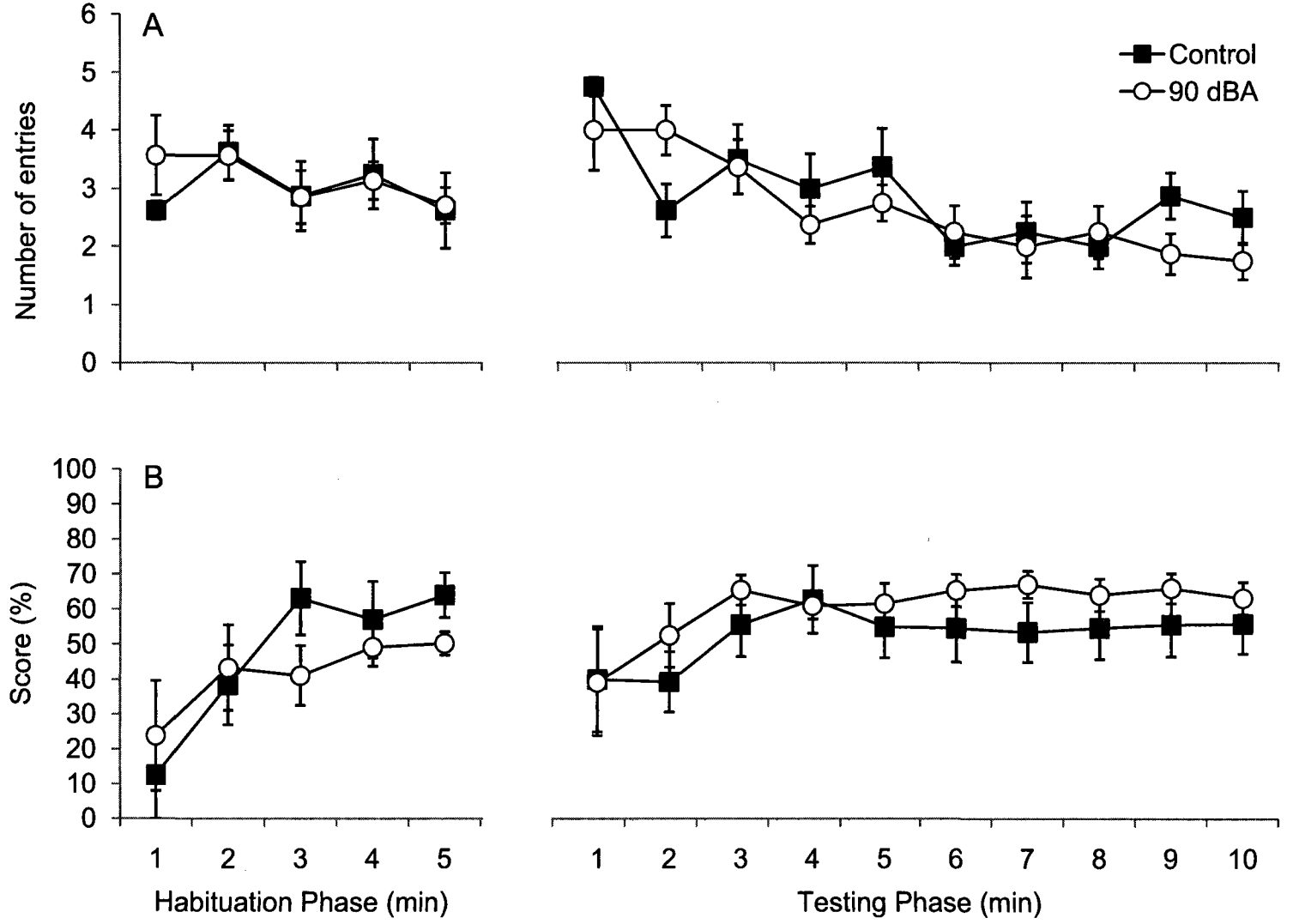

A
B
Control
90 dBA
Number of entries
Score (%)
Habituation Phase (min)
Testing Phase (min)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
100
7
8
9
10

Figure D2

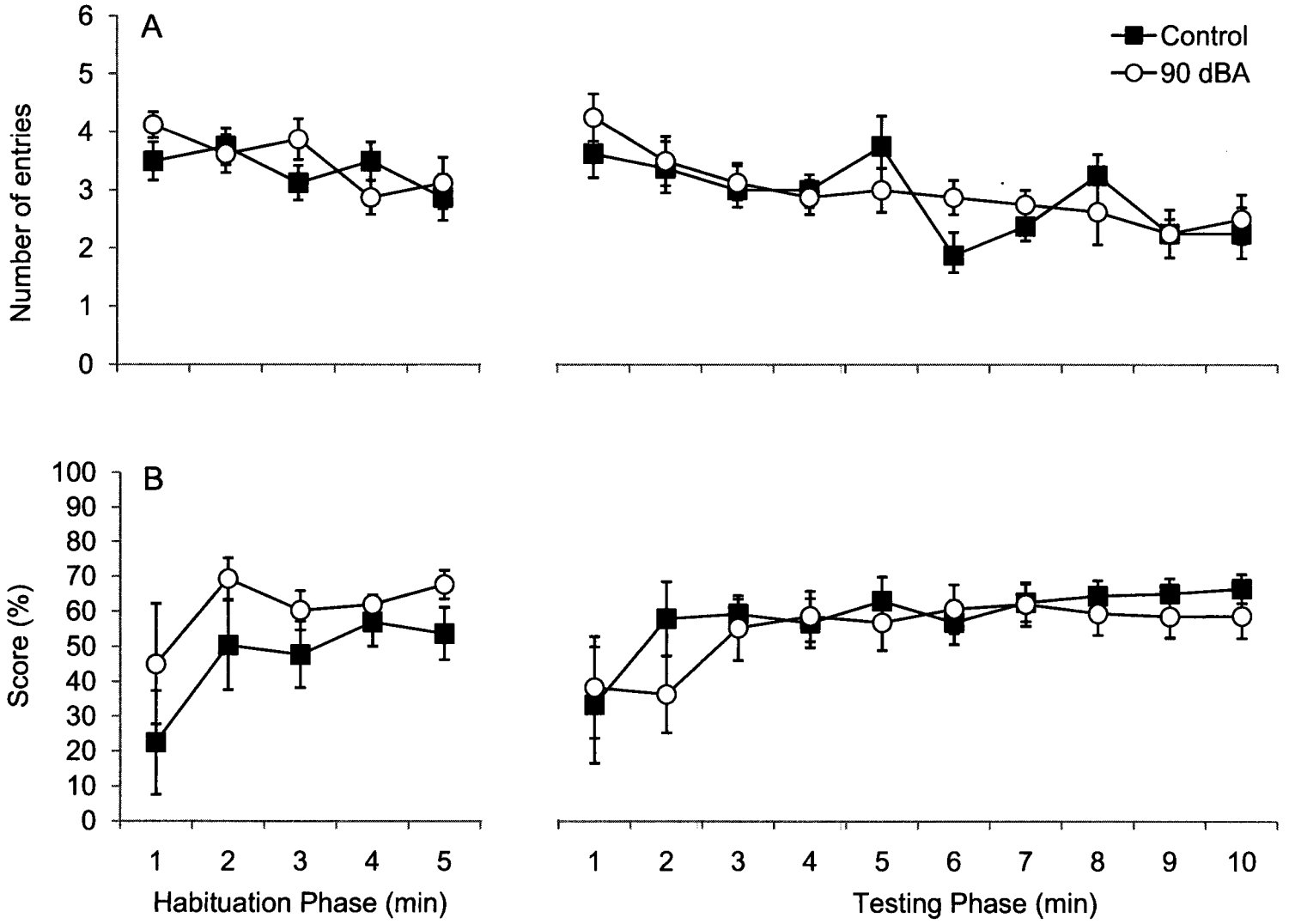

A
B
Control
90 dBA
Number of entries
Score (%)
Habituation Phase (min)
Testing Phase (min)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
100
7
8
9
10

Figure D3

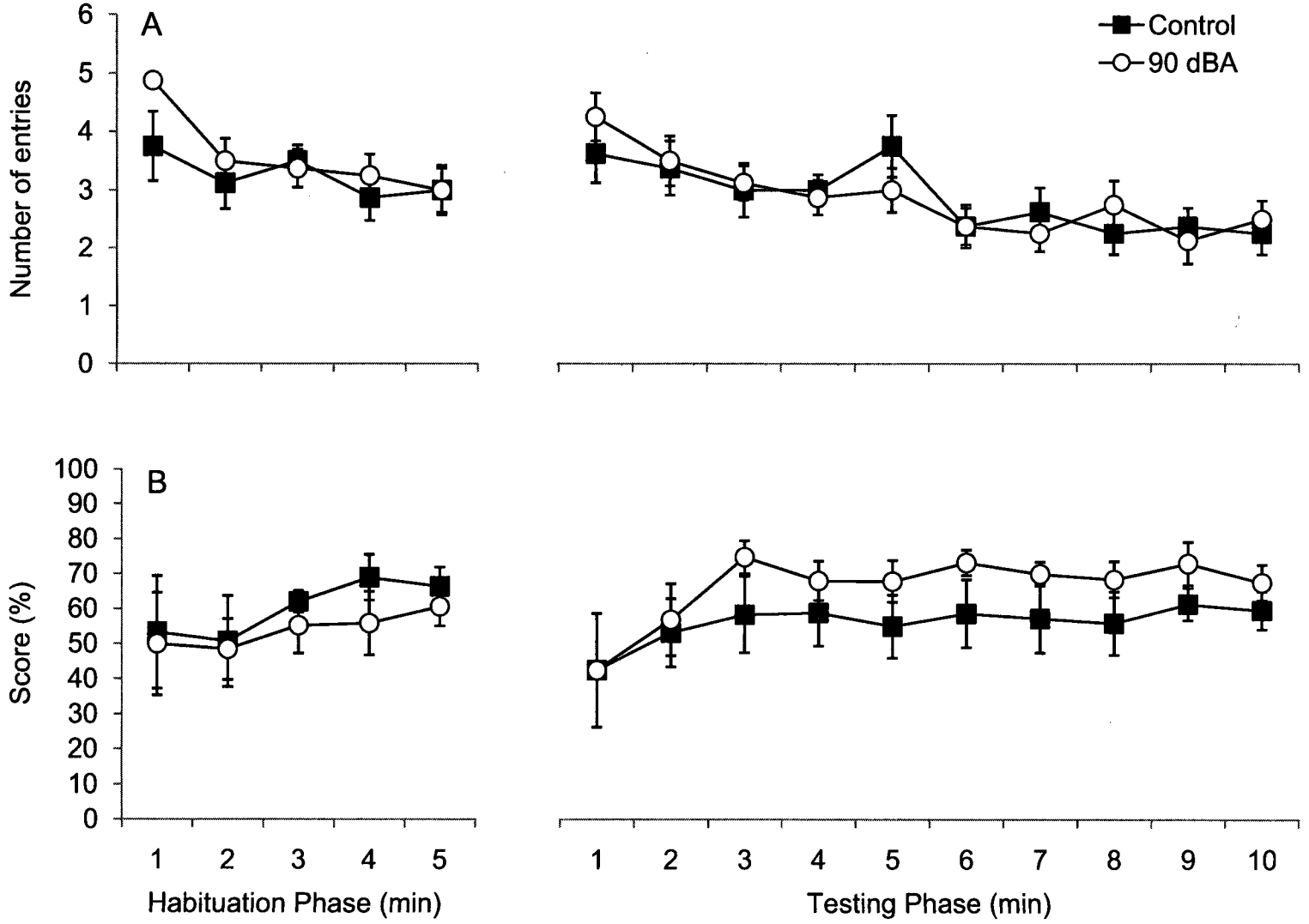
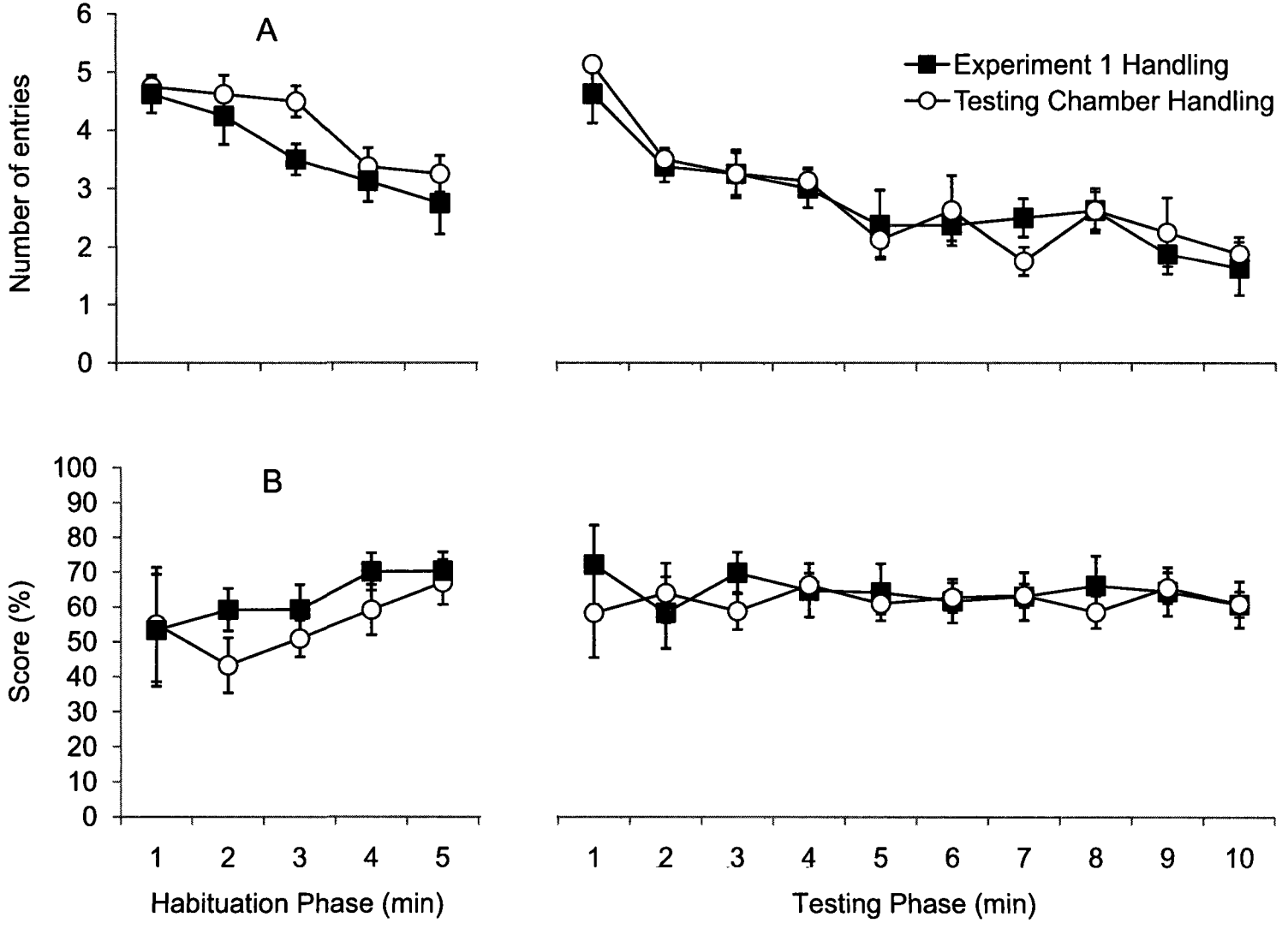

A
B
Control
90 dBA
Number of entries
Score (%)
Habituation Phase (min)
Testing Phase (min)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
100
7
8
9
10

Figure D4


A
B
Number of entries
Score (%)
Experiment 1 Handling
Testing Chamber Handling
Habituation Phase (min)
Testing Phase (min)