EFFECTS OF ANISOMYCIN, A PROTEIN SYNTHESIS INHIBITOR, ON DISRUPTING A FEAR MEMORY IN A PREDATOR STRESS SITUATION

KIRBY J. STRASSER

Effects of Anisomycin, a Protein Synthesis Inhibitor, on Disrupting a Fear Memory In a Predator Stress Situation

by

Kirby J. Strasser, B.A. (honours)

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

Master of Science

Department of Psychology

Memorial University of Newfoundland

2005

St. John's Newfoundland

Abstract

Posttraumatic stress disorder (PTSD) is an incurable psychological condition that develops as a result of being exposed to an extraordinary traumatic event. Many aspects of this affective disorder have been successfully initiated in animals through experimental shock, classical conditioning, pharmacological and predator stress procedures. This has led to the tentative hypothesis that all aspects of PTSD may be reduced to functional alterations in specific pre-existing neural nuclei or circuitry. The theory is that these presumed alterations follow the induction of long-term potentiation (LTP), a model of long-term memory, within the amygdala and related circuitry. This is based upon evidence that implicates the phosphorylation of CREB within the amygdala following predator stress. The hypothesized result of the phosphorylation of CREB is ultimately the synthesis of new protein. If protein synthesis is necessary in order to consolidate a predator stress (cat exposure) memory, then subcutaneous administration of anisomycin, a protein synthesis inhibitor, just after predator stress should prevent the memory from being formed. The effects of treatments were tested using various behavioural measures of rodent affect (i.e. hole-board, elevated plus-maze, light/dark box and social interaction tests; and, acoustic startle) 7-8 days post-exposure. Protein synthesis-dependent consolidation was demonstrated for open-arm exploration in the elevated plus-maze. A reconsolidation condition was added in order to probe whether or not a consolidated memory, once reactivated (i.e. exposed to a cat twice), was again susceptible to protein synthesis

inhibition. In this instance, anisomycin was given just after the second cat exposure. For some tests (elevated plus-maze) there was no evidence for protein synthesis-dependent reconsolidation. The results were less clear for the other tests. Due to the effects of vehicle injection, i.c.v. administration of anisomycin in future work may clarify the role of protein synthesis in reconsolidation.

Acknowledgements

I am certain that my degree from MUN will provide me with many interesting career opportunities in the near and distant future. Yet at any given point along this imaginary arc, I know now that I will always possess the fine sense to trace my good fortune back to a Science man taking a wild risk on a toddler of the Arts. I am truly grateful that Professor Adamec (and his proteins) elected to gamble so. He, along with Dr. J. Evans, spent many hours with me in a heroic attempt to help me understand the various applications of statistics to neuroscience. With, I am sure, divine patience; neither one at any time showed any visible signs of strain. I thought that was quite wonderful, really.

I would like to thank Paul, Jackie and David for helping to collect my data. I would especially like to thank Paul for keeping the ship afloat in general, and for having such phenomenal Zen-like patience with such an amateur as myself.

I would like to thank Dr. C. Harley and Dr. C. Malsbury for agreeing to sit on my Supervisory Committee, and for offering such fresh insight into my research question.

I would like to thank my father for his tender love, (cash!), and his great faith in such a tiny fellow to grow robust cucumbers. Indeed, the Cosmic Powers That Be have been especially kind to me in providing two such wonderful role models in him and in my older brother, Andrew.

Alas, on a quite necessary final note, I would like to thank my dear mummy and Mr. R. Dylan for generously sharing their timeless pearls of wisdom. At long last I am finally beginning to realize that the answer really is blowing in the wind…

Table of Contents

List of Figures

Effects of Anisomycin, a Protein Synthesis Inhibitor, on Disrupting a Fear Memory In a Predator-Stress Situation

Posttraumatic Stress Disorder (PTSD), defined by the Diagnostic and Statistical Manual (fourth edition), is a mental disorder that affects people who have been exposed to a perceived traumatic event. In the face of threats of terrorism, there is a growing need to find reasonable preclinical animal models of this incurable disorder. An animal model, to aid in pharmacological testing, must model some or all aspects of PTSD. To evaluate animal models requires a sense of the disorder. There are six criteria of PTSD: (A) the person has experienced a traumatic event; (B) the traumatic event is re-experienced in at least two of the following ways: intrusive memories invade consciousness; dreams, whereby the plot involves reliving the trauma; have a sense or feeling that the traumatic event is reoccurring; experiencing psychological anguish and or physiological arousal upon exposure to internal or external cues that are subjectively associated with the trauma; (C) a numbing of emotional experience; (D) the individual continuously experiences abnormal levels of arousal relative to his or her pre-trauma condition; (E) items B, C and D persist for at least one month plus a day; (F) the disturbances noted above cause subjectively significant distress and interfere with work and personal life. PTSD is subdivided into two categories: acute PTSD lasts for less than three months while chronic PTSD endures for a period greater than three months. A secondary consideration is if the symptoms came into being immediately after the trauma or if PTSD occurred with delayed onset, defined as the appearance of symptoms at a period of (at least) 3 months post-trauma (American Psychiatric Association, 2000). Presumed

neurological differences underlie the different periods of onset in this subset, which would inevitably require differing therapeutic interventions. It is possible, however, that there are essential neurological changes in the development of the disorder that are common to all types of PTSD.

Through the Neurological Looking Glass - A Potential Reduction of PTSD to an Altered Noradrenergic-Dependent Memory Network

The release of norepinephrine in the amygdala modulates the consolidation of fear memories (Liang, Chen, & Huang, 1995). Administering *propranolol*, a β-noradrenergic receptor blocker, to PTSD victims within a short period of time post-trauma attenuates PTSD symptom severity (Pitman, Sanders, Zusman, Healy, Cheema, Lasko, Cahill, & Orr, 2002; Vaiva, Ducrocq, Jezequel, Averland, Lestavel, Brunet, & Marmar, 2003). Complementing this research, Elzinger and Bremner (2002) have proposed that the various threads of PTSD may be reduced to an altered memory network. They propose that dysfunction in the hippocampus produces a decline in declarative memory that explains the trauma-related amnesia. Altered noradrenergic function in the amygdala increases sensitization to emotional stimuli, which enhances the subjective import of the traumatic memories. Dysfunction in the prefrontal cortex causes a decline in working memory, which produces deficits in concentration and an inability to inhibit cognitions not pertaining to an immediate task. This contributes to a decrease in its ability to inhibit emotional responses. This increases the number of intrusions and "flashbacks" and adds to the by now increased sensitization of the amygdala and its

consequent propensity for conditioning. Emotional sensitization symptoms associated with PTSD may be created experimentally in animal models, which provides an opportunity for understanding aetiology and developing various treatments.

Animal Models of PTSD

There are several potential animal models of PTSD.

Inescapable Shock Model

The inescapable shock model involves placing an animal in an enclosed environment with no opportunities to escape. The shock is traditionally delivered through a wire grid on the floor of the chamber. The rat will initially attempt to escape. Over the passage of time the rat, presumably sensing that the punishment is unavoidable, will consistently display a number of characteristics, including impaired cognition, signs of emotional discomfort and lethargy (Rosen & Fields, 1988). Enduring inescapable shock for lengthy periods will result in increased plasma catecholamine levels, increased norepinephrine turnover, a relatively low supply of central norepinephrine and increased production of MHPG (Van der Kolk, Greenberg, Boyd, & Krystal, 1985). There are two striking objections raised by Yehuda and Antelman (1993) that challenge the wisdom of using inescapable shock as a model for PTSD. The effects of inescapable shock on the rat are gradual in nature. It cannot easily account for the fact that PTSD may manifest itself in people after a delay of three months or more post-trauma. Nor can it easily explain how PTSD may develop after a single, intense exposure.

Classical Conditioning Model

The classical conditioning paradigm was developed by Pavlov (1927) in order to provide an explanation as to how a previously neutral stimulus (conditioned stimulus) may assume the psychic properties of an unconditioned stimulus to yield an unconditioned response. Applying this concept to the development of PTSD, it must be taken as a given that an exposure to a traumatic event yields an innate anxious response. An association may then be assumed to have developed between the innate anxious state and the numerous quite arbitrary cues present at the time and place of the trauma (Kolb & Multalipassi, 1982). This model shares a striking criticism with the inescapable shock model. When Pavlov conditioned a dog to salivate upon hearing the activation of a tone, the response on the part of the dog was immediate. If PTSD victims are indeed classically conditioned to cues present at the time of the trauma, then the anxious response to those cues post-trauma should be immediate. However, this is not the case, as there clearly is evidence for a sub-population of victims who have delayed-onset PTSD (Shalev, 1993). As in the case of the inescapable shock model, the effects of classical conditioning may aid in illuminating certain aspects of the disorder, but ultimately it fails to encompass the phenomena of PTSD in its entirety.

Pharmacological and Predator Stress Model

There are certain brain structures, particularly limbic structures, which are associated with well-defined emotional states. Emotive biasing, generally stated, involves the sensitization of a particular (limbic) structure, with the result being an enhanced response to normal inputs which persists for a period of time long after the stimulation

has ceased to be applied (Adamec, 1978). Adamec (1994) found that this result might be achieved pharmacologically in cats by administering the anxiogenic compound (or inverse benzodiazepine agonist) FG-7142 (N-methyl-beta-carboline-3-carboxyamide). The same circuitry sensitized by chemical and electrical stimulation is also sensitized by carefully engineering a predator stress exposure for a rat. What is lost in specificity is gained by a more naturalistic, immediate sensitization of emotional circuitry. This is the foremost consideration that places the predator stress model on a richer plane - sensitization is invoked via the exposure of the animal to a natural, species-relevant trauma. The inclusion of this crucial external component should provide a more realistic view of the neuronal changes taking place, as the nature of the external stimulus would more closely parallel those encountered in the normal development of PTSD. Researchers in the field have endorsed this view, claiming that emotive biasing does approximate the emotional disturbances found in those individuals suffering from PTSD (Pitman, Orr, & Shalev, 1993).

Long-Term Potentiation

Long-term changes in behaviour following predator stress likely persist as a result of some reorganization of pre-existing neural connections. One such mechanism is long-term potentiation (LTP). Originally conceptualized by Bliss and Lomo in 1973 to explain long-term changes in excitability in the hippocampus, LTP has up until the present day

retained the description of a mechanism that produces a long-lasting increase in synaptic efficacy by way of high-frequency stimulation of afferent fibres.

The paradigm of *associativity* is often evoked to explain the fundamental requirements of LTP induction. Excitation of a neuronal pathway, associated with a representation of the conditioned stimulus, produces posttetanic potentiation (PTP) that may last for a number of minutes (Chapman, Kairiss, Keenan, & Brown, 1990). This action (e.g. a brief train of pulses, 20 ms duration at 100-400HZ), facilitates the release of neurotransmitter (glutamate), but alone cannot easily produce activation of NMDA receptors on the post-synaptic cell (Maren, 1996). The pre-synaptic release of glutamate must be accompanied by a post-synaptic depolarization in the same pathway (via a repetition of PTP) that causes the removal of magnesium from ion channels. This allows the influx of calcium, which is crucial to LTP induction. Prolonged excitation of the perforant pathway eventually leads to intra-cellular genomic activation in the post-synaptic neuron which, in turn, encourages an increase in the number of active synapses and a synaptic restructuring from, as an example, non-perforated, relatively undifferentiated synapses to perforated, fully partitioned ones that have segmented post-synaptic density zones (Geinisman, de Toledo-Morrell, Morrell, Heller, Rossi, & Parshall, 1993). A unique contribution of these mechanisms is a long-lasting facilitation in communication between neurons that lasts, on average, 1.4 hours (early phase) to a late phase lasting from 5.1 days (Racine, Milgram, & Hafner, 1983) to one year (Abraham, 2003).

Long-Term Potentiation in the Amygdala

The existence of LTP has been established in the amygdala (Chapman & Bellavance, 1992; Watanabe, Ikegaya, Saito, & Abe, 1995). It can also be induced experimentally via seizure activity (Adamec, 1998). Dividing the thirty-five sub-units of the amygdala into functional polarities produces an image of sensory convergence in the basolateral nucleus and behavioural divergence in the central nucleus (Maren, 1996). The central nucleus, lacking intra-amygdaloid projection neurons, is more in the service of receiving amygdalar input and organizing information to send to the mid and hindbrain, frontal cortex and the bed nucleus of the stria terminalis for the purpose of executing adaptive behavioural patterns. The basolateral nucleus has a greater need for plasticity being the locus at which relevant sensory data is imposed on existing fear circuitry. Neurons in this nucleus exhibit increased firing rates to a recently conditioned stimulus. They also demonstrate receptive field plasticity (Rogan & LeDoux, 1996). There is also evidence of increased functional coupling of action potentials between cells in the lateral nucleus, which may explain the phenomenon of fear recovery, after extinction exhibits all behavioural signs of having occurred (Rogan & LeDoux, 1996). The stimulation of the amygdala via afferent inputs from the endopiriform cortex produces a long-term increase in EPSPs in monosynaptic connections within the amygdala. This would appear to indicate that the amygdala, in and of itself, is capable of long-term plasticity (Maren, 1996). Long-term potentiation in the amygdala is dependent on NMDA receptors (Goosens & Maren, 2004). Given that increases in post-cat exposure anxiety-like

behaviour (ALB) is mediated by an altered memory network; and that this alteration is presumably dependent on LTP, it therefore follows that the long-lasting effects of predator stress may potentially be blocked by the administration of an NMDA receptor antagonist. Indeed, a systemic injection of MK-801, AP7 or CPP, 30 minutes prior to, but not after, a predator stress exposure, prevents increased anxiety-like behaviour measured 7 days later in the elevated plus-maze (Adamec, Burton, Shallow, & Budgell, 1999). Relevance of the efficacy of NMDA receptor antagonists for our current investigation assumes there is a contribution of the predator stress experience to the induction of LTP. This assumption appears to be accurate, as research on predator stress supports this hypothesis.

Predator Stress Produces Long-Term Potentiation

LTP has been induced pharmacologically in cats using FG-7142. This anxiogenic agent produced LTP in transmission between the amygdala and the dorsolateral column of the periaqueductal gray matter (PAG) in the right hemisphere. This potentiation resulted in an increase in ALB (Adamec, 1994). Exposing a rat to a cat for 5 minutes produces an effect on ALB in the elevated plus-maze similar to that of pharmacological potentiation in the cat (Adamec & Shallow, 1993). Adamec et al. (2003) found a direct link between predator stress and an increase in the degree of phosphorylation of cyclic AMP (adenosine monophosphate) response element binding protein (CREB) expression in cells in the right lateral column of the PAG (Adamec, Blundell, & Burton, 2003). It has

been proposed that *p*CREB expression is critical for the induction of LTP (Paynes, Goldbart, Gozal, & Schurr, 2004). Predator stress also increased potentials evoked in the right lateral column of the PAG by stimulation of the central amygdala. LTP was observed to persist for at least 11-12 days post-exposure in the right hemisphere only (Adamec et al., 2003). This lateralization is consistent with the increase in activation of the right amygdala found in combat veterans upon being exposed to combat cues (Shin, McNally, Kosslyn, Thompson, Rauch, Alpert, Metzger, Lasko, Orr, & Pitman, 1997).

The ability to model PTSD using animals by way of a predator stress exposure may be valuable for developing pharmacological therapeutic interventions for PTSD sufferers. The blockade of NMDA receptors is one possible intervention, but is restricted to a brief time-window prior to stress and hence has little therapeutic potential. The maintenance of the enduring alterations in ALB produced by predator stress depends upon post-stress activation of CCK_B receptors, but not CCK_A receptors, in the amygdala (Adamec, Kent, Anisman, Shallow, & Merali, 1998; Adamec, Shallow, & Budgell, 1997). The neurotransmitter serotonin plays a modulating role in the predator stress potentiation of ALB. Administration of the 5-HT (1A) agonist Vilazadone (20-40mg/kg) to a rat, 10 minutes post-exposure to a cat, selectively blocked the stress-induced increase in the acoustic startle response measured 7 days following the predator stress exposure (Adamec, Bartoszyk, & Burton, 2004). Administration of a 5-HT (2A) antagonist within ten minutes post-cat exposure prevented increases in open arm avoidance in the elevated plus-maze and increased acoustic startle response measured 7 days later (Adamec, Creamer, Bartoszyk, & Burton, 2004). These findings suggest the predator stress model

not only produces alterations that are similar to those found in PTSD; it also provides evidence that the effects of predator stress are interruptible post-exposure. This makes it a potential model for screening post-trauma prophylactic interventions.

These neural-chemical series of events, required for the trauma to maintain its long-lasting psychological effects, may culminate in an alteration in the architecture of the synapses involved in the formation of a fear memory. Any long-term change in the synaptic framework is ultimately realized by the synthesis of proteins. If consolidation of a predator stress memory hinges upon a corresponding synaptic change, it would be highly likely that the formation of this memory would depend on protein synthesis. Disruption of protein synthesis, and hence consolidation, may therefore have therapeutic potential as a pharmacological blockade in attenuating the effects of a trauma.

Consolidation Theory and Protein Synthesis

The concept of protein synthesis playing a vital role in consolidation is not new. Katz and Halstead, in 1950, proposed that protein molecules are likely involved in the creation of memory traces (Katz & Halstead, 1950). J.B. Flexner et al. were the first team to demonstrate this by inhibiting protein synthesis in mice with puromycin (Flexner, Flexner, & Stellar, 1963). Protein synthesis is required to alter the efficacy of synapses (Abraham & Williams, 2003). The alteration of synapses is necessary to achieve and maintain a high degree of differentiation and specificity of stored information (Davis & Squire, 1984). Barondes and Squire (1972) noted that the tasks the newly synthesized

proteins were involved in included (1) enzymes that regulate the synthesis or termination of neurotransmitters, (2) the creation of receptor molecules on the post-synaptic neuron, (3) providing support so that a neuron may maintain its new structural integrity and, (4) providing molecular markers to give direction to altered intercellular processes. The diverse nature of the intra-neuronal chemical cascades and the ultimate use that protein synthesis is put to is highly dependent on the species, task and brain structure stimulated (Lee, Hung, Lu, Chen, & Chen, 1992).

Pharmacologically blocking the effects of fear in the laboratory is restricted to three intra-cellular biochemical targets: the site of protein kinase activation, CREB phosphorylation and *m*RNA translation. Protein kinase A is implicated in the early stages of LTP induction (Huang & Kandel, 1998). The inhibition of protein kinase A in the lateral nucleus of the amygdala impairs the consolidation of auditory fear conditioning (Schafe & LeDoux, 2000). It was suggested that this type of conditioning is also dependent on the activation of mitogen-activated protein kinase (MAPK) and the synthesis of new protein (Schafe & LeDoux, 2000).

The CREB protein has been proposed as the "molecular switch" that controls the expression of the protein synthesis dependent element of long-term memory formation. In one study, mice lacking the *alpha* and *delta* isoforms of CREB exhibited significant impairment in long-term memory for cued and contextual fear conditioning (Scharf, Woo, Lattal, Young, Nguyen, & Abel, 2002). Another study found that local microinjection of *phosphorothiate-modified oligodeoxynucleotides antisense* to CREB into the amygdala several hours before training impaired conditioned taste aversion when

measured at 3 and 5 days post-training (Lamprecht, Hazvi, & Dudai, 1997). It has also been found that the two periods of protein synthesis that occur after learning, the one immediately, and the other three to six hours post-training, overlap considerably with the two periods of effectiveness of CREB transcriptional inhibitors (Igaz, Vianna, Medina, & Izquierdo, 2002).

Protein synthesis inhibitors characteristically exert their effects post-transcription by disrupting RNA translation at multiple sites. Cycloheximide inhibits *r*DNA transcription, phosphorylation of Rrn3 and causes dissociation from RNA polymerase. Puromycin disrupts the translation of RNA by binding to the carboxyl end of a growing peptide chain, thus causing the premature dissociation of the peptidyl fragment from the ribosome. Actinomycin and anisomycin inhibit the synthesis of *m*RNA (Davis & Squire, 1984). Anisomycin, injected i.c.v., successfully blocked consolidation of contextual and auditory fear conditioning measured 24 hours later (Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999). In a similar paradigm, infusion of the drug directly into the basolateral nucleus of the amygdala immediately after fear conditioning training, during the initial consolidation phase, consistently disrupted performance on memory tasks administered a few days later (Schafe & LeDoux, 2000). It is also successful in disrupting fear memories during reconsolidation after undergoing reactivation 1-14 days post-training (Nader, Schafe, & LeDoux, 2000a). Amygdalar LTP, the mechanism proposed to perpetuate the fear memory, has been shown to be dependent on new protein synthesis (Okulski, Hess, & Kaczmarek, 2002). It is highly probable that behavioural sensitization of circuitry (i.e. LTP) that occurs as a result of predator stress is also dependent on the synthesis of new

protein. Although this dependency may be stated with great certainty, caution should be exercised in assuming that this synthesis occurs exclusively for new learning episodes. The post-translational modification of proteins theory (PTM), proposed by Routtenberg & Rekart (2005), posits that the necessary proteins required for the formation of any given memory are being continuously synthesized, irrespective of learning tasks. It further differentiates itself from consolidation theory by placing, at the time of learning, the primary locus of activity at the level of pre-and post-synaptic communication, with the primary action being one of protein rearrangement only. Confirmation of a consolidation effect in this experiment may prove to be a viable platform from which to further investigate any subtle variations within this scheme that may well result in elucidating existent processes highlighted in seemingly opposing models.

The eventual development of a protein synthesis inhibitor for clinical application may potentially be used in exposure therapy for PTSD. Exposure therapy requires the victim to confront their episode directly in a relaxing setting. This theoretically causes the formation of a new association between their felt relaxation and the imagery associated with the trauma. This approach is based on the premise that the emotional aspects of trauma are generated by classical conditioning (Mowrer, 1960) and maintained by operant conditioning (Saigh & Bremner, 1999). *Imaginal* therapy, one therapeutic tangent of exposure therapy, involves the patient recalling the suppressed imagery of a trauma. This reactivation of a consolidated memory may potentially cause it to return to a labile state before it undergoes reconsolidation (Nader, Schafe, & Ledoux, 2000b).

Administration of a protein synthesis inhibitor at this time may prove advantageous in reducing the established emotional valence of the trauma memory.

Implications for Blocking the Effects of Predator Exposure - Justification for the Present Study

Post-traumatic stress disorder is produced in humans by exposure to a traumatic event. Exhibited changes in behaviour that follow its onset are mediated by neural mechanisms. The alteration at this level of functioning may be partially reproduced in animal models, most notably in the predator stress model. Its perpetuation is theoretically maintained by LTP, a putative mechanism of learning and memory. The amygdala is a structure that is highly plastic, a characteristic mediated by NMDA-dependent LTP in a number of its nuclei. Research has elucidated an important role for this structure in anxiety and in the initiation and continuation of PTSD. Predator stress produces LTP in the amygdala and a number of its efferents, which presumably underlies the sensitization of defensive behavioural programs. As discussed above, many amygdala-based learning tasks require the synthesis of new protein as does the induction and maintenance of amygdalar LTP, which provides support for the idea that an alteration of synaptic architecture is involved in the manifest behavioural changes. For any given memory, oscillations between stability and more labile states exhibit themselves as a consequence of the memory undergoing reactivation. Given that "amygdala" learning and the maintenance of behavioural changes associated with predator stress may both hinge on neuro-plastic changes that likely culminate in the induction of LTP, and amygdalar

learning is known to be dependent on new protein synthesis, it may be hypothesized that the sensitization of behavioural change associated with predator stress endures a similar labile period during its initial consolidation as new proteins are being synthesized.

The process of consolidation for multiple amygdala-based learning paradigms is blocked by the administration of a protein synthesis inhibitor. The present study was designed to determine if consolidation of lasting changes in affective response following predator stress is interruptible by protein synthesis inhibition after stress. In addition, the idea that re-exposure to the stress might involve a reconsolidation process also interruptible by inhibition of protein synthesis was tested. Clinically, reactivation may be achieved by re-exposing the victim to cues associated with the trauma. It is at the point - during the act of reconsolidation - that therapeutic pharmacological intervention may prove viable. One justification for the present study is to provide evidence, using animal models, that a drug may potentially curb the anxiety associated with a species-relevant trauma. If positive results are found in animals, this may have interesting implications for PTSD victims by providing a means of erasure of a fear memory through the administration of a protein synthesis inhibitor during exposure therapy.

Methods

Animals

One hundred and sixty Long-Evans male rats (*rattus norvegicus*) were used in this experiment. Eighty rats were used in the consolidation paradigm of the study. Eighty rats

were used in the reconsolidation paradigm of the study. The rats were housed singly in polycarbonate cages measuring approximately 46 centimetres (cm) in length, 24 cm in height and 20 cm in width and fed *ad lib*. Rats were kept under a 12 hr-light/dark cycle; with lights on at 7 a.m. Rats were housed at the extreme opposite end of the building from the cats to ensure olfactory isolation. The rats in the consolidation and reconsolidation paradigms weighed approximately 90-100 and 50-60 grams, respectively, at the time of their arrival. This ensured that all rats weighed approximately the same at the time of their initial injection of anisomycin. The rats in the reconsolidation paradigm, however, were 50 grams lighter on average than the consolidation paradigm rats at the time of the initial handling. Upon arrival, the rats were removed from the traveling case and placed into their cages. They were allowed 1 day of adaptation to their home-cage. In the following 3 days, they were handled once per day for approximately 45 seconds to 1 minute between the hours of 10a.m. and 1p.m. The consolidation rats weighed approximately 140-160 grams at the time of their first cat exposure. The reconsolidation rats weighed approximately 80-100 grams at the time of their first cat exposure and 140-160 grams at the time of their second cat exposure.

Groups

The 80 rats in the consolidation paradigm were randomly assigned to four groups of 20 rats: SEXP (a rat was exposed to a cat once), SHD (a rat was handled once), SEXPV (a rat was exposed to a cat once and given a vehicle injection) and SEXPA (a rat was exposed to a cat once and given an injection of anisomycin). The 80 rats in the reconsolidation paradigm were randomly assigned to four groups of 20 rats: DEXP (a rat

was exposed to a cat twice), DHD (a rat was handled twice), DEXPV (a rat was exposed to a cat twice and given a vehicle injection) and DEXPA (a rat was exposed to a cat twice and given an injection of anisomycin). Therefore there were a total of 8 groups in the two paradigms. Rats were tested in blocks of 16 rats, 2 from each group of the eight groups.

Drug Administration

The dosage of anisomycin used was 210 milligrams per kilogram, suspended and sonicated in Tween 80 vehicle solution (2 drops sonicated in 10 ml of sterile saline). The dosage used was taken from the protocol established by Nader et al. (2000a), who used a similar experimental paradigm. The drug was prepared fresh daily with a maximum sitting time of 2.5 hours. All injections of the drug and vehicle (in a volume of 1 ml) were subcutaneous. Injections were administered 1 minute after cat exposure. In the case of DEXPA and DEXPV rats, injections were given after the second cat exposure.

Testing

Four male cats were used and were counterbalanced among rats in the various groups to ensure that there were no cat effects. The cat exposures took place between 9a.m. and 1p.m. All rats were weighed prior to testing.

Predator Stress

The dimensions of the cat exposure room were 2 meters by 1.3 meters. The height of the windowless room was approximately 3.5 meters. The cat was transported in

the arms of an experimenter to the room before the rat. Next, a different experimenter (who was free of cat odour) travelled to the opposite end of the building to collect the rat. The rat was then placed into a grey plastic container measuring 18.5 cm in height, 19 cm in length and 14.5 cm in width. The rat container was fitted into an opening into the cat testing room. The door of the rat container was opened and the rat slid in to the room on a slide within the container. The cat-rat encounter was videotaped remotely for 10 minutes.

The cat exposure room did not contain any barriers. The cat had direct access to the rat. The cat normally approached the rat initially for inspection. Then, depending on the actions of the rat, the cat chased, pawed, bit, sniffed or circled the rat for a period of seconds, characteristically followed by inactivity. This cycle of inactivity and activity on the part of the cat was repeated until the test ended. At the end of the test the rat was removed from the room. This was accomplished by gently pushing the rat with a soft broom to the entrance of the rat container. The lid of the container was slid open and the rat was pushed inside. The rat was then returned to its cage in the room adjacent to the cat exposure room. The rat was left alone for 1 minute post-exposure to recover. Sufficient recovery was defined as a lack of: erect fir, bulging eyes, and excited loco-motor activity. When the rat recovered it was removed from its cage by a gloved hand and placed on the table. It was gently wrapped in a white cloth with the back one third of its body protruding. The rat was then given an injection of the drug or the vehicle and returned to its cage. Given the reported potential side effects of the drug (Davis & Squire, 1984), injected rats were watched carefully for evidence of debilitation. Based upon visual inspection of the rats at various times post-injection, zero cases of sickness were found.

The rat was then returned to its original room at the opposite end of the building. Depending on the sequence of cats, the cat in the cat exposure room was either returned to its home cage or left in the cat exposure room in preparation for the next rat. The cat activity measures scored included the number of: paw bats, bites, pursuits, cat approaches, head approaches, sniffs, near "orienting"s, and near "not orienting"s. Each of these were paired with three rat activity measures; active (defence), passive (defence), and escapes. The number of rat approaches was scored separately.

Handled Control

The control rats were handled on the same day as the cat exposure. At no time did the handled rats come into contact with the cats. A different experimenter with a clean lab coat handled the rats to ensure that the rats were not unintentionally exposed to cat odour/hair. The handled rats were weighed at the beginning of the day along with the exposed rats. A table was wheeled into the holding room and the rat, in his cage, was placed on top. The rat was removed from his cage by a gloved hand. He then was cradled on the left forearm of the experimenter. Squirming was discouraged by a loose grip along the rat's body (generally unnecessary after the rat nestled in). The rat remained in this position for 45 seconds to 1 minute. The rat was placed back into his cage and then placed back onto his shelf. For this exercise, none of the rats left their holding room.

Behavioural Testing

Behaviour was assessed in the hole-board test, elevated plus-maze test, light/dark box test and social interaction test. These are tests commonly used to assess rodent

anxiety (File & Wardill, 1975a & b; File 1993). Cameras mounted over the various apparatus videotaped all tests. There were no experimenters present in the room while testing was taking place. The videotapes were analysed at a later time. All behavioural tests were 300 seconds in duration. The acoustic startle test involved measuring the startle amplitude of the rat upon being exposed to a loud burst of noise. This was analysed separately by a computer program (discussed below). All behavioural testing occurred between 8a.m. and 1p.m.

Hole-Board Apparatus

This apparatus provided measures of exploratory behaviour and activity independent of the elevated plus-maze (File & Wardill, 1975b). The apparatus was painted light grey and measured 60 cm in width (on all sides) and 35 cm in height. The floor of the apparatus was 12 cm from the floor of the behavioural testing room. There were four evenly spaced holes on the floor of the apparatus. They were large enough for the rat to poke its head through to explore. The holes were approximately 14 cm from the wall.

Hole-Board Behavioural Measures

Six behavioural measures were taken: (1) frequency of rearing; (2) total time spent in motion of any kind; (3) total number of head dips – a head dip occurred when the rat poked its head through any one of the four holes in the bottom of the apparatus; (4) total time spent near the wall; (5) total time spent in the center of the

box. The total time spent near the wall and in the center of the box was differentiated by observing where the rat was positioned relative to a piece of white tape surrounding the perimeter of the four holes. All four paws inside the tape counted as in the center, all four paws outside counted as near the wall. If any two paws were on the tape, the rat was considered neither near the wall nor in the center. And (6) number of fecal boli was also counted. The time spent moving and the number of rears was indicative of the relative level of activity. The number of head dips indicated the level of exploratory behaviour. The time spent near the wall relative to the time spent in the center was used to measure thigmotactic tendency.

Elevated Plus-Maze

The elevated plus-maze had four arms arranged in a plus sign design. Each arm was 10 cm in width and 50 cm in length. The four arms were joined in the centre by a 10 cm square platform. There were two "closed" arms and two "open" arms. The two closed arms opposite each other had walls that rose approximately 40 cm from the floor of the apparatus. The two open arms did not have walls; but did have a three cm high ledge surrounding the perimeter of the arms to raise baseline open arm exploration (Treit, Menard, & Royan, 1993). The maze arms and its centre were positioned 50 cm from the floor. Immediately after the five-minute hole-board test, rats were transferred by gloved hand to the center of the plus-maze facing the same open arm of the maze. Behaviour was videotaped for five minutes. Rats were then returned to their home cages.

Elevated Plus-Maze Behavioural Measures

Six measures were taken for this test. They were as follows: The number of arm entries into any arm (a rat was considered to be in an arm of the maze when it had all 4 feet within the arm); the number of entries into either closed arm (tallied together); head dips (head dips occurred when the rat dipped its head over the side of an open arm); the number of rears, with the rat standing up on its hind legs, either freestanding or using one of the closed arms for support. Both head dips and rears were further subdivided into protected (within the closed arm), center or unprotected (within the open arm). Open arm exploration was quantified in two ways: Ratio time was the time spent in the open arms divided by the time spent in any arm of the maze. The smaller the ratio time, the more anxious the rat was considered to be. Ratio entry was the number of entries into either open arm divided by the number of entries into any arm of the maze. As was the case in the previous ratio, the smaller the ratio, the more anxious the rat was considered to be. Frequency of risk assessment was measured by examining the number of times the rat extended its head and forepaws into either open arm of the maze (the hind legs of the rat would remain inside one of the closed arms). This was divided by time in the closed arms of the maze to yield a ratio frequency of risk assessment measure. Finally, the number of fecal boli was counted.

Light-Dark Box

The light-dark apparatus was a rectangular box constructed of plywood 2.5 cm thick. The dimensions of the box were: 31.75 cm in length, 10.48 cm in width and 14.06

cm in height. There were two openly connected chambers within the box, both of equal size. The roof of the box was covered with Plexiglas to permit videotaping of behaviour. There were ventilation holes on the roof of the box. One side was white and the other black. Unlike the wooden floor on the white side, the floor on the dark side was constructed of wire. There was a 100-watt lamp mounted above the chambers, thus fully illuminating the white side. The light intensity that was produced on the floor of the light side was 850 Lux (or 79 foot candles). The light intensity that was produced on the floor of the dark side was 0 Lux. At the beginning of the test, rats were placed in the white side facing away from the dark chamber.

Light-Dark Box Behavioural Measures

There were 6 measures taken for the light-dark box test. Measures include the time spent in the light and time spent in the dark. Timing of presence in a chamber commenced with the crossing of all four paws into a particular chamber and terminated with the front two paws exiting the chamber. The number of entries into the light and dark chambers was scored separately. An entry was defined as the crossing of all four paws into a particular chamber. The latency to enter the dark was the time it took the rat to enter the dark from the beginning of the test. The number of fecal boli was also recorded.

Social Interaction Test

The social interaction test involved two rats from the same testing group. One rat was marked with a black marker on its side and the other was unmarked. The rats were placed into the box at the same time. The square box was 35 cm in height and 60 cm in width. It was painted black. Red lighting was used to simulate nocturnal conditions in order to maximize social activity. The light intensity on the floor of the apparatus was 30 Lux (2.7 foot candles).

Social Interaction Test Behavioural Measures

Seven measures were taken for this test. Duration of social interaction was defined as any physical contact between the two subjects with an obvious orientation towards each other. Withdrawal was defined as the termination of a social interaction on the part of one of the rats signified by clearly changing his orientation and moving away from the other rat. "Pursuit" was defined as one rat chasing after the other either after the termination of an interaction or before the initiation of a new one. The number of fights was recorded. Time immobile was the total amount of time a rat remained frozen. "Time near" was the total amount of time a rat remained near the other rat. This was defined as physically touching the rat or in very close proximity to the rat. "Time far" was the total amount of time a rat remained apart from the other rat. This was defined as not being in close proximity to the rat and clearly not oriented towards the other rat.

Acoustic Startle Chamber

A San Diego Instruments Startle Chamber was used. The San Diego Instruments S-R Lab Startle Program controlled startle testing. The rat was placed into a Plexiglas cylindrical container measuring 20.3 centimetres (length) by 10.2 centimetres (diameter). The cylinder was placed atop a platform that was connected (underneath) to a piezo electric transducer motion detector that fed sample readings to a computer. The entire apparatus was placed inside a soundproof chamber. Initially, there was a 5-minute acclimation period to a 60-decibel white noise background. Then testing began. The startle stimulus was a 120-decibel burst of white noise 50 milliseconds in duration. It rose out of the 60-decibel background of white noise. There were 40 trials in total, with 20 trials in the light and 20 trials in the dark. Light and dark trials were in random order. The inter-trial interval was 30 seconds. The inter-trial period was spent in the dark. For a light trial, the total amount of time the light was on was 3.0 seconds. At 2.95 seconds into this period, the 120-decibel stimulus was delivered for the remaining 0.05 seconds (50 milliseconds). At the conclusion of this 3.0-second period, the lights were turned off.

Acoustic Startle Chamber Measures

Two measures were taken; (1) V-max, and (2) V-start. V-max was the highest peak of "movement" detected over the span of 250 milliseconds (i.e. the record window) per trial. Movement was measured in voltage by a piezo electric transducer whose output was sampled by computer. V-start was the baseline movement just prior to the startle

stimulus. Peak startle amplitude for each trial for each rat was calculated as V-max minus V-start.

Testing Across Days

In the consolidation paradigm, the cat exposures occurred on day 1. Behavioural testing took place on days 7 and 8 after treatment (handling or predator stress). The social interaction test followed in 75 min by the acoustic startle test occurred on day 7. The light/dark box test, followed in 30 min by the hole-board test, immediately followed by the elevated plus-maze test occurred on day 8.

In the reconsolidation paradigm, the two cat exposures (or handling for controls) were separated by 7 days. The second cat exposure served as the reactivation of the stress memory established by the first exposure. Anxiety and startle testing took place on days 7 and 8 after the second treatment (handling or cat exposure) as described for the consolidation paradigm.

Results

Prior to analysis data were tested for normality. When there were deviations (D'Agostino Omnibus Test $\geq$ 19.89, $p<.001$), data were either transformed to reduce deviation from normality or analyzed non-parametrically. Transformations and non-parametric analyses will be noted where appropriate.

Effect of Cat Exposure on Time Immobility and Defensive Responses

It was of importance to determine if rats in the different groups exposed to a cat experienced similar predator stress. In order to do this several ANOVAs were done on cat responses to the rat and on rat defensive responses to the cat. The first examined differences across three groups on first cat exposure and across paradigms (consolidation condition – consolidation versus reconsolidation). The groups were: cat exposed only, and cat exposed plus vehicle or anisomycin. There were no main effects or interactions. Another ANOVA was done to contrast first and second cat exposure experience in reconsolidation paradigm rats over the first and second cat exposures. The design examined groups as above with repeated measures on cat exposure (first or second). There were no effects on any measure of cat or rat behaviour except one, time spent immobile. On second cat exposure, reconsolidation paradigm rats spent more time immobile than in their first exposure (main cat exposure effect only, no interactions; $F\{1,38\}= 42.71$, $p<.001$). For illustration, rat defensive responses and time immobile for first and second cat exposures appear in Figures 1 and 2. Rats exposed to a cat twice showed greater time immobility than rats exposed once (Tukey-Kramer, multiple comparison test, all $p<.05$) (Figure 1). The style of defence (active, passive or escape) did not differ between once and twice exposed groups (Figure 2).

Initial Analyses of Elevated Plus-Maze, Hole-Board, Light/Dark Box and Social Interaction Measures

It was of interest to determine if two cat exposures produced a differential effect on rodent affect. To accomplish this, a two way ANOVA was executed assessing stress effects (three levels: cat exposure alone or with vehicle versus no cat exposure – handled control) and testing effect (one or two treatments – exposure or handling). There were only stress and testing main effects, no interactions. Of primary interest are those measures in which handled controls differed from stressed rats. Only these results will be reported in detail. Only four measures in two tests showed effects of predator stress. The two tests were the elevated plus-maze and the social interaction test.

In the elevated plus-maze predator stress affected ratio time and ratio entry (square root transformed, all $F\{2,114\} \geq 3.74$, $p<.03$). Mean contrasts revealed that stressed groups (stressed and stressed plus vehicle) did not differ, but were less than control (Figure 3, Fisher's LSD, $p<.05$). There was a trend for ratio frequency risk assessment to show a stress effect (square root transform, $p<.11$). In this case the two exposed groups did not differ, but were less than the handled group ($t\{114\}=2.12$, $p<.037$). In addition, one of these measures showed a test effect, ratio time ($F\{1,114\}= 7.56$, $p<.001$). Handled and cat exposed animals that were tested twice displayed greater ratio times than animals that were tested once (Figure 3).

There were no stress effects on measures of exploration or activity in the elevated plus-maze or hole-board. Therefore, the anxiogenic effects of predator stress cannot be attributed to changes in activity or exploratory tendency.

Frequency of fighting was reduced by predator stress in the social interaction test, but vehicle injection reversed this effect (stress effect, $F\{2,114\}=5.71$, $p< .005$, $t\{114\}=3.34$, $p<.002$: upper panel, Figure 4).

Anisomycin and the Effects of Predator Stress on Social Interaction and Behaviour in the Elevated Plus-Maze

The effects of anisomycin on those behaviours changed by predator stress were assessed. For social interaction, vehicle injection single and double exposed groups were combined (combined vehicle injection), as were single and double exposed only groups (exposed only), because they did not differ in the initial analyses. All handled groups (control) were also combined. Analysis of fight frequency in the social interaction test revealed a group effect ($F\{4,159\}=2.87$, $p<.025$). Predator stress reduced fights. Curiously, combined vehicle injection and injection of anisomycin after a second cat exposure (reconsolidation + anisomycin group) reversed the reduction in fighting. It is possible that the anisomycin effect in the reconsolidation + anisomycin group is an injection effect like that seen with both vehicle alone groups. However, injection of anisomycin after a single cat exposure (consolidation + anisomycin group) returned fighting levels to that of predator stressed only rats (Figure 4, lower panel, all $t\{159\} \geq 4.92$, $p< .001$), conceivably by reversing the vehicle injection block of predator stress suppression of fighting.

For the elevated plus-maze, all cat exposed and cat exposed given vehicle were combined (combined cat exposed, i.e. SEXP, SEXPV, DEXP, and DEXPV), because they did not differ in the initial analyses. A one way ANOVA was used to compare the

four groups. The groups were: all handled controls (single and double handled), combined cat exposed, cat exposed given anisomycin after the first exposure (consolidation) and cat exposed given anisomycin after the second cat exposure (reconsolidation).

In the elevated plus-maze, there were group effects for ratio entry, ratio frequency risk and ratio time (all $F\{3,156\} \geq 2.90$, $p<.037$, all square root transformed, Figure 5). Planned mean contrasts revealed different patterns for the various measures (all $t\{156\} \geq 2.25$, $p<.026$). Predator stress reduced all measures relative to control. Anisomycin given after one cat exposure (consolidation + anisomycin group) returned ratio entry to control levels, elevated ratio time to a level between control and predator stressed and was without effect on the predator stress induced reduction of risk assessment (Figure 5). Anisomycin given after the second cat exposure (reconsolidation + anisomycin group) did not alter the predator stress induced reductions in any measure used.

Because of the test effect in ratio time, the consolidation mean was compared to the control mean of handled controls of the single cat exposure groups. The means were nearly identical (control versus consolidation mean $\pm$ SEM: $0.30 \pm .04$ versus $0.29 \pm .05$). So like ratio entry, ratio time was returned fully to control levels.

A similar analysis was conducted on measures of activity and exploration in the plus-maze (closed arm entries) and hole-board (rears, head dips) to determine if anisomycin was having an effect on these measures. There were no group effects. Therefore, activity and exploration tendency changes cannot account for drug effects in the plus-maze. This is a critical distinction to observe, as anisomycin has been reported to

alter the locomotor activity of animals, albeit restricted to a few hours post-injection (Davis & Squire, 1984).

Initial Analyses of Response to Acoustic Startle

Groups were compared with respect to body weight prior to analysis of startle. Larger rats may produce greater startle responses simply because of weight. Two analyses were conducted, one for rats exposed once and associated groups (consolidation paradigm rats) and one for rats exposed twice and associated groups (reconsolidation paradigm rats). Each analysis assessed group effects for four groups: handled controls, cat exposed only, cat exposed plus vehicle, and cat exposed plus anisomycin. There were group effects in both analyses (all $F\{3,76\} \geq 3.40$, $p < .022$). Both sets of groups differed in the same pattern. Handled controls differed from anisomycin groups and the remaining groups fell in between (Figure 6, Tukey-Kramer multiple comparison test, $p < .05$). Comparable groups in the consolidation and reconsolidation groupings did not differ (all t, $p > .05$).

The difference in weight was accommodated in the analysis of peak startle amplitude by dividing peak startle amplitude by body weight (in kg). Relative peak startle amplitude (in arbitrary units) was then analyzed further.

Startle data were not normally distributed, so Kruskal-Wallis one way non-parametric analysis of variance on medians was used. To make these analyses manageable, consolidation and reconsolidation paradigm rats were compared in separate analyses. Responses in the light and dark for these groups were also analyzed separately.

Anisomycin and the Effects of Predator Stress on Amplitude of Response to Acoustic Startle

One way Kruskal-Wallis ANOVA was used to separately compare four groups in two conditions, consolidation and reconsolidation conditions. Relative peak startle amplitude data were collapsed across 20 trials. In all analyses there were significant group effects ($X^2(3)>17.97$, $p<.001$). Planned comparisons within treatments were performed with Kruskal-Wallis multiple comparison test (*z* test). Comparisons of comparable groups in light versus dark conditions or across consolidation versus reconsolidation conditions were made with Wilcoxon Rank Sum Test for median differences. Significant differences ($p<.05$) are displayed in Figure 7.

Within the consolidation paradigm rats, startle in the dark and in the light displayed similar patterns of differences across groups except for the anisomycin groups. Cat exposure alone increased ratio peak startle amplitude over controls, which is consistent with previous research. Vehicle injected immediately following cat exposure blocked this increase in startle amplitude, reducing it below control and cat exposed (alone) groups. For startle in the dark, anisomycin appeared to block the vehicle injection reduction in startle amplitude, returning it to the cat exposed only elevated levels (Figure 7). A parallel effect of anisomycin was observed for startle in the light, except that anisomycin tended to block both vehicle and cat exposure effects, returning startle amplitude to a level between cat exposed alone and control groups (Figure 7).

Within the reconsolidation condition, startle in the dark and in the light showed the same patterns of differences across groups. Unlike the consolidation condition, cat exposure alone had no effect on startle amplitude, though it tended to reduce it relative to

control levels in the dark. Like the consolidation paradigm rats, cat exposure plus vehicle reduced startle amplitude below control and anisomycin reversed this effect of injection. Suppression of startle amplitude by vehicle was comparable to that seen in the consolidation paradigm rats. Unlike the consolidation paradigm rats, however, anisomycin given after the second cat exposure actually potentiated startle response above control in both the dark and light tests (Figure 7).

Comparisons of light and dark conditions revealed light-potentiated startle response in the consolidation and reconsolidation controls and the reconsolidation cat exposed only groups. Comparing consolidation and reconsolidation paradigm rats revealed the following: cat exposed only groups in light and dark tests in the consolidation paradigm rats (exposed once) had greater startle responses than their counterparts exposed to a cat twice (reconsolidation, Figure 7). The only other difference across groups was the anisomycin group startle response in the light. Consolidation + anisomycin group startle amplitude in the light was less than its counterpart in the reconsolidation + anisomycin group.

Anisomycin and the Effects of Predator Stress on Habituation of Startle

Predator stress has been shown to prolong habituation to startle (Adamec, 1997). Given these past findings, habituation to startle in the different groups was determined and compared. For this analysis, the 20 startle trials were condensed into 10 blocks of 2 trials each (average of 2 trials). Exponential decline functions of the form:

$$y = y_0 + ae^{-b/\tau}$$

were fit to the relative peak startle amplitude mean data from each group using Jandel Table Curve V4.0. In the equation, y and y_0 are ratio peak startle amplitude, a is a constant, e is the base of the natural logarithm, b is trial block and *tau* is the trial constant, or the number of trial blocks to decline to 37% of the maximal peak startle amplitude. Data were smoothed to improve fit. An FFT smoothing function provided in the program (15% FFT smooth) was applied to means from each group to improve fit. Special care was taken to ensure the smoothing did not distort the data (Figure 8). All fits were good (degrees of freedom adjusted r squared range: 0.75 to 0.98; all Fit $F(2,9) \geq 17.2$, $p < .001$; $t\{9\} \geq 2.00$, $p < .05$ for all t tests of difference from zero of *tau*). The estimate of *tau* included a standard error of estimate. These standard errors were used to perform t tests between the trial block constants of the different groups of rats. Planned comparisons between *tau* values estimated for each group were executed using two-tailed t tests (Figure 9).

Pattern of findings were the same for Consolidation and Reconsolidation paradigm rats. In both conditions, cat exposure decreased habituation rate or increased trial block constant, but only when measuring startle in the light. There were no effects of injection of vehicle or anisomycin on the delay of habituation (all $t\{18\} \geq 2.32$, $p < .033$, Figure 9). Additionally, there were no differences in values of *tau* between comparable consolidation and reconsolidation paradigm rats. There were, however, differences between light and dark startle within conditions. All groups (except control) showed greater *tau* in the light than in the dark (all $t\{18\} \geq 2.12$, $p < .05$, Figure 9).

Discussion

The Predator Stress Experience

With one exception there were no group differences in cat response to rats and rat response to cats. This suggests drug and injection effects cannot be attributed to differential stress experiences. Predator stress did cause greater immobility during cat exposure in the double exposed rats on second exposure in comparison with the single exposed rats (Figure 1). This suggests an enhanced impact on defensive response of the second cat exposure. It may also suggest an element of contextual fear conditioning to the cat exposure room. Without an exposure to room alone control, however, this must remain a speculation. The enhanced immobility, however, may be relevant to the interpretation of the startle data discussed below.

Anisomycin and the Effects of Predator Stress on Anxiety Measures

Elevated Plus-Maze

The elevated plus-maze results offer the most compelling data that anisomycin blocks consolidation, but not reconsolidation of predator stress induced ALB (Figures 5). Administration of the drug resulted in values of ratio-entry and ratio-time that were statistically identical to, or approaching, control values. Risk assessment was unaffected by the drug. It is likely that different circuitry is at play with these two behaviours. For example, it has been shown that blockade of the NMDA receptors in the lateral nucleus of the amygdala with NMDA receptor antagonist MK-801 abolishes the decrease in risk

assessment following a predator exposure, but is without effect on ratio-time or ratio-entry (Adamec et al., 1999).

Social Interaction Test

It was presumed that predator stress caused an increase in anxiety, as the experience reduced fighting in the social interaction test (Figure 4). A vehicle injection following a single exposure returned predator stress fight levels to those of the control groups. The effects of the vehicle injection may be attributed to a rapid increase in the circulation of glucocorticoids. This release may indirectly provide hormonal prophylaxis by heightening activity in the HPA stress axis (Roozendaal, 2002) immediately after the predator stress. This claim is speculative, as glucocorticoid levels were not recorded in this experiment. However, in support of this idea, it was found that administration of cortisol to humans immediately following a traumatic experience significantly attenuated re-experiencing symptoms by interfering with the formation of memory (Aerni, Traber, Hock, Roozendaal, Schelling, Papassotiropoulos, Nitsch, Schnyder, & de Quervain, 2004). One line for future research may be to cannulate the drug i.c.v. to avoid the stress of systemic injection.

Anisomycin given in the consolidation condition appeared to reverse the effect of the vehicle injection block of predator stress suppression of fighting (Figure 4). This suggests anisomycin is interfering with a protein synthesis dependent process engaged by the vehicle injection. Anisomycin had the same effect as the vehicle injection in the reconsolidation paradigm. Thus, it cannot be concluded that protein synthesis disruption

by anisomycin was effective in blocking reconsolidation. It is likely that anisomycin interferes with protein synthesis dependent effects of the vehicle injection on a first cat exposure, but vehicle injection effects on subsequent cat exposures are no longer protein synthesis dependent. The mechanisms are unclear.

Acoustic Startle

In consolidation paradigm rats there was a difference between the effects of anisomycin in the dark versus the light condition (Figure 7). In the dark, anisomycin blocked the vehicle effect of suppressing startle amplitude, bringing startle back to predator stressed (only) levels, as it did in the social interaction test. In the light, anisomycin blocked both the effects of the injection and of the predator stress exposure, bringing levels back to those of controls. The addition of an anisomycin (only) group without predator stress may be included in future experimentation to eliminate the possibility that anisomycin increases startle independently of predator stress. However, given the above results of anisomycin in the light, this does not appear to be a probable effect.

Vehicle injection interfered with cat exposure amplification of startle amplitude in the consolidation and reconsolidation paradigm rats (Figure 7). Anisomycin interferes with this effect of vehicle, suggesting vehicle effects involve protein synthesis. In the consolidation paradigm, the block of vehicle effects by anisomycin is complete with startle in the dark but may only be partial in the light. Alternatively, the effects of anisomycin in the light might also reflect a block of both vehicle and predator stress

effects. The results at present are ambiguous and might be resolvable (again) with an i.c.v. cannulation of anisomycin.

In the reconsolidation paradigm, predator stress marginally reduced startle amplitude; vehicle injection reduced startle amplitude and anisomycin not only blocked vehicle injection effects, it enhanced startle. This suggests that anisomycin may be interfering with a protein synthesis dependent process that reduces startle enhancement on re-exposure to a cat in both light and dark startle conditions. In this regard, it is of interest that rats exposed to a cat twice become more immobile during the second cat exposure (Figure 1). If these rats are adopting an enhanced immobility defensive response to threat, it might interfere with the startle response, producing the slight decline in startle amplitude observed in predator stressed reconsolidation rats. Moreover, if the decrease of predator stress potentiation of startle is due to response interference (more immobility when threatened), then this process may be protein synthesis dependent as anisomycin relieves this suppression allowing a normal enhancement of startle in light and dark. If true, this argues in favour of the interpretation of consolidation data that startle enhancement in the dark at least is not protein synthesis dependent. Startle findings suggest different neural substrates and possibly neural mechanisms mediate effects of predator stress on habituation of startle and on potentiation of startle response in the dark and in the light. It is of interest in this regard that different forebrain substrates, including the central amygdala and bed nucleus of the stria terminalis may contribute differentially to light and fear potentiation of startle (Davis & Shi, 1999; Walker & Davis, 1997). Moreover, recent findings implicate different amygdala efferents in stress-induced

potentiation of startle and delay of habituation (Adamec, Blundell, & Burton, 2005). Fear potentiated startle and light potentiation of startle may also be modulated differently (DeJongh, Groenink, VanderGugten, & Olivier, 2003).

Habituation of Acoustic Startle

As expected, cat exposure decreased habituation (Figure 9). Neither anisomycin nor injection of vehicle had significant effects. The startle reflex is unique amongst the anxiety measures, as the stimulus is auditory. The neural circuitry mediating this behaviour may potentially be entirely different from the circuitry mediating all other behavioural tasks used in this study. The auditory component may implicate a tract from the cochlear root neurons to the nucleus reticularis pontis caudalis inclusive, but possibly exclusive, from the amygdala (Davis, 1992). If the latter scenario prevails, it is unlikely that habituation results would parallel those found in measures assessing an amygdala-based form of fear, such as in the elevated plus-maze or startle amplitude.

A possible mechanism of habituation could be a homosynaptic depression mediated by AMPA trafficking away from appropriately located synapses in the startle pathway (Kittler & Moss, 2001). Predator stress might interfere with this by interfering with the AMPA trafficking. Such a mechanism would not require long-term synaptic structural modification, but would require a long-term interference with the normal habituation process (Weber, Schnitzler, & Schmid, 2002).

Light-Dark Box

Predator stress was without effect on light-dark box behaviour. Nor were there effects of injection of vehicle or anisomycin. These findings are consistent with the view that different neural substrates mediate changes in ALB measured in different tests (Adamec, 2001). Under the present testing conditions, the question of the necessity of protein synthesis in the circuitry mediating the behaviour in the light-dark box is unanswerable.

Conclusions

The purpose of this experiment was to investigate the possibility that long-term fear sensitization by predator stress requires the synthesis of new protein. One interesting implication of this idea is that fear memories associated with PTSD may be susceptible to disruption upon reactivation, if the act of reconsolidation is essentially a recapitulation of the neuro-chemical events associated with the initial consolidation. Thus, administration of a protein synthesis inhibitor post-reactivation may curb anxiety associated with PTSD.

It is evident that consolidation of some, but not all, of the behavioural effects associated with predator stress requires the synthesis of new protein. This corresponds with previous research on the initial formation of fear memories, which have reported a necessity of the activation of PKA and the expression of *p*CREB. The results for reconsolidation are more ambiguous. There are at least three possibilities concerning the necessity of protein synthesis for consolidation of changes of those behaviours unaffected by anisomycin (i.e. risk assessment) and for reconsolidation. First, protein synthesis may

be unnecessary as there is little new information that must be recorded for reconsolidation (but in any case it argues against the proposition that all reactivation puts memory in a labile form susceptible to disruption, so has implications for McGaugh (2004) versus Nader et al. (2000b) arguments). Changes in defence may be regulated by a more cost-saving mechanism, such as the redistribution of extra-synaptic AMPA receptors (Tardin, Cognet, Bats, Lounis, & Choquet, 2003).

Second, in those cases where anisomycin was without effect, it cannot be unequivocally stated that protein synthesis is not involved. Protein synthesis may be necessary, but our drug administration schedule may not have overlapped successfully with the end of a second independent wave of protein synthesis. Work on hippocampal (CA1) *m*RNA synthesis for consolidating inhibitory avoidance training revealed two phases of gene expression: (a) activation of immediate early genes (*c-fos, c-jun*) immediately following training, and (b) at 3-6 hours post-training, structural genes are expressed (Igaz, Vianna, Medina, & Izquierdo, 2002). These windows closely parallel those found for the susceptibility of rats to PKA inhibitors (Huang, Martin, & Kandel, 2000) and the two peaks of CREB phosphorylation (Stanciu, Radulovic, & Spiess, 2001) for similar tasks. Although the dose of anisomycin used reportedly inhibits 80 percent of protein synthesis for 5-8 hours post-training (Davis & Squire, 1984), it cannot be stated with certainty that it successfully covered the second wave of gene expression entirely. Assuming a 5-hour period for the action of anisomycin and an upper limit of 6 hours for the synthesis of proteins for consolidation of predator stress effects on affect, there potentially could be a maximum window of 1 hour when long-term structural proteins

may have been synthesized, thus allowing for consolidation of behavioural changes dependent on these proteins to occur. Further experimentation could include more extended periods of protein synthesis inhibition.

Third, most of the literature examines the effect of a protein synthesis inhibitor on a strictly visual, auditory or tactile (i.e. shock) memory. Accordingly, this experiment was designed on the premise that for any given event, one single memory is formed that oscillates between a stable state and a fragile one, as a function of the number of times it undergoes reactivation. However, predator stress combines all three sensory modalities. Such unique complexity warrants a consideration of multiple trace theory (MTT), proposed by Nadel & Land (2000). MTT posits that every time a memory is recalled, a new trace is formed that is a replication of the original. If multiple traces of a single memory exist, then the very splintering of memory into whole new ones creates an inevitable condition whereby differing attributes of traces may be recalled in preference to others due to their relative subjective strength. This splintering would not necessarily be restricted to a given fear component, but to any conceivable attribute a rat may associate with a testing situation. A single reactivation may presumably activate numerous traces, yet others would inevitably lay dormant due to the likely failure of the reactivation to successfully eclipse all aspects of every trace. It is these lingering traces that may potentially contribute to the negative results of anisomycin for any given reconsolidation condition. Although at the present time purely speculative, this could be applicable to our current pattern of results.

Two major caveats must be addressed concerning further experimentation. It was stated above that an i.c.v. injection may avoid injection effects and their complication of the interpretation of the effects of anisomycin. If positive results for the drug are found with i.c.v. injection, then cannulation of anisomycin directly into the amygdala would further clarify the debate if this structure is necessary for consolidating and maintaining a predator-stress induced fear-related memory, as postulated by Fanselow and LeDoux (1999), or if the amygdala serves a modulatory influence, with such long-term memories being stored elsewhere (McGaugh, 2004).

The issue of a possible secondary effect of a simultaneous decrease in catecholamine synthesis as a result of the administration of a protein synthesis inhibitor has been raised by a number of researchers as a possible explanation of the drug-induced amnesia for a given learning task (Flexner & Goodman, 1975; Flood, Smith, & Jarvik, 1980). Although still considered a debatable point, Lundgren and Carr (1978) have concluded that the two processes may be successfully dissociated: the attenuation of the inhibitory effect of anisomycin by the administration of stimulants was not due to any obvious influence on catecholamine synthesis. Replication of Lundgren and Carr in the predator stress paradigm might clarify this issue.

While further experimentation on protein synthesis inhibition would certainly elucidate additional processes that are involved in the consolidation and reconsolidation of a predator stress memory, protein synthesis inhibition will not likely be a viable pharmacological adjunct to exposure therapy in the foreseeable future.

References

Abraham, W. (2003). How long will long-term potentiation last? Philosophical Transactions of the Royal Society of London B: Biological Sciences, 358, 735-744.

Abraham, W. & Williams, J. (2003). Properties and mechanisms of LTP maintenance. Neuroscientist, 9(6), 463-474.

Adamec, R. (1978). Normal and abnormal limbic system mechanisms of emotive biasing. In: K. Livingston and O. Hornykiewicz (Eds.). Limbic Mechanisms (pp. 405-455). New York, New York: Plenum Publishing Corporation.

Adamec, R. (1994). Modelling anxiety disorders following chemical exposures. Toxicology and Industrial Health, 10, 391-420.

Adamec, R. (1997). Transmitter systems involved in neural plasticity underlying increased anxiety and defence-Implications for understanding anxiety following traumatic stress. Neuroscience and Biobehavioral Reviews, 21, 755-765.

Adamec, R. (1998). Amygdala kindling and rodent anxiety. In: M. Corcoran , M., S. Mosche (Eds.). Kindling 5 (pp. 327-348). New York, New York: Plenum Press.

Adamec, R. (2001). Does long term potentiation in periacqueductal gray (PAG) mediate lasting changes in rodent anxiety-like behaviour (ALB) produced by predator stress? – Effects of low frequency stimulation (LFS) of PAG on place preference and changes in ALB produced by predator stress. Behavioural Brain Research, 120, 111-135.

Adamec, R., Bartoszyk, G., & Burton, P. (2004). Effects of systemic injections of Vilazodone, a selective serotonin reuptake inhibitor and serotonin 1A receptor agonist, on anxiety induced by predator stress in rats. European Journal of Pharmacology, 504(1-2), 65-77.

Adamec, R., Blundell, J., & Burton, P. (2005). Role of NMDA receptors in the lateralized potentiation of central amygdala (ACE) – periaqueductal gray (PAG) and ventral angular bundle (VAB) – basolateral amygdala (BLA) neural transmission produced by predator stress. Physiology and Behavior, (under review).

Adamec, R., Burton, P., Shallow, T., & Budgell, J. (1999). Unilateral block of NMDA receptors in the amygdala prevents predator stress-induced lasting increases in anxiety-like behaviour and unconditioned startle - effect on behaviour depends on the hemisphere. Physiology and Behavior, 65, 739-751.

Adamec, R., Creamer, K., Bartoszyk, G., & Burton, P. (2004). Prophylactic and therapeutic effects of acute systemic injections of EMD 281014, a selective serotonin 2A receptor antagonist on anxiety induced by predator stress in rats. European Journal of Pharmacology, 504(1-2), 79-96.

Adamec, R., Kent, P., Anisman, H., Shallow, T., & Merali, Z. (1998). Neural plasticity, neuropeptides and anxiety in animals-implications for understanding and treating affective disorder following traumatic stress in humans. Neuroscience and Biobehavioural Reviews, 23, 301-318.

Adamec, R. & Shallow, T. (1993). Lasting effects on rodent anxiety of a single exposure to a cat. Physiology and Behaviour, 54, 101-109.

Adamec, R., Shallow, T., & Budgell, J. (1997). Blockade of CCK_B but not CCK_A receptors before and after the stress of predator exposure prevents lasting increases in anxiety-like bahavior: implications for anxiety associated with posttraumatic stress disorder. Behavioral Neuroscience, 111(2), 435-449.

Aerni, A., Traber, R., Hock, C., Roozendaal, B., Schelling, G., Papassotiropoulos, A., Nitsch, R., Schnyder, U., & de Quervain, D. (2004). Low-dose cortisol for symptoms of posttraumatic stress disorder. American Journal of Psychiatry, 161, 1488-1490.

American Psychiatric Association. (2000). Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (Text Revision) (pp. 463-468). Washington, District of Columbia: American Psychiatric Association.

Barondes, S. & Squire, L. (1972). Time and the biology of memory. Clinical Neurosurgery, 19, 381-396.

Bliss, T. & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. Journal of Physiology, 232, 331-356.

Canteras, N. & Goto, M. (1999). *Fos*-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. NeuroReport, 10, 413-418.

Chapman, P. & Bellavance, L. (1992). Induction of long-term potentiation in the basolateral amygdala does not depend on NMDA receptor activation. Synapse, 11, 310-318.

Chapman, P., Kairiss, E., Keenan, C., & Brown, T. (1990). Long-term synaptic potentiation in the amygdala. Synapse, 6, 271-278.

Da Costa Gomez, T., Chandler, S., & Behbehani, M. (1996). The role of the basolateral nucleus of the amygdala in the pathway between the amygdala and the midbrain periaqueductal gray in the rat. Neuroscience Letters, 214, 5-8.

Davis, M. & Shi, C. (1999). The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differently involved in fear versus anxiety? Annual New York Academy of Science, 877, 281-291.

Davis, H. & Squire, L. (1984). Protein synthesis and memory: a review. Psychological Bulletin, 96(3), 518-559.

Davis, M. (1992). The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. Trends in Pharmacology Science, 13(1), 35-41.

Dejongh, R., Groenink, L., VenderGugten, J., & Olivier, B. (2003). Light-enhanced and fear–potentiated startle: temporal characteristics and effects of α–helical corticotrophin-releasing hormone. Biological Psychiatry, 54(10), 1041-1048.

Elzinga, B. & Bremner, J. (2002). Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD). Journal of Affective Dsorders, 70, 1-17

Fanselow, M. & LeDoux, J. (1999). Why we think plasticity underlying pavlovian fear conditioning occurs in the basolateral amygdala. Neuron, 23, 229-232.

File, S. (1993). The interplay of learning and anxiety in the elevated plus maze. Behavior Brain Research, 58, 199-202.

File, S. & Wardill, A. (1975a). The reliability of the hole-board apparatus. Psychopharmacologia, 44, 47-51.

File, S. & Wardill, A. (1975b). Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacologia, 44(1), 53-59.

Flexner, J., Flexner, L., & Stellar, E. (1963). Memory in mice as affected by intracerebral puromycin. Science, 141, 57-59.

Flexner, L. & Goodman, R. (1975). Studies on memory: Inhibitors of protein synthesis also inhibit catecholamine synthesis. Proceedings of the National Academy of Science, 72(11), 4660-4663.

Flood, J., Smith, G., & Jarvik, M. (1980). A comparison of the effects of localized brain administration of catecholamine and protein synthesis inhibitors on memory processing. Brain Research, 197, 153-165.

Geinisman, Y., deToledo-Morrell, L., Morrell, F., Heller, R., Rossi, M., & Parshall, R. (1993). Structural synaptic correlate of long-term potentiation: formation of axospinous synapses with multiple, completely partitioned transmission zones. Hippocampus, 3(4), 435-446.

Goosens, K & Maren, S. (2004). NMDA receptors are essential for the acquisition, but not expression , of conditioned fear and associative spike firing in the lateral amygdala. European Journal of Neuroscience, 20(2), 537-548.

Huang, Y., Martin, K., & Kandel, E. (2000). Both protein kinase A and mitogen-activated protein kinase are required in the amygdale for the macromolecular synthesis-dependent late phase of long-term potentiation. Journal of Neuroscience, 20(17), 6317-6325.

Huang, Y. & Kandel, E. (1998). Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. Neuron, 21, 169-177.

Igaz, L., Vianna, M., Medina, J., & Izquierdo, I. (2002). Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. The Journal of Neuroscience, 22(15), 6781-6789.

Katz, J. & Halstead, W. (1950). Protein organization and mental function. Comparative Psychology Monographs, 20, 1-38.

Kittler, J. & Moss, S. (2001). Neurotransmitter receptor trafficking and the regulation of synaptic strength. Traffic, 2(7), 437-448.

Kolb, L. & Multalipassi, L. (1982). The conditioned emotional response: a sub-class of the chronic and delayed post-traumatic stress disorder. Psychiatric Annals, 12(11), 979-987.

Lamprecht, R., Hazvi, S., & Dudai, Y. (1997). cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. The Journal of Neuroscience, 17(21), 8443-8450.

Lee, E., Hung, H., Lu, K., Chen, W., & Chen H. (1992). Protein synthesis in the hippocampus associated with memory facilitation by corticotropin-releasing factor in rats. Peptides, 13, 927-937.

Liang, K., Chen L., & Huang, T. (1995). The role of amygdala norepinephrine in memory formation: involvement in the memory enhancing effect of peripheral epinephrine. Chinese Journal of Physiology, 38(2), 81-91.

Lundgren, P. & Carr, L. (1978). Effects of anisomycin and CNS stimulants on brain catecholamine synthesis. Pharmacology, Biochemistry & Behavior, 9, 559-561.

Maren, S. (1996). Synaptic transmission and plasticity in the amygdala. An emerging physiology of fear conditioning circuits. Molecular Neurobiology, 13(1), 1-22.

McGaugh, J. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annual Review of Neuroscience, 27, 1-28.

Mowrer, O. (1960). Learning Theory and Behavior (pp. 63-91). New York, New York: Wiley Publishing.

Nadel, L. & Land, C. (2000). Memory traces revisited. Nature Review, 1, 209-212.

Nader, K., Schafe, G., & LeDoux, J. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature, 406, 722-726.

Nader, K., Schafe, G., & LeDoux, J. (2000b). The labile nature of consolidation theory. Nature, 1, 216-219.

Okulski, P., Hess, G., & Kaczmarek, L. (2002). Anisomycin treatment paradigm affects duration of long-term potentiation in slices of the amygdala. Neuroscience, 114(1), 1-5.

Pavlov, I. (1927). Conditioned Reflexes. London, England: Oxford University Press.

Paynes, R., Goldbart, A., Gozal, D., & Schurr, A. (2004). Effect of intermittent hypoxia on long-term potentiation in rat hippocampal slices. Brain Research, 1029(2), 195-199.

Pitman, R., Orr, S., & Shalev, A. (1993). Once bitten, twice shy: beyond the conditioning model of PTSD. Biological Psychiatry, 33, 145-146.

Pitman, R., Sanders, K., Zusman, R., Healy, A., Cheema, F., Lasko, N., Cahill, L., & Orr, S. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. Biological Psychiatry, 51, 189-192.

Racine, R., Milgram, N., & Hafner, S. (1983). Long-term potentiation phenomena in the rat limbic forebrain. Brain Research, 260, 217-231.

Rogan, M. & LeDoux, J. (1996). Emotion: systems, cells, synaptic plasticity. Cell, 85, 469-475.

Roozendaal, B. (2002). Stress and memory: Opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiology of Learning and Memory, 78, 578-595.

Rosen, J. & Fields, R. (1988). The long-term effects of extraordinary trauma: a look beyond PTSD. Journal of Anxiety Disorders, 2, 179-191.

Routtenberg, A. & Rekart, J. (2005). Post-translational protein modification as the substrate for long-lasting memory. Trends in Neuroscience, 28(1), 12-19.

Saigh, P. & Bremner, J. (1999). Posttraumatic Stress Disorder (pp. 376-387). Needham Heights, Massachusettes: Allyn and Bacon.

Schafe, G. & LeDoux, J. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. The Journal of Neuroscience, 20(RC96), 1-5.

Schafe, G., Nadel, N., Sullivan, G., Harris, A., & LeDoux, J. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learning and Memory, 6(2), 97-110.

Scharf, M., Woo, N., Lattal, K., Young, J., Nguyen, P., & Abel, T. (2002). Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. Journal of Neurophysiology, 87, 2770-2777.

Shalev, A. (1993). Post-traumatic stress disorder: a biopsychological perspective. Israeli Journal of Psychiatry and Related Sciences, 30(2), 102-109.

Shors, T. & Matzel, L. (1997). Long-term potentiation: what's learning got to do with it? Behavioral and Brain Sciences, 20(4), 597-655.

Shin, L., McNally, R., Kosslyn, S., Thompson, W., Rauch, S., Alpert, N., Metzger, L., Lasko, N., Orr, S., & Pitman, R. (1997). A positron emission tomographic study of symptom provocation in PTSD. Annals of New York Academy of Science, 821, 521-523.

Stanciu, M., Radulovic, J., & Spiess, J. (2001). Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to *Fos* production. Brain Research Molecular Brain Research, 94(1-2), 15-24.

Tardin, C., Cognet, L., Bats, C., Lounis, B., & Choquet, D. (2003). Direct imaging of lateral movements of AMPA receptors inside synapses. The European Molecular Biology Association, 22(18), 4656-4665.

Treit, D., Menard, J., & Royan, C. (1993). Anxiogenic stimuli in the elevated plus-maze. Pharmacology, Biochemistry and Behavior, 44, 463-469.

Vaiva, G., Ducrocq, F., Jezequel, K., Averland, B., Lestavel, P., Brunet, A., & Marmar, C. (2003). Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. Biological Psychiatry, 54, 947-949.

Van der Kolk, B., Greenberg, M., Boyd, H., & Krystal, J. (1985). Inescapable shock, neurotransmitters, and addiction to trauma: toward a psychobiology of post traumatic stress. Biological Psychiatry, 20, 314-325.

Walker, D. & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. Journal of Neuroscience, 17(23), 9375-9383.

Watanabe, Y., Ikegaya, Y., Saito, H., & Abe, K. (1995). Roles of $GABA_A$, NMDA and muscarinic receptors in induction of long-term potentiation in the medial and lateral amygdala in vitro. Neuroscience Research, 21, 317-322.

Weber, M. Schnitzler, H. & Schmid, S. (2002). Synaptic plasticity in the acoustic startle pathway: the neuronal basis for short-term habituation? European Journal of Neuroscience, 16(7), 1325-1332.

Yehuda, R. & Antelman, S. (1993). Criteria for rationally evaluating animal models of posttraumatic stress disorder. Biological Psychiatry, 33, 479-486.

Figure Captions

Figure 1. Mean + SEM of time immobile during cat exposure are plotted for three cat exposed groups. The groups were all cat exposed rats exposed to a cat once (Single Exposure) and all cat exposed rats exposed to a cat twice, plotting responses on first and second cat exposure separately (Double Exposure Groups First and Second Exposure). Means marked similarly do not differ but differ from means marked differently.

Figure 2. Mean + SEM of rat defensive response to cats over three cat exposed groups. The groups were all cat exposed rats exposed to a cat once (Single Exposure) and all cat exposed rats exposed to a cat twice, plotting responses on first and second cat exposure separately (Double Exposure Groups First and Second Exposure). Plotted are frequencies of active, passive and escape responses. Unmarked means do not differ.

Figure 3. Mean + SEM of elevated plus-maze behaviours (square root transformed) collapsed over one and two treatment conditions are plotted over three groups: handled controls, cat exposed with and without vehicle. Upper right panel shows the test effect for ratio time. In any given plot, means marked similarly do not differ but differ from means marked differently.

Figure 4. Plotted in the upper panel are mean + SEM of number of fights in the social interaction test collapsed over one and two treatment conditions for three groups: handled controls, cat exposed with and without vehicle. The lower panel shows the group effect for this measure comparing controls, cat exposed only and vehicle collapsed over one and two treatment conditions, and groups exposed to a cat and given anisomycin after the first exposure (consolidation) or after the second cat exposure (reconsolidation). In any given plot, means marked similarly do not differ but differ from means marked differently.

Figure 5. Mean + SEM of elevated plus-maze behaviours (square root transformed) are plotted over four groups: handled (Control), cat exposed (once or twice with or without vehicle) (Predator Stressed), cat exposed once and given anisomycin just after exposure (Consolidation) and cat exposed twice and given anisomycin just after the second cat exposure (Reconsolidation). In any given plot means marked similarly do not differ but differ from means marked differently. Means with two letters fall between means marked with either letter.

Figure 6. Mean + SEM of body weight (g) are plotted over four groups: handled controls, cat exposed with and without vehicle and cat exposed and given anisomycin. Data are plotted separately for rats in the consolidation (upper panel) and reconsolidation (lower panel) paradigms. In any given plot, means marked similarly do not differ but differ from means marked differently. Means with two letters fall between means marked with either letter.

Figure 7. Median peak startle amplitudes as a ratio of body weight (kg) are plotted over four groups: handled controls, cat exposed with and without vehicle, and cat exposed and given anisomycin. Data are plotted separately for rats in the consolidation (upper panel) and reconsolidation (lower panel) paradigms. Within a paradigm plot, data from startle in the dark and in the light are also plotted separately. Within a panel, medians marked similarly do not differ but differ from medians marked differently ($p<.05$). Medians with two letters fall between means marked with either letter.

Figure 8. Mean peak startle amplitude in the dark as a ratio of body weight is plotted over trial block for cat exposed only rats in the reconsolidation paradigm (raw data means). The means after 15% FFT smoothing are also plotted (smoothed data) along with the fitted exponential decline function. This example fit was good ($p<.001$, df adjusted $r^2=.92$).

Figure 9. Mean + SEM of block constants (*tau*) over four groups: handled controls, cat exposed with and without vehicle, and cat exposed and given anisomycin. Data are plotted separately for rats in the consolidation (upper panel) and reconsolidation (lower panel) paradigms. Within a paradigm plot, data from startle in the dark and in the light are also plotted separately. All means in the startle in the dark condition are unmarked and do not differ. For startle in the light, within a panel, means marked similarly do not differ but differ from means marked differently.

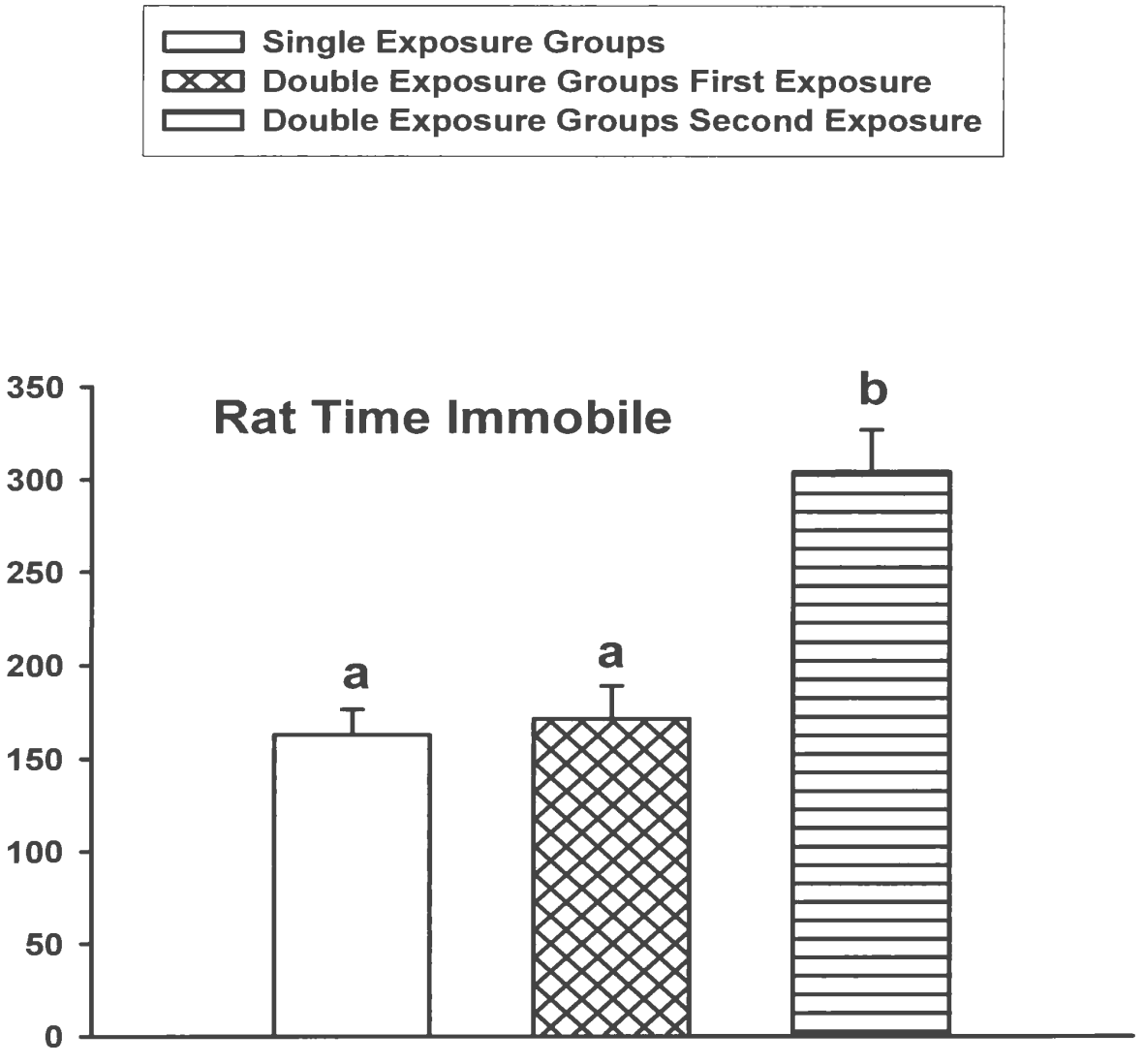

Single Exposure Groups
Double Exposure Groups First Exposure
Double Exposure Groups Second Exposure
Rat Time Immobile
350
300
250
200
150
100
50
0
a
a
b

Figure 1

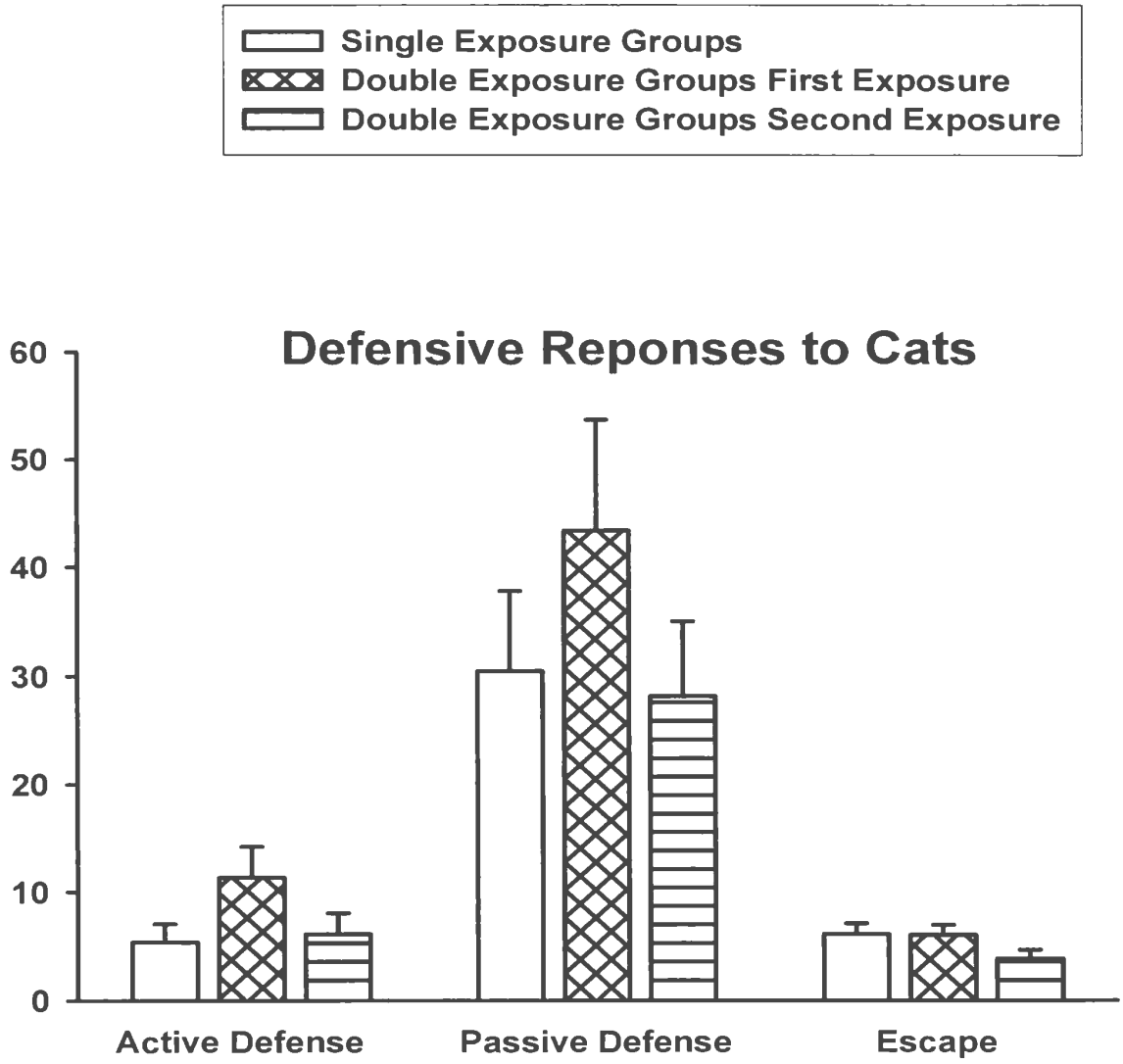

Single Exposure Groups
Double Exposure Groups First Exposure
Double Exposure Groups Second Exposure
Defensive Reponses to Cats
60
50
40
30
20
10
0
Active Defense
Passive Defense
Escape

Figure 2

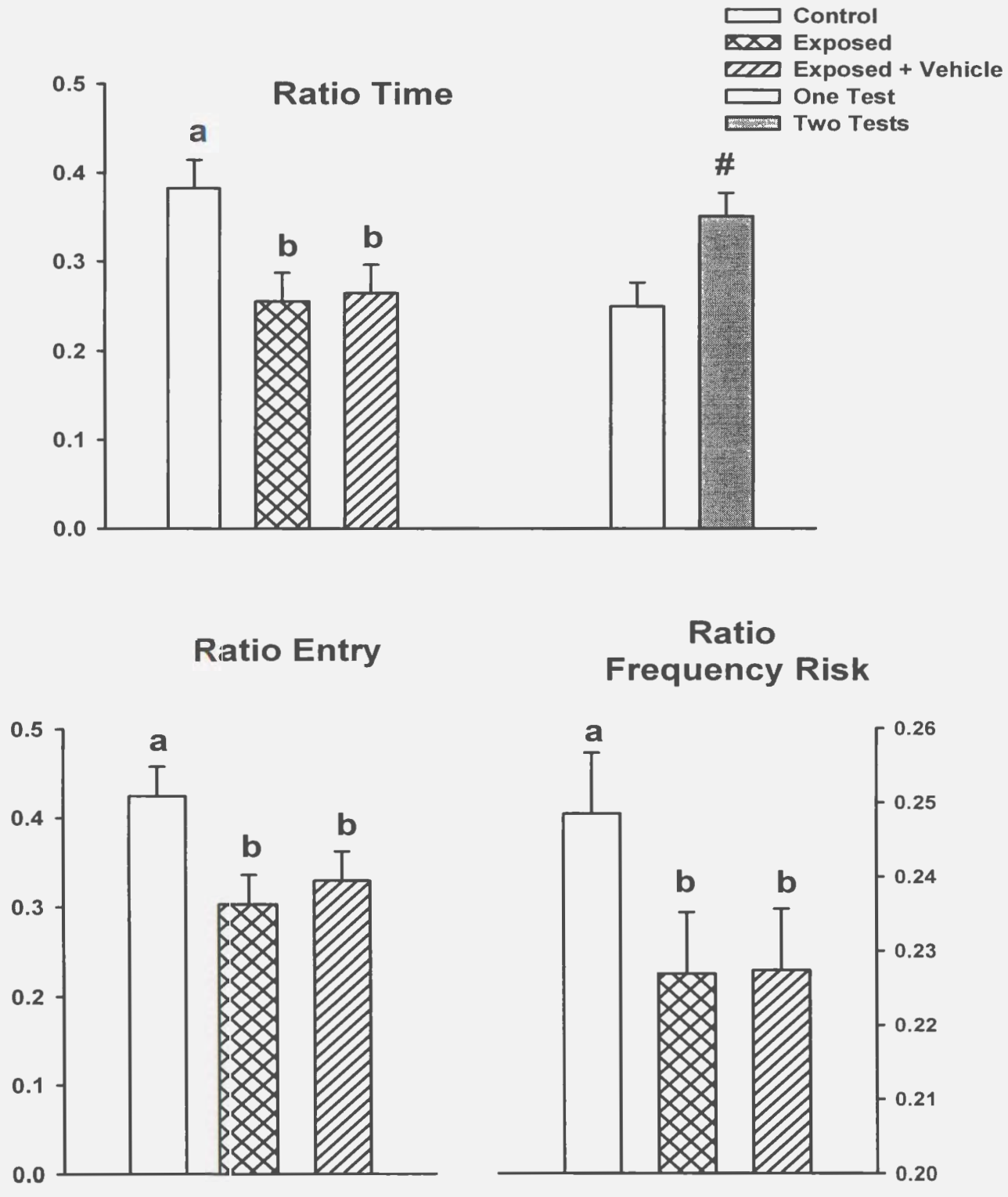

Control
Exposed
Exposed + Vehicle
One Test
Two Tests
Ratio Time
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
#
Ratio Entry
Ratio
Frequency Risk
0.26
0.25
0.24
0.23
0.22
0.21
0.20

Figure 3

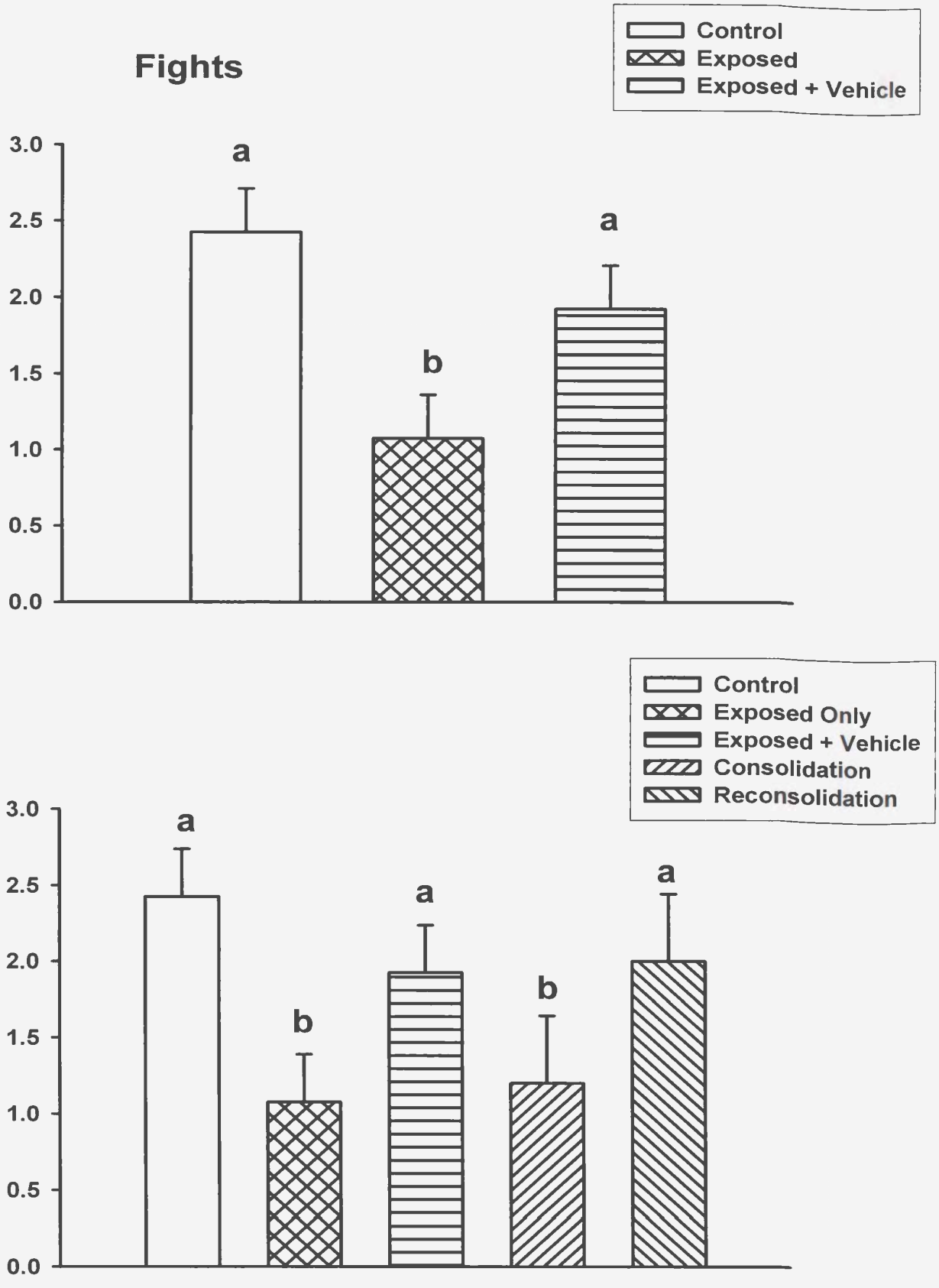

Fights
Control
Exposed
Exposed + Vehicle
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
a
Control
Exposed Only
Exposed + Vehicle
Consolidation
Reconsolidation
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
a
b
a

Figure 4

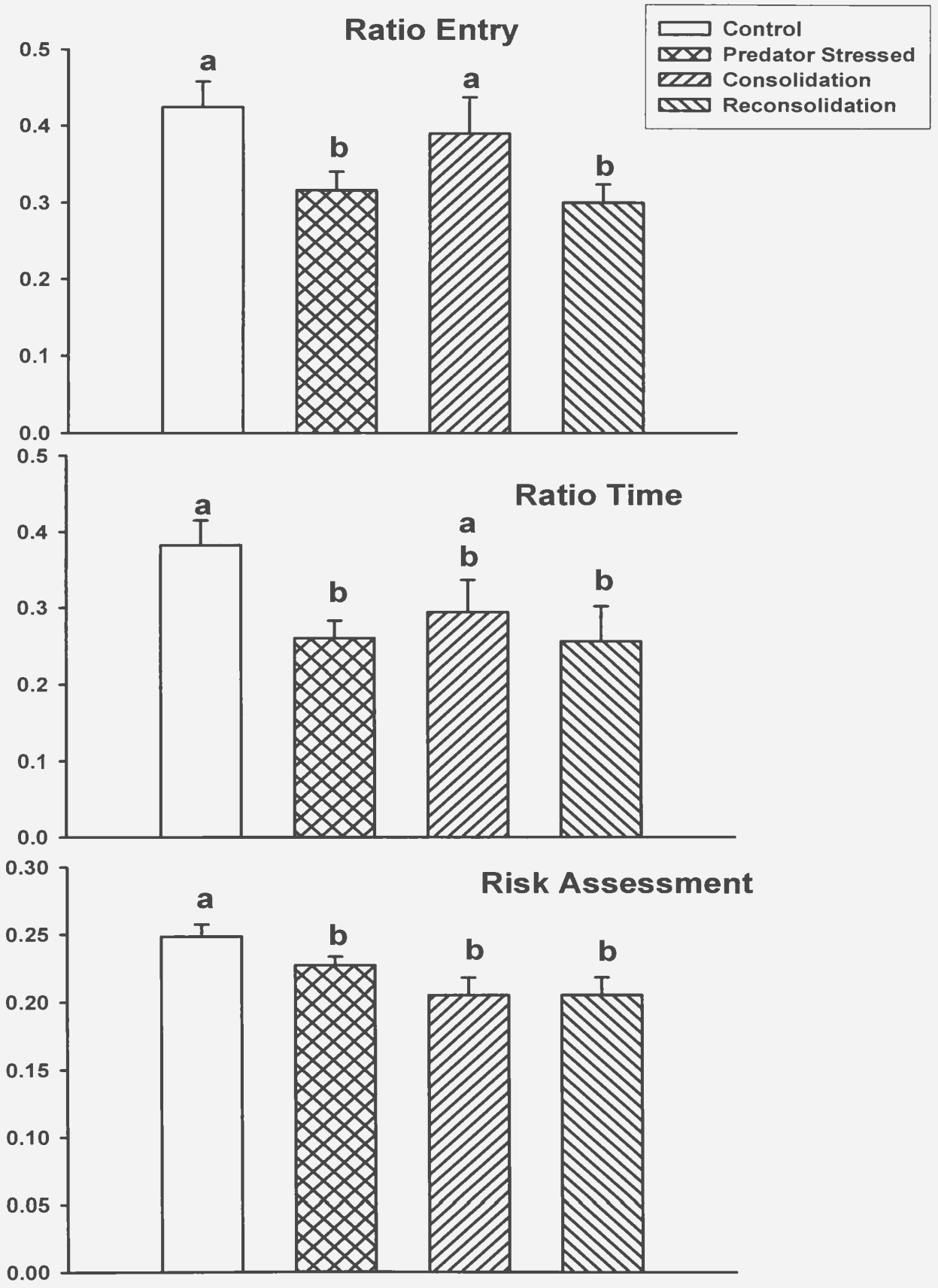
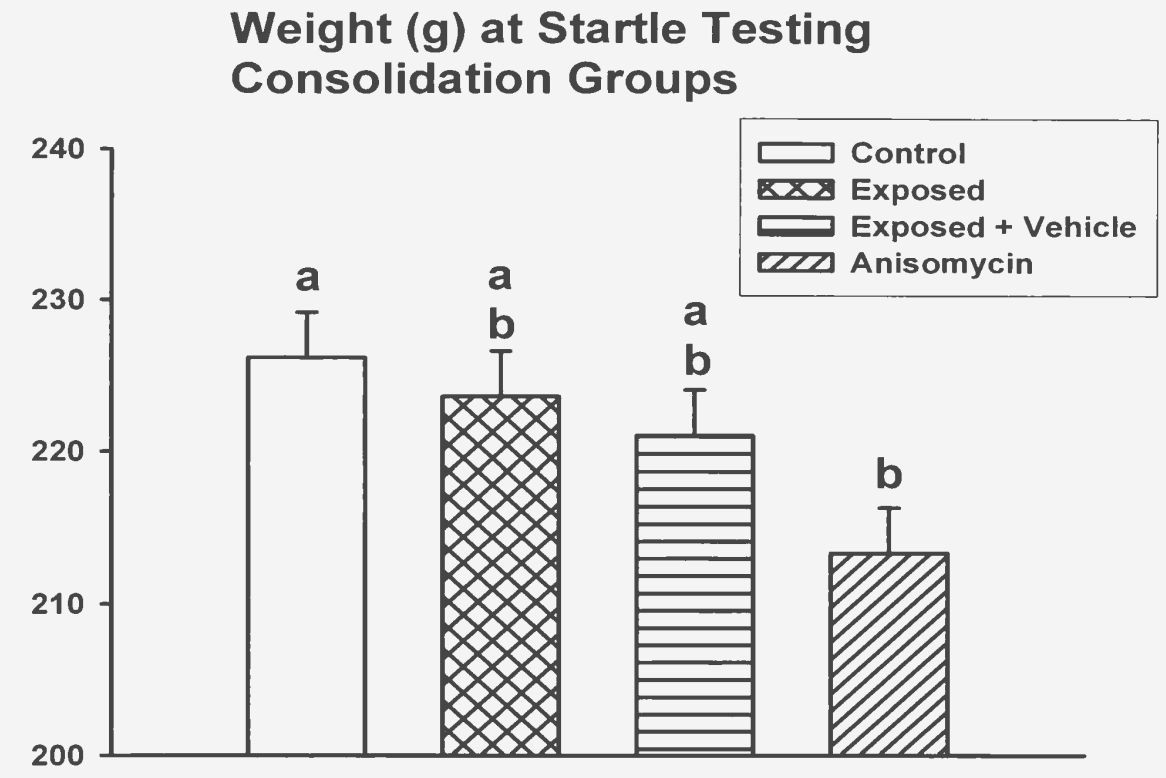

Ratio Entry
Control
Predator Stressed
Consolidation
Reconsolidation
0.5
0.4
0.3
0.2
0.1
0.0
a
b
a
b
Ratio Time
0.5
0.4
0.3
0.2
0.1
0.0
a
b
a
b
b
Risk Assessment
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
b
b
b

Figure 5


Weight (g) at Startle Testing
Consolidation Groups
Control
Exposed
Exposed + Vehicle
Anisomycin
240
230
220
210
200
a
a
b
a
b
b

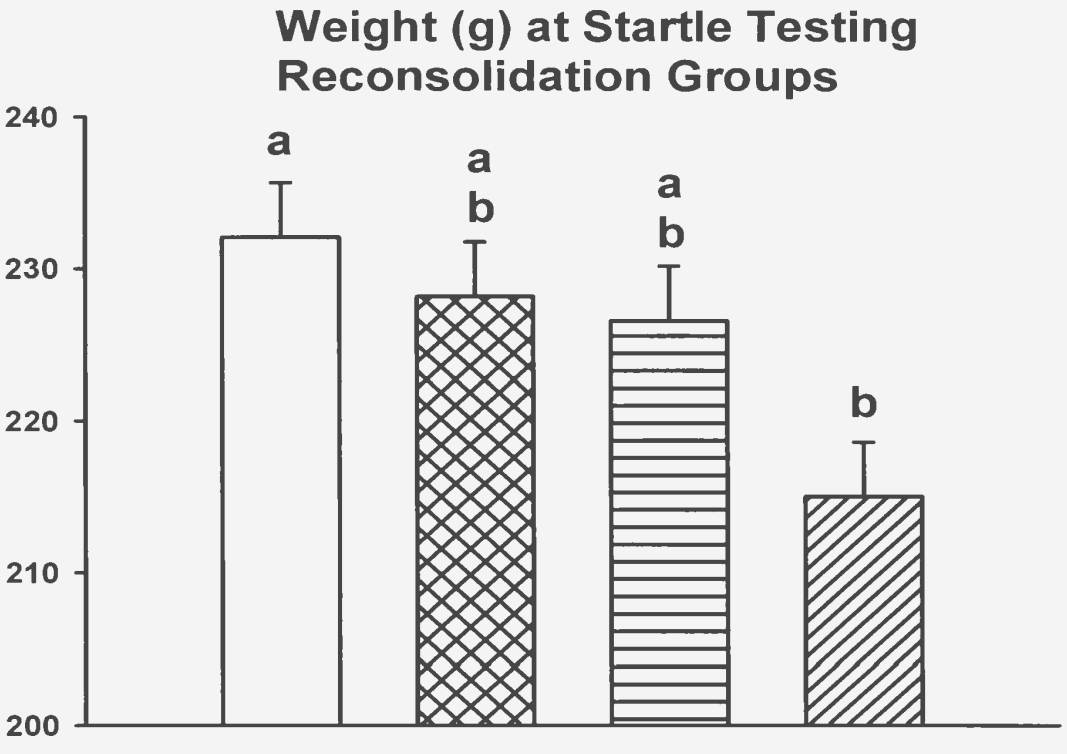
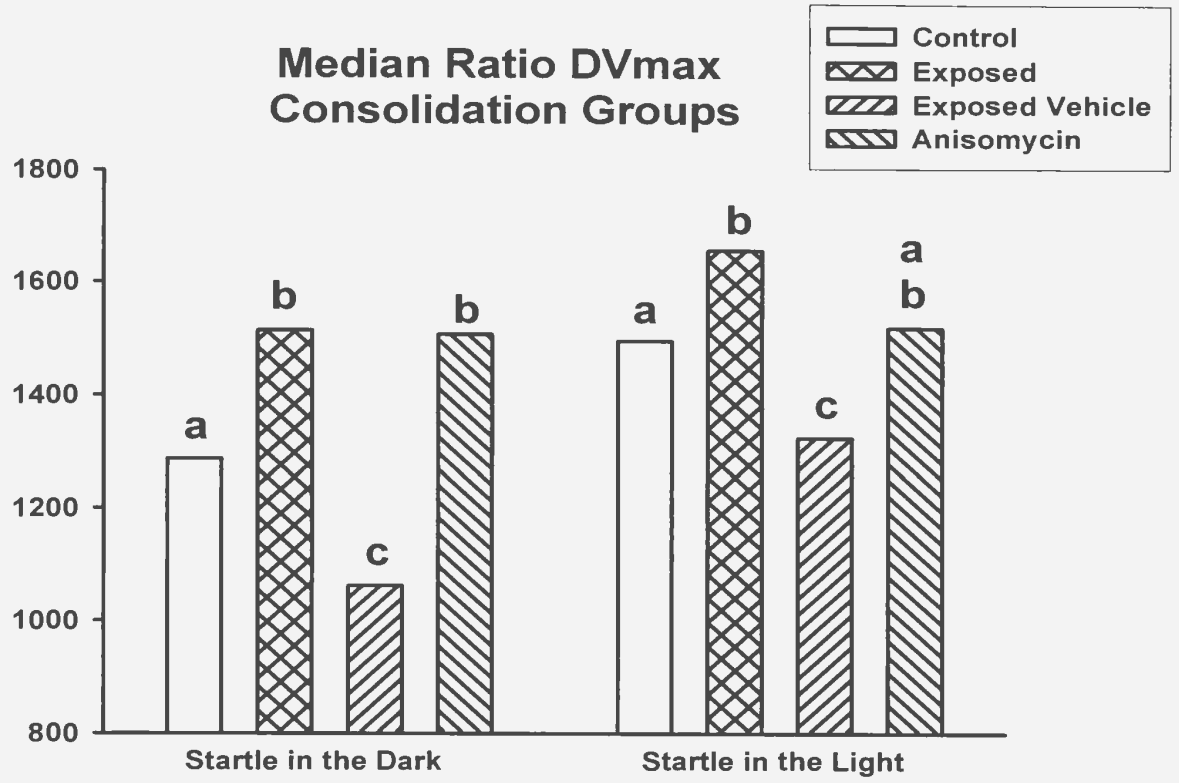

Weight (g) at Startle Testing
Reconsolidation Groups
240
230
220
210
200
a
a
b
a
b
b

Figure 6


Median Ratio DVmax
Consolidation Groups
Control
Exposed
Exposed Vehicle
Anisomycin
1800
1600
1400
1200
1000
800
a
b
b
c
b
a
b
c
a
b
Startle in the Dark
Startle in the Light

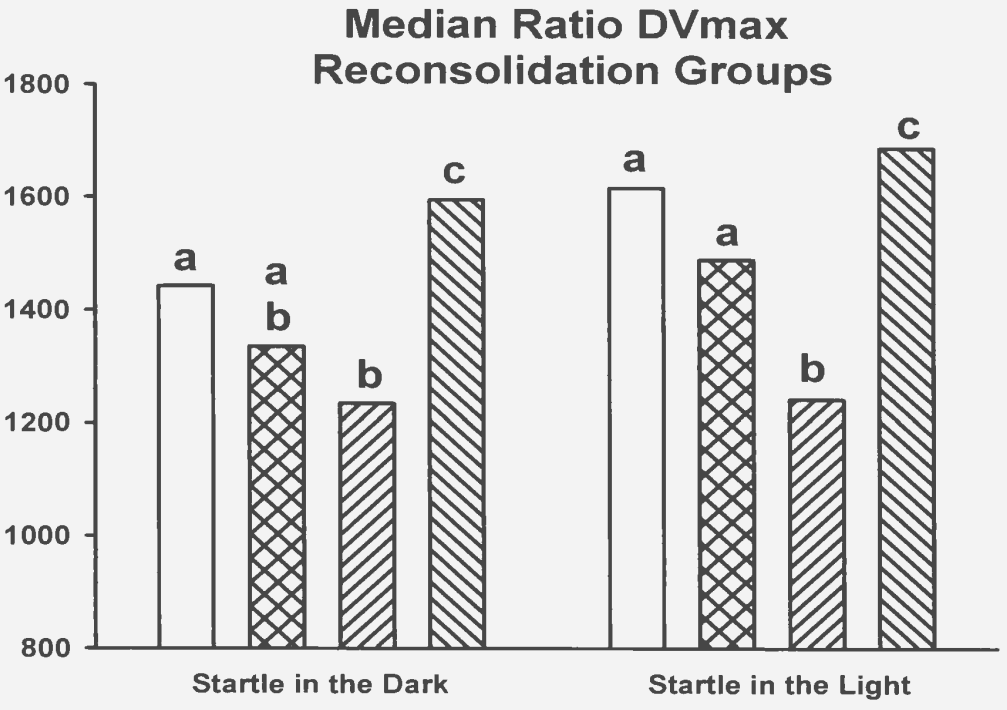

Median Ratio DVmax
Reconsolidation Groups
1800
1600
1400
1200
1000
800
a
a
b
b
c
a
a
b
c
Startle in the Dark
Startle in the Light

Figure 7

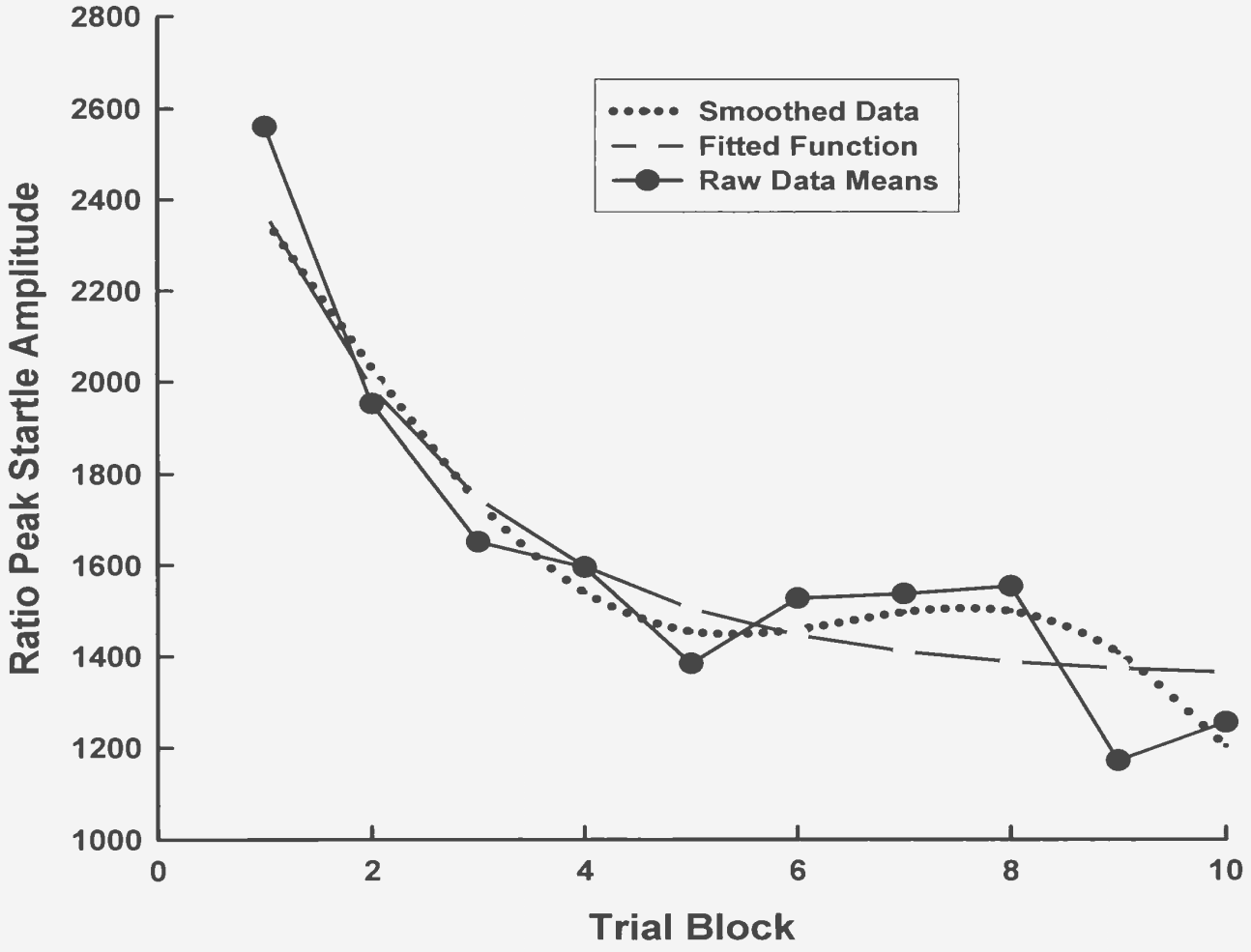

Smoothed Data
Fitted Function
Raw Data Means
Ratio Peak Startle Amplitude
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
0
2
4
6
8
10
Trial Block

Figure 8

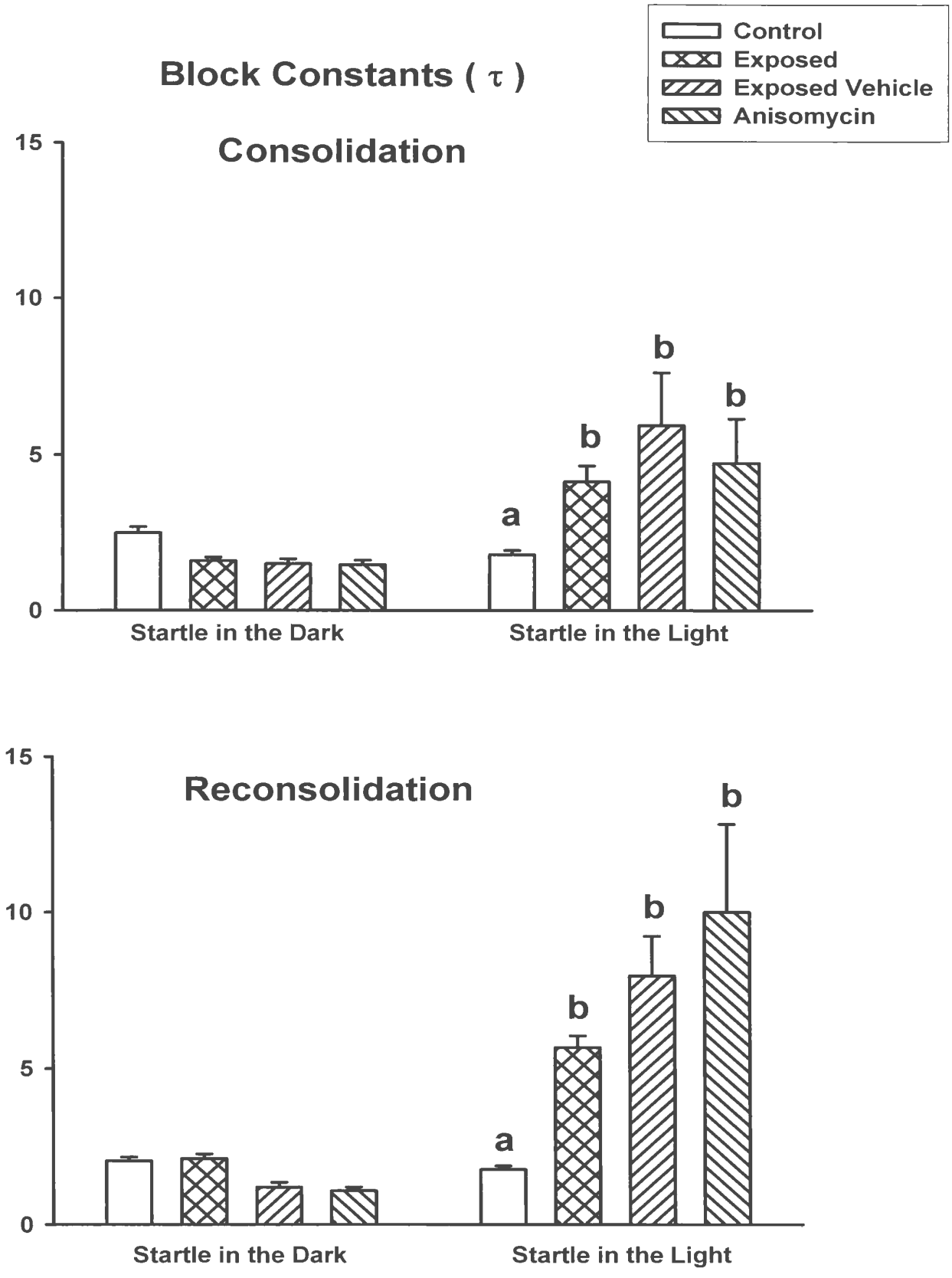

Block Constants (τ)
Consolidation
Control
Exposed
Exposed Vehicle
Anisomycin
15
10
5
0
a
b
b
b
Startle in the Dark
Startle in the Light
Reconsolidation
15
10
5
0
a
b
b
b
Startle in the Dark
Startle in the Light

Figure 9