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The Effects of Early Maternal Deprivation on Adult Behavior in Sprague Dawley Rats.

By

Catherine J. Morgan

A dissertation submitted to the Graduate Faculty in Psychology (Biopsychology Subprogram) in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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This manuscript has been read and accepted for the Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

THE EFFECTS OF EARLY MATERNAL DEPRIVATION ON ADULT BEHAVIOR IN SPRAGUE DAWLEY RATS

by

Catherine J. Morgan

Advisor: Professor Gerald Turkewitz

Early Maternal Deprivation reliably derails the developmental trajectory of adult stress responses. Without early environmental intervention Hypothalamic-Pituitary Axis function is permanently altered. This work investigates maternal deprivation procedural mechanisms and seeks to uncover some underlying relationships between procedure and resulting shifts in adult behavioral outcome. Male and female rats were exposed to one of two different Isolation Treatment (IT) procedures both utilizing a 1-hour for 8-days (PND 2 thru PND 9) deprivation from the dam and siblings, either with or without a standard auditory white noise mask. Before/after treatment pup weights were measured; adult weights at two ages were related to perinatal weights. Also, influence of psychostimulant drug was examined.

Utilizing a 2-Isolation x 2-Drug x 2-Sex Design, two adult 30-minute tests were videotaped: (1) Open Field Box; and (2) Post-baseline. Either a "challenge" 2.0 mg/kg dose of Amphetamine or .09% physiological Saline was administered 15-minutes prior to placement in the test apparatus; anxiety behavior was scored.

All IT subjects receiving Amphetamine Challenge exhibited significantly higher stress behavior scores, across all measures, compared with No-Isolation Animal Facility Reared Controls. Direction and strength of response to Amphetamine pre-stressor depended on type of IT received: No-Auditory IT subjects had exaggerated, higher drug responses compared with all other subjects regardless of condition, while Amphetamine Auditory IT subjects had activity levels (1) lower than Amphetamine Controls, (2) lower

than No-Auditory IT Amphetamine Conditions. Only No-Auditory IT subjects exhibited significantly different scores across all measures when receiving only Saline pre-test.

Significant differences existed in pup, and in adult weights, between: (1) Treatment and Controls, (2) Drug Conditions, (3) types of IT, (4) before/after deprivation. All IT pups gained less weight than Controls during 8-day treatment, and before-after treatment on PND 5; unexpectedly, pups experiencing No-Auditory IT compared to Auditory IT's, gained less weight PND 2-PND 9, and before-after treatment on PND 5. This relationship in Treatment v. Control weight differences, and between types of IT, persisted into adulthood.

Behavioral measures revealed a differential impact on adult stress-responses, on pup-adult weight, dependant on Isolation Treatment procedures.

PREFACE

Depending on the current *State* of the organism (animal or human), increasing stress factors can result in either a positive or negative change in homeostatic state. Since this is true for not only discrete time points in development, but also for the lifetime developmental process, identifying and understanding what those individual stress factors are (as well as the degree of application necessary for change), could have beneficial utility. In a very limited fashion, that is the purpose of this study: Using behavioral measurements in adulthood to assess the experimental manipulation of several discrete stress factors during a limited, but particularly plastic, perinatal time period. Why use a behavioral measure? Because even though you identify the individual parts (be they environmental or genetic components) contributing to the whole, you still cannot begin to predict *whole organism function* without testing its behavior, and that involves testing at a behavioral level.

Acknowledgments

The construction of this Degree required steadfast work over the past thirteen years, but that alone did not suffice. It took the support of numerous individuals, and some unfortunately will go without proper acknowledgment.

First I would like to thank Dr. and Mrs. Gerald Turkewitz for their tenacity, if not faith; for not giving up, giving in or giving out, throughout this rather arduous Time-Journey.

I also wish to thank my doctoral Committee for agreeing to serve and for, finally validating. I thank my classroom professors who, in the earlier part of the decade, kindled the flame of developmental epigenesis - a view which has become to me more touchstone than looking-glass..

Without the timely support of my immediate family, especially my sister Mary Ann Boger and her son Christopher Martino, I would not have had the financial support to have completed this work, particularly over the last year and a half–the time necessary to research, purchase and/or build the bigger budget equipment, collect the data, do the analysis and write the results; and had it been necessary-- she was available 24/7. Fortunate I am to have a brother who provided, in the last half of the decade, "mundane" things such as --- health insurance and aging transportation.....but transportation none-the-less, carrying me forward, ensconced in a mechanized cocoon, in the echoing, earliest morning hours along abandoned New York City Streets destined to study naturally nocturnal lab life forms (and semi-literate alliteration!). I thank my middle sister, Theresa, for being crazy enough to (against her own wishes and desires) understand my inability to not finish.. I thank my children for buying my books when the where-with-all went elsewhere; for choosing to keep me as their Mother–even at long distance–and for providing offspring for me to care about, wonder at, love and visit.

Many thanks to the personnel of the Hunter College Animal Facility; they

provided the space to house my animals, conduct my research, and even offered occasional guidance in the handling of my animals: Barbara Wolin, Sony Acavedo, Sally Soccs, Lynn, and their crew. Of paramount importance and value to me was the knowledge that they shared a view of animal care which is critical to conducting *valid* research; I appreciate their desire to promulgate by teaching conscientious, antiseptic surgical technique, post-surgical aftercare, and their promotion of healthy, more humane housing and colony environments.

Also, there have been friends and guides that have, like Gibraltar, seen me through: my friend Ellen Breheny, my friend and guide Tam Spitzer, my personal family physician Jeffrey Harp who kept the faith--(God help him!), and my cohort student and friend Emily Carrasco. Even some genuinely concerned neighbors, and various inventors at the part-time job that did and does provide a working means to an end —this has been so long an endeavor, and it has required so much from everyone. For lack of knowing the names of all of the various Committee Members and Gardners, some will go unthanked here but not in my memory and heart

I acknowledge that, after all this education has cost in terms beyond money, I have an obligation to apply that knowledge. I will be working on it!

Eppur si muove....

(Galileo Galilei 1564-1642)

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Males/Amph subjects only, measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions. A significant effect for Time $F(1, 47) = 63.539$, $p < .0001$; for Time x Isolation $F(1, 40) = 47.141$, $p < .0001$; and for Time x Sex $F(1, 40) = 3.825$, $p < .057$; and for Time x Isolation $F(1,40) = 5.272$, $p < 027$. Further, there was a Between-Subject Effect for Sex $F(1, 40) = 719.559$, $p < .0001$.

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Figure 42(d): Bar graph for EXP#3,5 v. CG2,3, compares the mean weights for Females/Amph subjects only, measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions. A significant effect for Time $F(1, 47) = 63.539$, $p < .0001$; for Time x Isolation $F(1, 40) = 47.141$, $p < .0001$; and for Time x Sex $F(1, 40) = 3.825$, $p < .057$; and for Time x Isolation $F(1,40) = 5.272$, $p < 027$. Further, there was a Between-Subject Effect for Sex $F(1, 40) = 719.559$, $p < .0001$.

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Figure 43(b): Bar graph for EXP#5 v. CG3, compares the mean weights for

Males/Amph subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 43(c): Bar graph for EXP#5 v. CG3, compares the mean weights for Female/Saline subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 43(d): Bar graph for EXP#5 v. CG3, compares the mean weights for Female/Amph subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 44(a): Bar graph for EXP#5 v. CG3, compares the mean weights for Males/Saline subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 44(b): Bar graph for EXP#5 v. CG3, compares the mean weights for Males/Amph subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) =$

32.858, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 44(c): Bar graph for EXP#5 v. CG3, compares the mean weights for Female/Saline subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Figure 44(d): Bar graph for EXP#5 v. CG3, compares the mean weights for Female/Amph subjects only measured at original test and at postbaseline, for the No-Isolation and Isolation Conditions, shows a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$.

Part I.

1.0 General Introduction.

The *Early Handling Procedure* (EH) in developmental research involves some form of handling of neonatal pups daily for a short amount of time, usually no more than 15 minutes, in the home cage with siblings and dam present, generally for several hours or days during the "Stress Hyporesponsive Period" (SHRP). This term is more fully explained in the following sections, however, briefly, it is the critical period between postnatal day 4 through postnatal day 14 in a newborn rat wherein the dam controls the pups' hormone response to environmental stressors by providing maternal care, i.e., feeding, licking, thermal regulation, etc. Various studies have shown that this mild form of stress during this period of development in the rat, reliably produces increases in plasma and tissue levels of several neurohormones during the procedure. More importantly, Early Handling during this perinatal period reliably down regulates the levels of stressor hormones secreted to an environmental stressor when tested in adulthood. This "Hypothalamic Pituitary Axis" (HPA) hyporesponsiveness, when compared to controls, carries with it a more efficient glucocorticoid negative feed-back loop, and the expression of increased number of glucocorticoid receptors in the hippocampus, all of which are or can be considered positive adaptive changes. This procedure also permanently alters the production of Corticotropin Releasing Factor (CRF), Arginine Vasopressin (AVP), Gamma-amino-butyric Acid (GABA), and several other neurotransmitters which regulate anxiety and stress responses, and generally has a positive physiological function, increasing coping mechanisms to environmental stressors and preventing some of the negative responses seen in the aging processes. This could be thought of as a type of "inoculation" effect in that increasing levels of physical stimulation (which is a mild stressor - usually within the first two weeks of life) produces a reorganization of the HPA in a way that allows the animal to produce less of a stressor-

hormone response later in life.

Depending on which model you ascribe to, the Early Handling Procedure can be contrasted with the *Early Deprivation Procedure*, which has been posited by several researchers as generally having the opposite effect on development compared to the Early Handling Procedure. The Early Deprivation Procedure produces a negatively reorganized HPA stress response both after the procedure and into adulthood. During the SHRP the pup is removed from the Dam, with further removal from its litter mates depending on the criteria fixed by the individual researcher. Pryce and Feldon (2003), have proposed that the term "Early Deprivation"(ED) be made the standard in the literature for the situation where the pup is removed from the Dam and *also its litter mates* and placed into another environment other than the homecage in isolation for a specified period of time. The term "Maternal Deprivation"(MD) would indicate that the whole litter was removed from the Dam but kept together in a new environment other than the home cage, for a specific period of time. In either MD or ED, the pup will experience physiological changes in levels of circulating glucocorticoid, such that the level of increase of circulating plasma Glucocorticoid (GLUC) is positively correlated with the level of increase in procedure severity. Procedure severity can be increased by: (1) Prolonging the length of separation, and/or (2) Removing the litter mates. The deprivation takes place at a period of time when normally the Dam would be actively, downwardly regulating glucocorticoid levels in her pups by using various methods including feeding (i.e., style of nursing the pups), licking and grooming, and utilizing various ways of controlling pup temperature levels in the early days after birth when pups cannot maintain body temperature outside of the huddle. This MD procedure reliably results in a dysregulation, negatively reorganized HPA axis and stress response (i.e, a changed hormone release and changed binding mechanisms). Multitudes of studies have been done to identify exactly which hormones, receptors, neurotransmitters

are changed and more recently studies are focusing on which gene regulators are changed, identifying the Immediate Early Gene cascades, and, at a cellular-environment level, identifying how such modifications by proximate effectors takes place. Further studies have been conducted to identify other external environmental variables necessary for the deprivation procedure to effect dysregulation, including nutritional status of the dam, the effects of pup age when separated, and under what types of environmental housing conditions and during which times in the daily circadian cycles (i.e., light/dark cycles among others). Deprivation studies have utilized 24 hours of separation at various postnatal ages, or shorter periods (i.e., 1 to 3 hours) for continuous periods of anywhere from 9 to 14 days. Currently PND 4-14 seems to be designated as the generally agreed upon length for the SHRP However as Table 1 indicates there have many been other combinations used in deprivation procedures.

Time or number of days is usually varied within a deprivation procedure in an attempt to discover critical change-points in HPA function, for instance, notice has been taken that on PND days 12-14 a different stressor hormone response can be elicited from the hormone response produced by the same tests when administered on PND 2-10. One possible explanation for a developmental change linked to days PND 12-14, is that functional coupling between areas in the HPA that act as inhibitory circuits do not become physiologically-connected, that is they are not fully functionally active as inhibitory circuits until PND 12-14 (Nair & Gonzales-Lima, 1999). All of the same neurohormones and neurotransmitters are present from birth, but the Limbic network circuitry (important in stress-HPA and the *Hypothalamic-Pituitary-Adrenal-Steriodial Axis* [HPAS] regulation) is not functionally connected until after PND 12. Also, recent research has provided information regarding the functional timing of other developmental "switches"; for example, both the neurotransmitter GABA, and certain specific subunits of AMPA/Glutamate receptors (Kumar, Bacci, Kharazia & Hugenard,

2002) have excitatory properties only during early development, and once a certain stage of neural organization has been reached, GABA and at least one form of its receptor types, and at least one form of the Ampa/Glutamate receptor subunits then become predominantly inhibitory in function.

Generally, it is accepted that a shorter deprivation time period for several consecutive days, or longer time (up to 8 hours) for non-consecutive days, will produce an immediate effect on the HPA , such that there will be an activation of the stressor hormones ACTH and Glucocorticoid during the SHRP period when in normal pups those hormone levels are kept suppressed by the pup-sibling-dam interaction. The question then evolves to: "Is this particular deprivation intervention 'strong enough' to overcome the array of epigenetic, compensatory mechanisms that the individual *may* have in its developmental arsenal, (during infancy, puphood, and juvenile years) which would allow this postnatal manipulation of the HPA and HPAS to have an effect of "long-term" duration. "Long-term" means that the changes in HPA function produced by the experimental manipulation of maternal deprivation exposure during the perinatal period are still detectable in adulthood when measured physiologically and/or tested behaviorally. Depending on which aspect of the pup-dam-sibling interaction or developmental functional outcome the researcher is attempting to manipulate (i.e., specific variables to provide answers to specific research questions under investigation), the deprivation procedure has been modified towards producing that specific outcome. To this end then, what background information could a researcher in this field rely upon in making the choice of which variables to manipulate and test?

A LIMITED HISTORY OF THE DEVELOPMENT OF THE CONCEPT OF HOMEOSTATIC STATE

By developing a theoretical model of the processes involved in physiologically-based stress reactions in the body, Hans Selye, along with his colleagues Fortier and Ingle, sought to describe and explain the multi-varied set of nonspecific and indiscriminate physiological state changes instituted in responses to a range of physiological/environmental contingencies. According to Weiner (1985), this work arose out of various laboratory and clinical/medical observations which were descriptive and correlational in method and spanned the years from approximately 1936 through 1954. Seyle's choice of definitional terminology does evidence that he was writing during the zeitgeist of the Behaviorist School. But contrary to strict S-R behaviorist thinking, Seyle sought to describe multiple, fuzzy-boundaries *sets* of physiological symptoms which comprised the state-change, and which he felt constituted a generalized adaptive response to internal/external (and often multi-context dependent) environmental stressors. These symptoms, which could appear to be an amorphous, collectively-negative functional change in bodily state, were felt by Seyle to actually be the body's first-stage-attempt to muster and effectuate an adaptive response. In effecting a change from its no longer successfully-regulated homeostatic state (due to the onset of some internal or external stress [or perceived threat of stress] encountered but not immediately surmountable, the body was instituting a state-change-process which, if functioning correctly, would lead to the body's recovery and reinstatement to its former homeostatic set point, a/k/a: The State of Wellness. Foreshadowing future theoretical developments in Behaviorism (a movement from strict S-R thinking to an acknowledgment of behaviors elicited by a nexus of context dependent cues; R-S-S-R-S-R), Selye defined stress as the seemingly indeterminate group of physiological and/or psychological antecedent *stimuli* that illicit an indeterminate group of physiological responses (Johnson, Kamilaris, Chrousos & Gold, 1992).

1.1 Selye's "General Adaptation Syndrome Theory" (GAS).

Diseases produced by stress Seyle termed: *"Diseases of Adaptation"*. As Munck, Guyre and Holbrook (1984), and later Munck and Naray-Fejes-Toth (1992) observe, however, if one were to make a strict application of the evolutionary term to Seyle's process, it would be better phrased as "*Diseases resulting from Adaptation-Gone-Awry*" since Seyle was not intending to indicate that pathology was the result of a successful Darwinian adaptational process. By utilizing the then already popular theory in developmental psychology of stage, and biologically oriented state theory motif, Selye went on to describe how the organism incurred a systematic state change under the following three, linear, progressive conditions embodied in his General Adaptation Syndrome Theory, briefly summarized as follows:

(1) The first-stage response was termed by Selye as the "*Alarm Reaction Stage*". Physiologically registering some threat to systems equilibrium, the body initiates "an immediate sympathoadrenomedullary discharge" (Johnson, et al, p 117) or change in hormone production. The purpose of this change is to move the body into a defensive state of countershock.

(2) The second or "*countershock*" stage, is called the "*Stage of Resistance*" and is marked by further adaptive-defense, physiological responses by the body (i.e., activation of the HPA axis, steroid productions/secretions concomitant with increased neuronal activity, among others). These defensive responses, which are adaptive in short term but dam aging to the body in the long term, serve to hold the progression of further, possibly dam aging, stress resistance reactions at bay long enough for the body to physiologically successfully meet its current environmental challenge, and eventually return to its former homeostatic steady-state. In the event that the body lacked the ability to muster a successful second stage resistance, the maladaptive, negative state-change-process would spiral down through successive stages of

generalized, non-specific, maladaptive response states, ending in a third state termed: The "*Stage of Exhaustion*".

(3) In this third stage-that of *exhaustion*, the body relapses out of the second-stage defensive, stress holding pattern, back into the first-stage, the Alarm Reaction Stage (thus expressing all of the mired physiological symptoms circumscribed/constrained by that stage); but this time there is no possibility of effecting a reinstatement of the countershock/second-stage defensive state. This extremely pathological form of the first-stage state terminates in "death-by-disease of [*mal*]adaptation". As stated before, Seyle's Diseases of Adaptation describes the failure of the body to utilize homeostatic mechanisms to defend its cells, tissues, organs and systems successfully against the effects of both short-term and long-term, stress-induced state change. Seyle himself noted that some defensive state changes if held too long by the body without successfully shifting it back to the originally held homeostatic condition, create its own pathological state. In effect, the resistance to stress state instituted by the body has a time-limited positive effect -which could be measured in terms of minutes to months to years, depending on individual variability within the organism or species. Weiner (1985) cites Seyle's example: A raise in aldosterone levels increases the body's natural defenses against infection and injury, but this state change *held for too long a time* also can lead to hypertension and myocardial necrosis eventually resulting in death.

Seyle's General Adaptation Syndrome Theory model is a very general theory which arose from correlative experimental data/observations and not from closely controlled experimental manipulations of specific variables which would allow for the determination of a systematically defined causal relationship It does *not* answer most questions arising from the model in terms of mechanistic specificity. Therefore, it gives us clues as to why but it is vague as to how, or exactly what or when. Seyle's theory is a

simplified process theory. If one were to describe where GAS Theory belonged on a historical time line of the development of current homeostatic process theory, it might be safe to say that in a Behaviorist world of successive approximations, it falls several steps short of the penultimate response. Even so, it was for many years the touchstone; a viable framework for evaluating what and how to look at the endocrine system and which questions would, should or could be asked to discover how it functioned.

However, GAS Theory is mired in linearity and lacks acknowledgment of multiple co-activational levels, process-sensitive, time-dependant, set-point-reorganizable plasticity (not to mention the focus on relationships between systems' components at different levels of organization perspective), which could afford it the more sophisticated degree of acute theoretical analysis seen in some of newer, modern theories. For instance, the later derived Gottliebian Probabilistic-Epigenetic Theory is a 20th century, second-generation, *plastic* synthetic product of the fusion-synthesis of certain theories in the fields of Biological Development and Psychobiology. Making application of the developmentally framed Probabilistic Epigenetic Theory to the current body of knowledge of anatomical structure and physiological function of the neuroendocrine/endocrine systems would allow for a level of description and understanding of specificity of physiological function, at both a systematic and mechanistic level, that was not possible for Selye and his colleagues to attain, especially at the then current state of technology (Turkewitz & Devenny, 1993).

1.2 Probabilistic Epigenesis

"*Probabilistic Epigenesis*" is a concept often ascribed to Gottlieb but the tenets of the theory were further elucidated by both Kuo, and Schneierla, among others. In their view, the mature adult/end-product of development is determined by a probabilistic process in that, although it is influenced by an underlying genome, exactly what, which, how and how much of those genes will be expressed is determined by the genes'

interaction with its immediate environment. Further, this relationship is bidirectional due to the fact that the environment is impacted on and changed by such gene product production (by both the process itself and the products produced from the process). In developmental time, the genome never exists in a vacuum and any interaction is actually *co-directional* from the onset, and relative to the ongoing level of analysis (i.e.: Interactions between cell-tissue-organs-systems, and all levels in between). This is not to imply an ongoing parallel process approach; it is parallel plus. As Lerner states, the components within the levels have an ongoing dynamic interaction *between* levels of organization and they are "reciprocally influential over the course of human ontogeny." (In Turkewitz, et al, p 41). Structural maturation and physiological function are also co-directional in that the ongoing developmental processes in one plane can serve to anchor, activate and maintain certain developmental processes in the other plane, and visa versa (Gottlieb, 1993). In this fashion development progresses gradually and continuously, but not necessarily linearly, within a system that allows for a certain degree of individual variability in the mature organism, even if the entire population were genetic clones (Storrs & Williams, 1968; Kuo, 1976). This is partly due to the fact that every individual, to some degree, encounters its own unique environment. An example of this is the study by Storrs, et al (1968), of 9-banded armadillos. Nine-banded armadillos normally produce monozygous quadruplets which are genetic clones. These researchers measured 20 physical parameters immediately after birth (including the spleen, brain and adrenals) and found that 12 out of 20 parameters evidenced differences, some up to 140 fold. Co-actional processes among the extra genetic cytoplasmic environment and the inherited genome at the level of proliferation of various differentiated cells is held by the authors to be the source of such variation.

Just as it can be said that ontogeny recapitulates phylogeny--but only to a point--it can also be said that baring genetic mutation or some cataclysmic reorganization (for

further reference see Gould, 1982), the developmental end-product of a lizard zygote over the time span of a single generation is going to be an adult lizard, and *NOT* a chicken. But, *THIS* lizard has a *range of* possible variability in physiological and behavioral outcomes for its mature/adult state. This adult outcome will be probabilistically determined during the developmental epigenetic process, even if it is still constrained by its genome during development to meet the overall phylogenic species criteria: "still a Lizard and *NOT* a chicken." To a degree even further constrained by what is currently developmentally adaptive relative to its immediate environment, the range of expression of genes (notwithstanding its stockpile of junk genes) is probablistically determined during the adaptive processes encountered *throughout* the individual's lifetime. This *slack* (range of probabilistic gene expression) in the developmental system is said to be *selected out* by evolution at the species level; at the level of the moment-to-moment adaptive processes experienced by an individual throughout its lifetime, it entails the regulation of the individual's genetically controlled production of amino acids (protein production) which is modified at the cellular level by numerous environmentally triggered homeostatic processes (i.e. neurotransmitters, hormones, enzymes, chemical/electrochemical secondary messengers and Immediate Early Gene gene cascades, temperature, pH, energy metabolism, catabolism, etc., etc.) is also termed "plasticity". It is not limited to anatomical structural and physiological function; the co-activation of dynamical systems within the brain/body is also plastic, although the degree of such plasticity (once again, baring genetic mutation and extreme changes in environment during development) is, again, endogenous/exogenous-environment-dependent, time-dependant and species-specific.

1.3 Time v. "Timing".

In any system where the individual components are co-actional (i.e. have mutual ability or capability to impact and change the other and, consequently its relationship to

and with the other), direction of change becomes relative to locus and moment of process, and to the temporal and causal order which we impose on it so that we can cognitively grasp and understand the phenomena (one could use a Kantian argument on the imposition of order, or an argument of constraint of human "wiring" to defend this assertion). The notion of causal events (like change), implies linearity -from something to something- within some designated time frame; but the time frame itself is relative to your instant point of reference, and the relationship between individual points of reference can be non-linear within a field of points [having an Einsteinian 4-dimensional distribution]. It is *not* that quantities and events cannot be measured in complex systems (just as it is *not* the case that everything is relative--but it is relative to the observer at a particular point in time and space) it is just that your measurement remains relative to your construct of time. Gottlieb himself has posited that since time is a construct, treating it as if it were an experimental variable on a par with physical and social variables (i.e., hormones, neurotransmitter, electrical brain activity) that can be manipulated in the same manner, has yet to be proven acceptable (Turkewitz & Devenny, 1993). Turkewitz et al., (1993) have argued that although time may be a construct, the "temporal relationships between the components of development that change during the course of the development of the organism" can be termed "Timing", which is treated in their theoretical analysis as a thing different from the relative construct "Time" and your criteria of field. Whereas, as Gottlieb says: Time might be treated only as a "background, scaffold on which other more concrete influences...are seen to make their contribution.", Timing -if seen as a measurable change in the relationship between systems- could be considered and treated as a distinct variable for use in theorizing and an independent, manipulable, causal variable in developmental studies. This difference in perspective could be seen as a matter of relative focus. It also could be said that, to (somewhat pompously) paraphrase Shakespeare, in studying systems from a

developmental perspective, Timing may not be the all; in fact, it may not even closely approximate the *Be all to end all*, but it certainly necessitates serious consideration of it as one of the most important causal agent(s) in a developmental system where "...coaction of multiple influences ...is the hallmark."(p x) From a utilitarian, pragmatic standpoint (a position not unfamiliar to Western scientific pursuit), valid, sophisticated statistical paradigms already exist to analyze data produced from studies where Timing is identified as having a relationship to other variables and the weight of this relationship among other co-existing relationships can be determined. It would seem that a circumscribed cost-benefit analysis might indicate that, based on the number and quality of comparative studies that have shown Timing to be extremely important variable, it would be more costly to ignore it than not (for further explanation, see: *Pascal's Wager.*) Meta-analysis by its own definition overarches and subsumes more linear, reductionistic, cause-effect relationships[1]. In doing so, it makes some linear thinkers extremely nervous; some may even adopt an attitude similar to that which William James expressed in his comment on metaphysics (defined as an abstruse branch of philosophy that in seeking first principles, must go beyond what can be learned by mechanical and physical analysis), in which he characterized it as "nothing more than an unusually obstinate effort to think clearly." Others, of course, see the use of a systems approach in Developmental Biopsychology as the *only* logical, valid way to handle something as complex as *process of becoming* (Turkewitz, 1993, p. 7, 8).

1.4 Developmental Time v. Real Time.

Probabilistic Epigenesis also brings to the forefront the idea that within developmental processes, developmental time is not necessarily equated to real time. Events (including change) happening within a developmental time frame may not carry

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[1]. According to Gottlieb, this traditional way of thinking--limiting explanation to *only* "sources-of-variation", may appear to be less unwieldily and unmuddled, but it is also invalid.(5, p x)

the same degree of impact (or weight) as those experienced in more strictly regulated, linear "real" time.(Lerner, Perkins and Jacobson, 1993). Seemingly small co-actions can have disportionately large consequences, and so, in this paradigm the nature of development is not direct, but indirect and probabilistic. In this vein, subtle changes between levels of organization of developing systems can have a major impact on the adult organism's structure and function (i.e., maternal effects, teratogen effects, cognitive processing differences-laterality, etc.,etc. in individual development; even to the point of generating new species development, i.e. neoteny, heterochrony (Gottlieb, 1993; Kuo 1976; Gould, 1982). How these ideas apply to this dissertation study is that, based on the above, one could propose that changes in stress induced homoeostatic hormone response magnitude[2] could be the result of components within different levels of organization within the overall system being expressed in greater than normal or less than normal amounts at sensitive periods of time in development, so as to cause a reorganization in later, adult homoeostatic regulation of hormone response *magnitude* to stress. Turkewitz and Devenny (1993), in their book Developmental Time and Timing, state:

> "...all qualitative changes in development occur as a result of heterochronic relationships (between components having different rates of development) in a system. Furthermore, we believe that reorganization occurs when a stable organization is disrupted by a component of a system changing at a rate that is different from others within the system. According to this view, slight differences in the relative timing of components can produce changes in a system, and reorganization becomes

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[2]. Changes in stressed-induced "homoeostatic hormone response magnitude" could be generated by changes in physiological interactions, changes in structural capacity to produce and sustain those interactions, (which are dose and time dependent), and/or a synergism between the two (i.e., an emergent property).

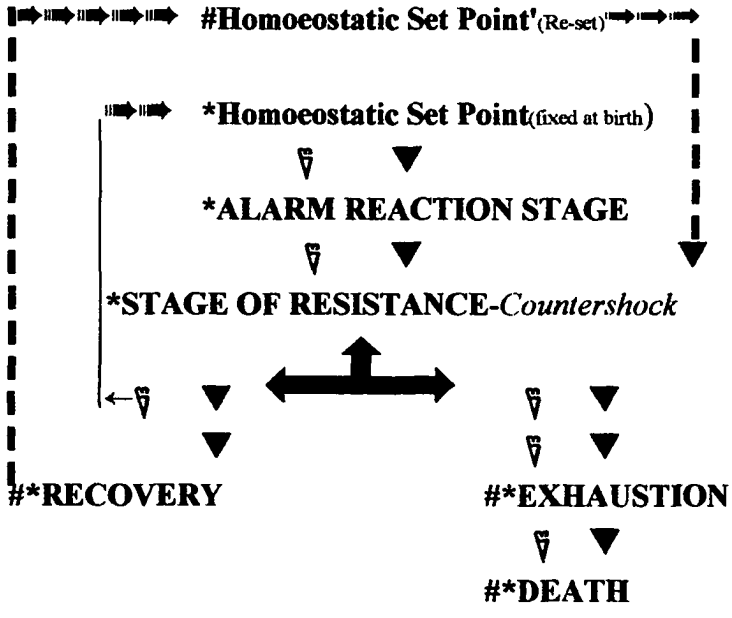
a consequence of new relationships between components."(p 9)

Furthermore, because plasticity exists before, during and after development in vertebrates, physiological function is actually a range of possible function, including the functional interaction between bodily systems and the overall homoeostatic regulation of those systems (Turkewitz, et al,1993; Gottlieb, 1993). Over time and throughout the entire course of the development of the individual organism, there are many homoeostatic state-shifts, temporally both temporary and permanent. A very limited aspect of this type of probabilistic-homoeostatic shift is what I attempt to describe and test at a behavioral level in this dissertation.

1.5 Tyhurst on GAS - Shift in Homoeostatic Set Point.

Seyle's contemporary, Tyhurst (1953), challenged the Selyian concept of homeostasis. Earlier in the 20th century, Walter Cannon originally coined the term to describe the body's attempt to institute and maintain the fight-or-flight response in situations of high physiological or emotional stress. However, Selye described homeostasis within the context of his concept of Stage of Resistance as contained in GAS Theory: Homeostasis is that state in effect prior to the onset of the (first stage) Alarm Stage. Homeostasis is that state to which the organism, in changing from the first stage to the second stage (the Stage of Resistance), is attempting to forestall the occurrence of any further negative state changes until the body, through various adaptive/defense mechanisms, can reinstate itself back to its former, most baseline homoeostatic state. Selyian homeostasis denotes a complete and unaltered return to an optimum, prior, fixed set-point range state in which the organism normally functions, and functions best (Weiner, 1995). Tyhurst was one of the first to watershed (what could be described as) an application of probabilistic epigenetic theory to Selye's seminal theory.

1.6 Tyhurst Introduces: Seyle(* ∇) to Gottlieb(# ▼):



Discussing this criticism, Weiner (1995) notes that Tyhurst and Rioch (1971) both proposed that any extreme perturbation in the current state of the organism would cause a permanent reorganizational shift of the primary/baseline state.

Three further changes in concepts dealing with homoeostatic control of the body by neuroendocrine mechanisms focused on the proposed dichotomies: (1) the idea of Hypothalamic-Pituitary-Adrenal Axis (HPA) v. central/local effects; (2)the concept of pharmacological v. physiological effects; and (3) the argument regarding permissive v. regulatory v. suppressive effects.

2.0. HPA v. Central/Local Effects:

Having long been the focus of intensive study, the HPA has been recognized as the premier controlling mechanism of the body's response to endogenous/exogenous environmental stress through its release of secretagogue hormones, neurohormone, and various releasing factors (Rang, Dale, Ritter & Gardner, 1995; Kandel & Schwartz, 1985; DeSouza & Nemeroff, 1990; Dunn & Berridge, 1990). It also serves to regulate normal

endocrine function although it is by no means the only regulatory system utilized by the body in the control or restoration of homeostasis, or in the regulation of various physiological responses to a range of different stressors, including immunological stress responses.

2.1 CNS v. Endocrine Activation of HPA Hormones.

The HPA uses cells contained in the paraventricular nucleus of the hypothalamus (PVN) (brain or central locus) to produce hormone releasing factors and various secretogues which are released via (1) the median eminence of the hypothalamus located inside of the central nervous system (CNS); (2) the anterior pituitary which contains the superior hypophyseal artery and the adenohypophysis which are all considered to part of the endocrine system (its structural tissue during ontogeny is derived from mesoderm not ectoderm) and is located outside of the central nervous system; (3) and via the posterior pituitary which contains the neurohypophysis and superior hypophyseal artery which releases oxytocin and vasopressin; this area is considered to be inside of the CNS (its structural tissue is derived during ontogeny from ectoderm not mesoderm (Storrs & Williams, 1968; Angulo & McEwen, 1994; Purves & Lichtman, 1985; and Pick & Howden, 1977).

2.2 CRF and Activation of HPA in Stress v. Extra-hypothalamic "Local" Effects and Activation of HPA in Stress.

Corticotrophin Releasing Hormone (CRH) (used synonymously with Corticotrophin Releasing Factor (CRF)), is a fully structurally characterized 41-molecule amino-acid peptide which by itself, and at times in conjunction or synergism with several other CRF-*like* hormones [i.e. AVP, ADH, CCK, a-MSH-various pairs of which are often co-produced, stored an released in the same neuron], is the primary hormone responsible for activation of the HPA in response to either psychological or physiological stress (Pitch, Lorang, Yeganeh, Rodriguez de Fonseca, Raber, Koob & Weiss, 1995; Hooks,

Jones, Liem & Justice, Jr., 1992; Walker, Perrin, Vale & Rivier, 1986). In recent times, it has been recognized that CRF, when administered centrally (directly into key sites of the brain) can initiate behavioral stress effects similar to those initiated by the HPA, even in hypophysectomy/adrenalectomized animals. Since CRF is a comparatively large peptide which does not readily cross the blood brain barrier, the behavioral stress-like effects produced by *local or central* administration of hormones into various brain nuclei are recognized to have their origin of initiation of ultimate net behavioral responses inside the brain and CNS (Dunn, et al, 1990; Menzaghi, Rassnick, Heinriches, Baldwin, Pich, Weiss & Koob, 1996; Angulo, et al., 1994). Such stress-effected overt and covert behavioral responses are similar to those resulting from HPA activated stress responses, presumably having a point-source of initiation of such net behavior primarily in the periphery via activation of hormone production at targeted end-organs (i.e., Glucocorticoid). What this [false] dichotomy omits is that some of the hormones produced in the periphery in response to HPA-stress-induced activation have their behavior producing effect in the central CNS. Central CNS is also where the main negative feed-back loops for the shut down of the HPA are located, blood brain barrier notwithstanding. As discussed later, this reductionistic dichotomy (central v. periphery) is a notion that may be valid but still not be *true*. It's utility lies as a means of organizing an approach to thinking about the problem that is less unwieldy, however as Gottlieb points out when speaking of the process of development, some phenomenon should not be treated within this paradigm especially when to do so ignores the between/multi-systems functionality which is embedded in the nature of hormones and homeostasis (Munck, et al, 1984, 1992; Rang, et al., 1995).

In the intact animal, central or extrahypothalamic administration of CRF has been shown (over time) to activate the HPA through central/local activation of several discrete afferent neural pathways running ultimately to either the paraventricular nuclei of the

hypothalamus or the median eminence of the anterior pituitary, or both (DeSouza, et al., 1990; Dunn, et al., 1990; and Menzagahi, et al., 1996). Which afferent pathway is activated is dependent upon which area(s) of brain nuclei receive the local application of CRF, or activation via several different efferent neuro pathways if administered by intracerebral ventricular (i.c.v.) infusion (Heinrichs, Mezaghi, Pich, Baldwin, Rassnick & Koob, 1994; and Stenzel-Poore, Heinrichs, Riveist, Koob & Vale, 1994). The discriminative information here is that similar stress-like, net behavioral effects can be produced via (1) activation of the HPA directly, or (2) by direct local activation of various central/local brain areas, or (3) by indirect ultimate activation of the HPA after stimulation of central/local brain areas via neural-chemical pathways. The chemical activating mechanism is the same in any of the routes: A specific group of neurohormones also having neurotransmitter functional capacity, that have the ability to elicit, mediate, modify, permit and regulate differential responses in a neuron/synaptic/tissue/pathway-dependent fashion which is also dose specific and time dependant (Rang et al., 1995; Kandel et al, 1985; DeSouza et al., 1990; Dunn et al., 1990; 1973; Tache & Rivier, 1995; Menzaghi et al, 1996; and Angulo et al., 1994). These neurohormones do not function independently; they, in turn, function in a micro/macrocosmic environment, populated by other hormones, dedicated neurotransmitters, excitatory amino acids, endogenous opioids and a proletariat of secondary messengers, anabolic and catabolic pathway products, protease, ligands, etc., etc., etc., all of which comprise and define the galaxy of systematic (but not necessary linear) interactions within the brain and body, functioning together to effect homoeostatic regulation of physiology.

2.3 <u>Endocrine v. Neuroendocrine</u>.

Over time and after reaching a certain dose/levels criteria, locally induced effects produced by stressor type hormones will elicit an endogenously activated HPA stress

reaction (resistance) effect; the fuzzy-boundary between the neuroendocrine and endocrine systems functionally disappear (Munck et al., 1984, 1992; Rang et al., 1995; and Kandel et al., 1985). This is a time and process dependant dynamic shift. What variables actually comprise and originate a shift of this order of magnitude? As J.A. Fodor (1985) writes, when speaking of "The Modularity of Mind" and *where* in human consciousness it might be predicted that bottom up perceptual (physiological) processes most likely meet top-down cognitive (psychological) processes:

> "No one in his right mind doubts that perception interacts with cognition *somewhere*. What's at issue in the disagreement between modularity theory and "New Look" Cognitivism (e.g. Bruner 1973) is the locus of this interaction."(emphasis added)

To paraphrase Fodor, no one in their right mind doubts that psychology interacts with physiology ***somewhere***... to produce mind/brain processes, i.e. Brain state, which is maintained by the co-active functions intrinsic to psychological/physiological homeostasis throughout the brain and body.

Developmental dysregulation of the homoeostatic mechanism acts to (1) change the underlying mechanistic physiological capability for response to environmental stressors; (2) this reorganization during development produces either a magnified stress response or a down-regulated HPA axis response function.

2.4 Adaptive v. Non-Adaptive Response.

An argument could be made, however, that the developmentally ontogenist dysregulation of the homoeostatic mechanism, if viewed from another level of analysis is not dysregulation but simply an example of the expression of the plasticity in higher vertebrate neurogenesis selected out by evolution. According to Darwin, the basic biological processes underlying adaptation of a species are one and the same with the

adaptation of the individual across lifespan; albeit different time frames (Grey, 1994; and Darwin, 1859). Although evolution is not teleological, the same processes may result in different ends and whether that end serves the survival of the species (inasfar as individual members live long enough to reproduce healthy offspring that are viable–the Darwinian definition of adaptation,) it is *not* functionally necessary that such processes serve survival of the individual after the point of reproduction (Storrs et al., 1968; Grey, 1994; and Darwin, 1859). Anthropomorphically speaking, Adaptation is the servant of Evolution and if a choice has to be made between serving two masters, the needs of the individual member of the species will loose out to the needs of the species' survival. The processes of adaptation are ongoing in utero: i.e. if a foetus is developing in an environment which contains stronger (quantitatively and qualitatively) than normal physiological stressors during critical/sensitive periods in the development of its homoeostatic mechanisms, then, and in that event, a change in the organization of the individual's individual components which serve to regulate homeostasis, to a new organization that will generate a larger magnitude-of-response to environmentally encountered stressor hormones **IS** an example of a positively adaptive response for that individual member of the species. And should the individual's environment continue to be -as Seyle stated- in the short term highly stressful, then this increased magnitude-of-response by the body to stress onset, would be functionally appropriate and adaptive - an example of adaptive processes working at the level of the individual to get him/her to live until it could reproduce. Where, then, could this reorganization which seems to meet the criteria for an adaptive response (immediately for the sake of the individual and eventually for the survival of the species) go so wrong as to cause the type of pathological dysregulation of homeostasis which ultimately results in the failure of the process which it evolved to serve, either at the level of the individual, or the species if the individual did not survive to the point of reproduction?

From the individual's perspective, (assuming it wished to survive past reproduction) it might not be necessary to be dysregulated to the point of being in a functionally pathological state to consider yourself in *systems failure*. It may be sufficient that the quality of their ongoing state be such that they are motivated to self-medicate; i.e. seek and self-administer abusive pharmacological substances in an attempt to regain a state more akin to that experienced in normalized homeostasis (Wolffgram & Heyne, 1995; Pitch et al., 1995; Menzaghi et al., 1993; Hooks et al, 1992; and Dunn et al., 1990). So, again, how could it be that an adaptationally-correct developmental shift in homoeostatic response to stress, ultimately serve a negative outcome?
Possible answers involve:

(A) The increased or decreased magnitude-of-response to environmental stressors is, as Seyle said, time-dependant as to positive function; and/or

(B) The negative feedback mechanism designed to protect the body from this hyporesponsive or hyperresponsive stress-hormone response -(i.e. the production of deficient or excessive glucocorticoid, glucocorticoid receptors or changed binding affinity) was not concomitantly reset, or reset insufficiently, or not adjusted proportionately to functionally counteract the (developmentally changed by adaptation-processes) increased or decreased magnitude-of-response to the onset of stressors in the fetal or perinatal environment (Takahaski, 1994; Sapolsky & Meaney, 1986; Piazza, Deroche, Deminiere, LeMoal & Simon, 1993; and Maccari, Piazza, Deminiere, Lemaire, Mormede, Simon, Angelucci & LeMoal, 1991).

2.5 Developmental Timing and Reorganization of Stress Response.

Timing in development has been shown to be a critical factor (Levine, 1993; Turkewitz, 1992; Takahashi, 1992; Sapolsky et al., 1986; and Walker et al., 1986). It could be the case that the structure of glucocorticoid, i.e. expressed levels of the hormone itself, receptor type, number, and binding affinity, are the same as in a "normal"

population prenatally, but a dysfunctional reorganization of the glucocorticoid negative feedback system could be produced due to the existence of a sensitive/critical period of development after birth. During this sensitive/critical period, it is necessary to restrict or dampen glucocorticoid levels in order to (1)protect another still-developing brain structure [see Turkewitz, 1992; 1993, on hemispheric differentiation] which is vulnerable at that specific time to damage by having high levels of the steroid present; or (2) to allow another part of the system to finish its development (Meaney, Bhatnagar, Larocque, McCormick, Shanks, Sharma, Smythe, Viau & Plotsky, 1993; Kenny & Turkewitz, 1982; Lickliter, 1993). There is a body of research evidence that supports the existence of such a critical period in development for the rat population. The *Brain Growth Spurt Period* (BGSP) which is a period that is said to roughly equate to the last trimester of human development (Goodlet, Leo, O'Callaghan & Mahoney, 1993; Napper & West. This is a period of accelerated neurogenesis and synaptic generation/pruning which coincides with The *Stress Non-Responsive Period* (SHRP) during which glucocorticoid production within the neonate is tightly regulated by a dam /pup interaction because it is necessary to keep the developing neural structures exposed to a very specific range of glucocorticoid to promote normal neural development (Hofer, 1981; Takahashi, 1994; Tache & Rivier, 1995; Walker et al., 1986; Meaney et al., 1993; and Walker et al., 1991). There is also work to support the notion that there is a "SHRP" period in humans which, although not identical to the rat, may prove to be a sensitive period of brain development (Hoyenga et al., 1993; and McEwen, 1987). To what extent these proposed answers apply to homoeostatic control of stress responses and reward/hedonic state interaction, is a question currently being asked by several researchers (Piazza et al., 1993; Maccari et al., 1991; and Deroche, Piazza, Casolini, LeMoal & Simon, 1993).

3.0. Pharmacological v. Physiological Effects:

Selye' perspective about the *pharmacological v. physiological* effects of hormones in the HPA and central/local sites are not currently held. With the realization that many hormones also have neurotransmitter capabilities depending on their locus of action and effect, the notion that some of the adrenal hormones (i.e. glucocorticoid) might have different types of actions and effects within the body generated a further discrimination of function (Rang, 1995; Kandel et al., 1985; and Munck et al., 1984; 1992).

Physiological effects of hormones are said to be an endogenous hormone's actions in regulating various aspects of the body's resistance to stress; according to Seyle, they require elevated levels of the hormone to be produced in order to regulate and protect against the negative effects of stress (Munck et al., 1992; Johnson et al, 1992; and Weiner, 1965). Permissive effects act to protect against certain physiological responses to stress but do not require an elevation of hormone from baseline levels in order to effect this protection; Ingle has been credited with this criteria and term. Physiological effects are those hormone functions carried on in all cells, tissues and systems of the body which are circadian-diurnal rhythm constrained and fall within the range of normalized function or steady-state homoeostatic process. Under this definition physiological effects, when acting in a regulatory fashion, would also include the function of the HPA in conferring resistance to stress (Gottlieb, 1993; Kandel et al., 1985; and Pitch et al., 1995).

Pharmacological effects have in the past been viewed as those effects that glucocorticoid have when they are exogenously administered, or when they are endogenously generated by the body in large amounts in response to inflammation or other immunoreactive processes. Pharmacological effects act to suppress currently ongoing, specific, negative threats to the body's steady-state homeostasis (i.e. infection, injury, extremely aversive external environmental changes, etc.). (Munck et al., 1996; Gottlieb, 1993; Kandel et al., 1985; and Hofer, 1981). Similar to medicinal drugs,

pharmacological effects function to correct a well circumscribed dysfunction or pathology.

In 1984, Munck, Guyre and Holbrook published a hypothesis that approached a Gottliebian-type understanding of endocrine/neuroendocrine interactive function and, in particular the *physiological v. pharmacological* dichotomy, from another, more systematics, probabilistic oriented level of analysis (Munck et al., 1996; Kandel et al., 1985.; and Pitch et al., 1995). Question: When does glucocorticoid simultaneously function in the pharmacological and physiological mode? Answer: When it n*eed*s to, at a particular point in time, and within the demands and constraints of the current ongoing states of all of the body's co-active systems.

4.0 Munck on Time/Dose Dependent Function of HPA Hormones.

Munck, and later others, proposed that glucocorticoid, have pharmacological effects that are, simultaneously, functioning in a physiological mode. Where previously it had been held that pharmacological effects have functions that are qualitatively different from physiological effects, Munck asserted a seemingly new idea that glucocorticoid have functions and effects that can be qualitatively different and distinct *at some times*, yet function in tandem at other times depending on certain quantitative criteria (dose/levels, in/between/across systems-HPA-CNS-immunosuppressive-central/local); the passage of time itself; onset of effect (timing); the homoeostatic state of the organism before, during and after the onset of stress, and change or perturbation in the system (Munck et al., 1985; 1992; Gottlieb, 1993; and Pitch et al., 1995). Timing is so important because, as the philosopher Woody Allen says: "Time is nature's way of keeping everything from happening all at once." (Lickliter, 1993, p 105). Who knew Woody ascribed to Newtonian Physics?

Munck held that generally glucocorticoid serves a *regulatory function* (using the term in the same manner as Ingle), and functions to stimulate the HPA under elevated

stress conditions in order to afford resistance to negative stress effects. Munck also accepts the notion of permissive effects, which do not require elevated levels to confer hormone protection. However where Munck becomes singular is when he asserts that very high levels of glucocorticoid, beyond what is produced by either regulatory or permissive functions, exist for the *sole* purpose of suppressing and protecting against the body's normal defensive reactions to stress itself. Left unchecked this high range production of glucocorticoid can cause tissue damage from run-amuck metabolic processing, suppression of the immune system, and retarded healing. The body will continue to operate at an increased level of resistance to stress functioning that eventually becomes destructive if maintained for too long a period of time; therefore, the original pharmacological action of glucocorticoid takes on a physiological role in that it "prevents overshoot of the body's immensely powerful defense reactions, which might otherwise themselves threaten homeostasis."(Rang, p 439).

Current research in this area has expanded on this Levels Theory to include the inverse relationship: HPA axis function when glucocorticoid response levels are reset during development to a level of hypofunctionality. In this case the amount of glucocorticoid released in response to stress-induced ACTH stimulation (which primes glucocorticoid release from the adrenal glands) is either insufficient to shut down CRF production (a time and levels-dependant negative feedback function of GLUC), or, the glucocorticoid receptors in the negative feedback loop are functionally deficient and the hormone cannot have its intended effect because of faulty receptor function. This approach to understanding glucocorticoid function under stress stimulation does not negate Munck's work. Instead it rounds out the search for understanding the timing and levels of release factors involved in properly functioning HPA axis set-point. More of this work is included in the discussion section below as it supports the dissertation findings (or visa versa).

This historical background material is relevant to the questions asked in this dissertation work. It has been said that *you cannot see where you are going if you don't know where you have been.* The general question underlying developmental studies can be categorized under the topic heading of "Nature v. Nurture". Even in the current Zeitgeist (particularly in the wake of the successful feat of mapping the human genome), there are some researchers who believe that the Nature v. Nurture controversy represents a false dichotomy which leads to spurious research. They insist that what is actually inherited from generation to generation is the lifetime developmental epigenesis process- - and nothing short of it. They assert that the minute to minute, dynamic functioning of the genome within its immediate internal and external environment is probabilistic in nature, with a range of possible outcomes ordered within a continunium, whose boundaries are constrained by its species specific parameters (baring some cataclysmic change in the environment). But within the individual's lifetime development, these same dynamic processes allow for a remarkable amount of plasticity which opposes any absolute biological determinism of behavior. The concept of "Levels of Reversibility", described by Hoyenga and Hoyenga (1993) state as follows:

> "Not only can environmental manipulations have behavioral as well as anatomical effects similar to those of hormone manipulations but the brain anatomical connections formed before birth are only one part of the cause of the complex behaviors....Although the effects measured at the level of brain anatomy *may* not be reversible, the effects measured at the behavioral level may be reversed by altering the organism's experiences (which presumably also change some part of the brain's anatomy but maybe not the same part)....*sometimes* postpubertal hormones can

> have relatively irreversible effects, and *sometimes* perinatal hormone effects can be reversed." *(p 126,* emphasis added*).*

This next-generation theory outdates the controversy debated in developmental biology hormone research of *activational v. organizational effects*, and replaces it with Levels of Reversibility Theory. The former dichotomy fails to reflect the most recent research findings on the brain plasticity throughout lifetime development (Luine, Grattan,& Selmanoff, 1997; Luine, Martinez, Villegas, Magarinnos & McEwen, 1995). As the Hoyengas' further point out, dose, liver and kidney clearance, levels of stimulus input or feedback levels of inhibition (regardless of source of generation-exogenous or endogenous) to the system, changes in ratios of hormone levels, transmitter levels, gene or environmental changes in cell's metabolism or cytoplasmic metabolic machinery,- in short, all ongoing physiological processes, including the homoeostatic regulation of those co-activational processes are all relative to the organisms's collective *State*, and State is time-dependant.

As was said earlier, critical time periods in development do constrain some organizational outcomes. The idea of Fixed Action Patterns occurring during fixed, unmodifiable Critical Time Periods in the course of normal development has long been abandoned by most developmental psychologists; however, there are time limitations and irreversible changes in brain function arising from either pathological developmental processes, or from those falling in the extreme end of the range of possible functional outcomes (borderline phenotypic/gene expression). Some brain anatomy is less plastic than others and even though "bathed in the same regulatory hormones and neurotransmitters" as adjacent brain tissue, their range of functional response to the presence of similar levels of those same regulatory/modifying substances (hormones, enzymes, metabolic metabolites) may have been permanently fixed during a critical time

period window (Hoyenga, 1993). Much of current brain research is focused on discovering plastic brain processes, as well as the limits of those processes, and the underlying co-activational mechanisms of control, such as the environmental triggers of gene activation, pre and post co-activational cellular cascades where *structure and function and time and levels together* define functional process.

Because some non-reversible Critical Windows do exist in development, a normally minor imbalance in brain chemistry during a particularly critical period[3] in brain development *can* permanently dysfunctional reorganize the individual's homoeostatic machinery and forever constrain the organism's capacity to initiate and effect physiological or organizational plasticity. Any serious negative developmental shift in the functional response of the Hypothalamic Pituitary Axis has the *potential* to be, in a sense, *the most devastating insult an organism can encounter within its individual lifetime short of non-viability.* Depending on the degree of response dysfunction, the inability to reinstate brain homeostasis can be the source of major depression, bipolar disorders, obsessive compulsive behavior and many hormone imbalances which can affect social adjustment and parenting behaviors (Gold, Licinio, Wong & Chrousos, 1996; Post, Weiss, Smith, Rosen & Frye, 1995; Stratakis & Chrousos, 1995; Kunovac & Stahl, 1995; and Bachus, & Kleinman, 1996). Research by several labs, including that of Meany-Plotsky shows that aspects of this negative reorganization can be passed to offspring by non-genetic or quasi-genetic inheritance (Francis, Diorio, Liu & Meaney, 1999; Champagne & Meaney, 2001; Meaney, 2001; and

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3. Any developmental time period that is cannulated, that is, it possesses a range of outcomes but existing within specific fixed parameters. The parameters are generated by the genome co-actionally functioning within its immediate environment. Any modification capability, or degree of reversibility is "capped" by a time-limited organizational state, and it is usually (but not exclusively) an early developmental process.

Monk, 1995).[4]

It has been said that it doesn't matter how often you perturb a system; what counts is the systems' ability to recover, and the time it takes to do so.

Following are the methods and statistical analysis of a numerically "small" group of experiments; what the realistic constraints of limited time, funding and human resources would or could allow. This researcher thinks of it as the Concrete Garage attached to that Theoretical Castle covered in my original Dissertation Proposal; or (hopefully) more accurately, a concrete foundation on which to build.

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4. Depending on the point of origin of your level of analysis—Similar to the human imposition of Time construct constraints–cross-levels, non-linear phenomena may, in fact, *be* 'happening all at once'–but human language alone, as it is presently constructed, fails in its ability to communicate this type of phenomena--the vocabulary as yet doesn't exist. Understanding relegated to "Gestalt", can not be quantified, measured, independently observed or reliably replicated; it can not be used to objectively describe, predict or control behavior. As one researcher in my program insists: "Research without data is philosophy and *not* Science." True, but, on the other hand, data alone-without the application of clear, valid, hypotheticodeductive reasoning (Logic has its origins in Philosophy) --is just a collection of numbers. .

PART II - INVESTIGATIONS

5.0 Statement of Questions Specifically Addressed by This Research

5.1 Main Inquiry: Experiment No. 1.

Does perinatal stress result in a functionally changed hypothalamic-pituitary-adrenal axis response to environmental stressors (i.e. a negative reorganization of homoeostatic regulatory capability) which persists to adulthood, and which can be experimentally tested (Experiment 1 - Auditory Isolation Treatment by Early Deprivation during PND 2 through 9; once adulthood is reached, subjects are then tested in an open field procedure and scored on five different measurements of stress/anxiety in response to a drug or saline challenge).

5.2 Experiment No. 2:

In several reviews of the Early Deprivation literature covering this field of inquiry, such as that published by Lehmann and Feldon (2000)(see Table 1), and others, a call is made to standardize methodological issues to reduce the number and sources of inconsistent findings. The use of an *auditory mask* is fairly standard in Early Deprivation paradigms. According to The Dictionary of Psychology, auditory masking is "....any process whereby a detectable or recognizable stimulus (called the target) is made difficult or impossible to detect or recognize by the presentation of a second stimulus (the masker) in close temporal or spatial proximity to it." (p 419).

Pups emit ultrasonic vocalizations when separated from their mothers. When tested simultaneously in the same location the pups' perception of being isolated might be voided if they were aware of the vocalizations of their siblings. Some form of *white noise*, if played continuously is usually somewhere between 70-80 dB, which is the standard used for this type of auditory mask. White noise is an auditory stimulus that contains all frequencies and such frequencies are in random fluxuation. However, in that the masking noise itself might be a variable which produces the Isolation Treatment

Effect, Experiment No. 2 was designed to test a subgroup of animals using an identical isolation paradigm but without any masking noise present in the environment. This was done in order to assess effects that the inclusion or exclusion of this variable in the isolation methodology may make in the adult behavior.

5.3 Subinquiry No. 1: Necessity of Drug as Stress Primer.

If subjects receiving Isolation Treatment perinatally are later tested in adulthood, and they exhibit behavior different from controls (indicative of a changed HPA or Hypothalamic-Steriodial-Axis [HPSA] response to stress), was it necessary to administer a psychostimulant (drug) stressor when testing them in order to elicit the behavioral changes? Or is it the case that animals Isolation Treatment subjects would exhibit more stress-related behavior than controls without the necessity of priming the stress response by administering psychostimulant drugs? According to Kehoe, Shoemaker, Triano, Callahan and Rappolt, (1998): "The question of whether a challenge is necessary to reveal enduring behavioral differences in the isolated animals remains open....Although the conditions of a saline injection and placement in a novel open-field environment constitute a mild environmental challenge, this level of challenge may not be strong enough to reveal statistically different behaviors due to prior isolation experience."(p 123) Are the effects of the Early Deprivation Treatment alone during this specific perinatal period strong enough, and the inferred changes in the HPA axis response to stress permanent enough, to be elicited by only a mild stressor, i.e. receiving an injection of saline and being placed in the open field test?

5.4 Experiment No. 3:

To investigate whether there was a prolonged physiological activation of the HPA axis, post-baseline open field tests were conducted. During post-baseline tests no drugs were administered (and a suitable washout period observed, i.e. no physiological trace of the exogenous drug was left in the subject's plasma). The Postbaseline behavioral tests

measure (1) for a second time the effects of the Isolation Treatment, (2) residual effects that could be attributed to the original open field procedure itself, and (3) tests for residual effects of the psychostimulant given at the original test; and/or (4) for any interactions between the separate variables.

5.5 Subinquiry No. 2:

To investigate the possibility that loss or gain of weight *during* the isolation procedure might somehow make a contribution to the behavioral outcome as hypothesized in the Main Inquiry, Experiment No. 1 (i.e., Isolated subjects exhibit behavior different from Control subjects), a small subgroup of pups were weighed *after* the daily isolation procedures. Note that all groups were weighed before the daily procedures. Also, adults were weighed immediately prior to the original open-field test (i.e. before the administration of any drug) and again just prior to the post-baseline open-field test (when no drug was administered) to assess any statistically significant, long-term, between-group differences in the weights which might be attributable to the either perinatal isolation treatment alone, or to the combination of the perinatal isolation treatment and drug effects.

6.0 Experiment No. 1 -Effects of Early Deprivation on Adult Stress Response (Measurement: Open Field Behavior).

Various comprehensive reviews of the history and development of the Early Handling and Early Deprivation research manipulations have been published. Table 1 is a comprehensive historical review and summary by Lehmann and Feldon (2000) of the multiple versions of these two methodologies existing in this field. Other referenced reviews include a more recent publication by Pryce and Feldon (2003) and multiple review articles by Meaney et al. (2002) and Levine, et al.(2002) . As emphasized in the Pryce-Feldon article, the Early Deprivation and Early Handling Procedure is a potentially robust methodology for manipulating and studying environment-gene interactions during specific developmental stages using this animal model.

Lehmann and Feldon argue that while much of the reviewed research has yielded important information with supporting data, and has generated prolific new research, the variability in the methodologies between laboratories has limited the generalizability of the data beyond the immediate study, and losing the ability to make comparison of these findings across studies is a loss to those working in the developmental area. Hormone research, particularly when using a behavioral measurement, presents numerable challenges due to the fact that the work is not limited to a slice of brain tissue confined to a Petrie dish where a researcher can, with patience and skill, identify, quantify then manipulate the individual chemical variables in an attempt to measure an intended interaction. Quantifying variables in a larger, more complicated test environment, and then holding the physiological state of a whole animal constant while manipulating another variable (or set of variables) which are intended to effect immediate change in hormone levels in the animal, but long-term changes in the developmental outcome of the animal's function (as researchers regularly do with this procedure), is *demanding* to say the least. Both the Early Deprivation procedure and the Early Handling procedure

TABLE No.: 1. Summary of Table by Lehmann & Feldon (2000), *Reviews in the Neurosciences*, 11, 383-408, by permission.

Parameters exerting critical influence on neuroendocrinology and/or behavior: Frequency; duration of MS; age at MS; temperature during MS; short separations, cross-fostering, culling; contact with littermates(physical ,auditory); prenatal stress; experience; sex of the animals; litter size, sex composition; maternal behavior; housing conditions (group housed/single; grid floor/sawdust); statistics; age at testing; strain differences; control groups.

Abbreviations: MS=maternal separation; EH=early handling; NH=non-handled; Not all studies mentioned here have been carried out in the context of maternal separation. References to individual studies listed below are given in the published article.

Strain/Sex	MS procedure	Control subject	Age at start of test	Culling/Cross-fostering/Cleaning	Pups per litter
Sprague-Dawley/both	1 hr/day;PND 2-9(only some pups per litter); pups placed individually in plastic chambers	NH controls and handled littermate controls	10 wks.	Culling on PND1	MS & handled controls; 63 pups from 10 litters; NH controls; 21 pups from 5 litters
Wistar/males	1 hr. on PND 5-20; in litter in new cages PND 5-10; individually PND 11-20	"normal" controls and EH controls (15 min PND 5-20)	8 wks.	Cross-fostering on PND 3; cage cleaning once a week	Details not given
Wistar/both	2 hr/day from PND 0-28	EH(3 min)& controls(no details	Beg. PND 43	Cull within 12 hrs.after birth	91 pups from 13 litters
Sprague Dawley/both	3 hrs, PND 2-21;pups placed individually into cups	NH & EH(15 min.)	6 wks.	Pooling on PND 1;cage clean 1 x per week	Pups pooled & randomly assigned to treatment groups
Wistar/both	3 hrs; PND3-12;litter in new cage on heating pad	"normal" controls	4 mos.	Cull on cross-fostering at PND1; cage clean 2 x /wk.	6 litters
Sprague-Dawley/males	4.5 hrs/PND1-21; litters in new cage	Undisturbed controls	7 wks.	Cross-fostered on PND0;clean cage 1x/wk.	60 pups from 14 litters
Sprague-Dawley/males	5 hrs/PND 2-6(pups individually in incubator)	NH&EH, no cage clean	PND 45	Culling/PND2	Max. 2pups/litter
Long-Evans/both	6 hrs/PND2-15;either Warm(34°C)or AGIT(warm&moved);indiv.pups/litter;MS individually	Controls were weighed, remained w/dams;(reduced litter size)	PND16,28 & 75	Sexing & cull on PND1	No litter effects
Wistar/males	16hrs./PND3-14; only half of litter & injected w/saline	Within litter controls, injected w/saline	PND80	Cull on PND0	Details no given
Wistar/both	2hrs/PND15,18 & 21	EH(3 min)& controls(no details)	Beg. PND43	Cull w/I 12 hrs.after birth	91 pups from 13 litters
Listar-Hooded/both	Obtained at 13th day of gestation; 10x6 hrs./PND 5-20(litter placed in wire baskets)	Control handled(placed in wire baskets/5 min.)	8-10 wks.	Details not given	Details not given
Listar-Hooded/both	Obtained at 13th day of gestation; 10x6 hrs./PND 5-20(litter placed in wire baskets)	Control handled(placed in wire baskets/5 min.)	11 wks.	Sex & cull/PND2	Experimental groups from 3 diff. Litters

Listar-Hooded/both	Obtained at 13th day of gestation; 10x6 hrs./PND 5-20(litter placed in wire baskets)	Control handled(placed in wire baskets/5 min.)	13 wks.	Sex & cull/PND2	59 pups from 6 litters
Strain not given/females	Obtained at 1week prior to parturition; 10x6 hrs./PND 5-20(litter placed in incubator)	Control handled(placed in incubator for 5 min.)	8 wks.	Cull after birth	30 pups from 6 litters
Wistar males & females(bred in-house)	6 hrs. on PND 12,14,16,18	"normal"controls	12 wks.	Sex and cull/PND0	Max.2 related subjects/group
Wistar males & females(bred in-house)	6 hrs. on PND 12,14,16,18	Relatively undisturbed controls(cage clean on PND10)	12 wks.	Sex & cull/PND0	Unrelated subjects in each exp Group
Wistar/males	8 hr./PND2,4,6,8,10(plus daily injectionsPND1-10 of vehicle)	"normal"controls (daily vehicle inje.PND1-10)	10 wks.	Cull 6 hr./birth	Details not given
Wistar/both(bred in house)	24 hrs./PND4 or 9 or 18; litters in home cage (34°C)	Undisturbed control	3 mo.	Cull on PND1; cage clean once on PND10	Unrelated subjects 48 litters
Wistar/?	24 hrs./PND10;litter remains in home cage 22-024°C	Details not given	7 wks.	No cull	11 litters
Wistar/both	24 hrs./PND3,6,or 9;litters in home cage	Controls were separated for 20 sec.	Exp 1,3:>9 wks. Exp 2:5 wks.	Exp 1 no details; Exp 2-4 cull on PND1	Exp 1:2-3 litters per treatment; Exp 2: details not given

have allowed researchers to test *in vivo*[5] the effects of environmental factors on hormonal function at a particular time point in development. It is then possible to infer from test measurements taken at various time points in the animal's life, what effect over time (no time passage, no process!) the experimental manipulation produced when measured against a standard or control. Using Time as a variable (more precisely, a Time-Tool) (Gottlieb notwithstanding) is as much intrinsic to the process of developmental research and discovery as it is to development itself[6]. The ability to make some reasonable comparisons of data from one project to another project, certainly could produce more consistently reliable information (more –as Lehman and Feldon phase it, "face value"), thus allowing for more rapid advances by the developmental research community. Considering the aims of this type of research, the potential for application to improvements in health and life expectancies, and the ultimate reduction in numbers of research animal lives, then promoting some standardization in methodology that results in "face and construct validity" seems reasonable and worthwhile. For most participants in research any proposed reformation which could result in savings in the very expensive cost of conducting research -much less redundant research- be it money, time, animal lives or human resources -elicits attention. Lehman and Feldon's proposed standardization of control groups and statistical methodology deserves consideration. They set forth a relevant argument on the use of an early Non-Handled (NH) group defined as the "complete absence of handling [both experimental and husbandry related] in the breeding cage between birth and weaning."(p 2). Basically, they state that after a comprehensive review of the data from those published studies that have used NH groups, and after reviewing their own work which utilized NH groups as control groups,

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5.In studies of developmental brain systems' interaction, this is often analogous to conducting *non-invasive brain surgery.*

6. THE virtual tool, perfect for performing that *non-invasive brain surgery*!

they have aligned themselves with a current move by some researchers in this field to abandon the inclusion of NH groups in future research. Aside from the bandwagon argument cited (i.e., Meany, Plotsky and Levine have all "moved away from it[NH]), it is noted that the NH procedure -since it deviates so drastically from most known, accepted Animal Facility husbandry practices- serves to produce an animal that would not be similar to any other laboratory rat and, therefore, it is really an instance of an experimental manipulation of standard maternity housing and practices." (p 19). In this view, NH groups are experimental groups not control groups. A similar argument was made in past years regarding pair feeding as a control measure, basically stating that restricting access to food under any circumstances is an experimental manipulation and not the standard experience of laboratory rats. It could be noted that not only does NH constitute an experimental manipulation of the parturition-to-weaning period for lab rats, but instances of confining a dam and pups to an extremely restricted location (equal to a 22"L x 12"W x 8"H maternity cage) without the ability to remove waste or to move the pups from accumulated waste would NOT often be found outside [in nature], as waste materials can signal the presence of the pups to predators and dams would act to avoid the possibility of exposing her young. The abnormal confinement within the NH procedure could possibly introduce an unintended stressor variable and an unintended, and therefore uncontrolled for, experimental manipulation. It may be that some researchers have provided special housing for NH that would overcome this in the published studies There is an animal model of depression currently in existence which makes use of a visible burrow system, but it is structured so as to *produce* stress not reduce it. Also, it should be noted that "control group" as used in these dissertation experiments designates a group of animals which received the standard Animal Facility husbandry practices followed by the Hunter College Animal Facility, and they are set forth in the "Methods" sections below.

This first experiment investigates if the particular Early Deprivation procedure used in this study will result in subjects with a changed HPA stressor response when tested in adulthood. It is hypothesized that the Main Inquiry Experimental Group (Litters No. 1, 3 and 5) which will receive the Auditory Isolation Treatment on PND 2 thru PND 9, when tested as adults will show significantly different behavior when compared to the Main Inquiry Control Group (Control Litters No. 1, 2 and 3). Specifically, it is predicted that: (1) the subjects receiving Auditory Isolation Treatment perinatally and then tested as adults, will, after receiving a 2.0 kg/bw, dose of Amphetamine, show significantly greater scores on all of the test measures when compared to each and every of the other combined-condition groups, that is, the Isolation/Saline, No-Isolation/Saline and No-Isolation/Amphetamine conditions; and (2) it is also predicted that the Isolation/Saline Condition would produce lower scores than the No-Isolation/Saline Condition across all measures.

6.1 METHODS

6.1a Subjects.

All animals were maintained in accordance with the NIH Guide for Care and Use of Animals. Six time-pregnant Sprague Dawley dams were obtained from Charles River Laboratories (at three separate intervals, two dams shipped at a time), and timed to arrive at the Hunter College Animal Facility on E15, allowing five to seven days for acclimation to the Facility before parturitions. The dams were inspected and weighed upon arrival at the facility and then singly in their colony room (occupied only by the subjects in this study). They were housed in clear plexiglass containers (measuring 18-1/2 in. L x 10-1/2 in. W x 8-1/2 in. H for double housed, and a 20 in. L x 12 in. W x 8-1/2 in. H plexiglass container for triple housed animals) with approximately 1-3/4 in. to 2 in. (3.1025 cm to 5.08 cm) Beta Chip bedding covering the floor. All animals were maintained on a 14:10 light/dark cycle with lights on at 5:00 AM. In accordance with the

standard husbandry practices of the facility, the room was entered once a day to check food levels, provide fresh water, record colony temperature and humidity, but after the initial arrival procedures and transfer to the maternity cages, no other person handled any of the dams or offspring subjects at any time over the length of the entire experiment, the one exception being that a technician aided in sexing the animals at weaning. Maternity cages were visually checked once a day and pups born prior to 5:00 PM were designated PND 0. Cage changing was performed twice a week in order to comply with the Facility's requirements. Purina Rodent Diet, Lab Diet 5001, and fresh water was available ad libitum. Colony Temperature was maintained by the laboratory building's heating and central air-conditioning system, and with the aid of the installation of permanent supplemental thermostatically controlled space heaters and humidifiers; the temperature in the colony was maintained in a range from 68° F to 75° F, with humidity between 33 % to 55 %. Temperature and humidity gauges were monitored during the light cycle. Dams produced between 11 to 13 pups, one pup was cross-fostered on PND 1 and one pup died before the isolation treatments were completed. Cross-fostering involves removing a pup from its natural dam (as soon after birth as possible) and placing it with another dam, usually to even out pup numbers within litters. The pup is rubbed with a slurry of the adoptive dam's feces and urine before placing in the pup huddle to facilitate acceptance. Table 2 sets out the number and composition of the litters.

Except for cage changing and testing, the animals were not handled, per the requirements of Isolation Paradigm. Aside from weighing the pups on PND 2 through PND 9, no additional weighing of the animals was done until (1) just prior to the first open field test procedure in adulthood; (2) just prior to the second open field test procedure (post-baseline) in adulthood; and (3) just prior to being sacrificed after all procedures had been completed.

Table 2. Subject distribution by conditions for Main Inquiry, Auditory Isolation Treatment Group (EXP 1,3,5) and No-Isolation Treatment Control (CG1,2,3) (Animal Facility Reared) Group

		Male	Fem	Tot	Tot
EXP-1			1		
	Amp	2	4	6	11
	Sal	1	4	5	
EXP-3	Amp	2	4	6	12
	Sal	3	3	6	
EXP-5	Amp	6	1	7	12
	Sal	3	2	5	
Tot		17	18		35

	Amp	Sal	Tot
EXP-1	6	5	11
EXP-3	6	6	12
EXP-5	7	5	12
Tot	19	16	35
CG-1	5	6	11
CG-2	7	5	12
CG-3	7	6	13
Tot	19	17	35

		Male	Feml	Tot	Tot
CG-1	Amp	2	3		11
	Sal	2	4	7	
CG-2	Amp	3	4	7	12
	Sal	2	3	5	
CG-X	Amp	5	2	7	13
	Sal	4	2	6	
Tot		18	18		36

	Isol.	No-Isol.	Total
Drug	19	19	38
No-Drug	16	17	33
Total	35	36	71

Weaning took place on PND 25 at which time the sex of the pups was determined and they were placed into same-sex group cage with their litter mates. The clear plexiglass cages were placed on two, 4-tiered shelves in the colony; cages were systematically rotated between shelves once a week at one of the cage cleans. Two further rehousings took place for each litter at similarly designated intervals to accommodate growth rates. When the dams were delivered they were randomly assigned as either experimental or controls. Random assignment was used for all grouping procedures thereafter and for assignment to all test procedures. Within each litter, animals were randomly assigned to cages with litter mates, by sex, by groups of two or three per cage. Table 3 sets out the cage distribution by litter, experimental group, number per cage and sex.

TABLE NO. 3 Number of Rats per cage

Sub. No	Cage No.	Grp No.	d/o/b	sex
1	1.0	CG1	02/15/02	m
2		CG1	02/15/02	m
3	2.0	CG1	02/15/02	f
4		CG1	02/15/02	f
5	3.0	CG1	02/15/02	m
6		CG1	02/15/02	m
7	4.0	CG1	02/15/02	f
8		CG1	02/15/02	f
9		CG1	02/15/02	f
10	5.0	CG1	02/15/02	f
11		CG1	02/15/02	f
12	6.0	CG1	02/15/02	f
13		CG1	02/15/02	f
14		CG1	02/15/02	f
15	7.0	EX1	03/13/02	f
16		EX1	03/13/02	f
17	8.0	EX1	03/13/02	f
18		EX1	03/13/02	f
19	9.0	EX1	03/13/02	m
20		EX1	03/13/02	m
21		EX1	03/13/02	m
22	10.0	EX1	03/13/02	f
23		EX1	03/13/02	f
24	11.0	EX1	03/13/02	f
25		EX1	03/13/02	f
26	12.0	EX2	03/14/02	m
27		EX2	03/14/02	m
28	13.0	EX2	03/14/02	m
29		EX2	03/14/02	m
30	14.0	EX2	03/14/02	m
31		EX2	03/14/02	m
32	15.0	EX2	03/14/02	m
33		EX2	03/14/02	m
34	16.0	EX2	03/14/02	m
35		EX2	03/14/02	m
36	17.0	CG3	04/03/02	m
37		CG3	04/03/02	m
38		CG3	04/03/02	m
39	18.0	CG3	04/03/02	f
40		CG3	04/03/02	f
41	21.0	CG3	04/03/02	f
42		CG3	04/03/02	f
43		CG3	04/03/02	f
44	22.0	CG3	04/03/02	m
45		CG3	04/03/02	m
46	26.0	CG3	04/03/02	f
47		CG3	04/03/02	f
48	19.0	EX3	04/04/02	f
49		EX3	04/04/02	f
50		EX3	04/04/02	f
51	20.0	EX3	04/04/02	m
52		EX3	04/04/02	m
53		EX3	04/04/02	m
54	23.0	EX3	04/04/02	f
55		EX3	04/04/02	f
56	24.0	EX3	04/04/02	m
57		EX3	04/04/02	m
58	25.0	EX3	04/04/02	f
59		EX3	04/04/02	f
60	26.0	CG3	07/03/02	m
61		CG3	07/03/02	m
62	27.0	CG3	07/03/02	m
63		CG3	07/03/02	m
64	28.0	CG3	07/03/02	m
65		CG3	07/03/02	m
66		CG3	07/03/02	m
67	29.0	CG3	07/03/02	f
68		CG3	07/03/02	f
69	29.5	CG3	07/03/02	m
70		CG3	07/03/02	m
71	30.0	CG3	07/03/02	f
72		CG3	07/03/02	f
73	31.0	EX4	07/02/02	m
74		EX4	07/02/02	m
75		EX4	07/02/02	m
76	32.0	EX4	07/02/02	m
77		EX4	07/02/02	m
78	33.0	EX4	07/02/02	m
79		EX4	07/02/02	m

80	33.5	EX4	07/02/02	f
81		EX4	07/02/02	f
82	34.0	EX4	07/02/02	f
83		EX4	07/02/02	f
84		EX4	07/02/02	f
85	35.0	EX5	07/02/02	m
86		EX5	07/02/02	m
87	36.0	EX5	07/02/02	m
88		EX5	07/02/02	m
89	36.5	EX5	07/02/02	m
90	36.5	EX5	07/02/02	m
91	37.0	EX5	07/02/02	m
92		EX5	07/02/02	m
93		EX5	07/02/02	m
94	38.0	EX5	07/02/02	f
95		EX5	07/02/02	f
96		EX5	07/02/02	f

6.2 Testing Room.

All tests were carried out in a small, immediately adjacent, well-ventilated, and temperature controlled room. Testing room temperature was invariably 72 °F (measured with more than one gauge). The room measured 73 in. x 65 in. x 8 ft (187.18 cm x 166.67 cm x 243.84 cm), just large enough to accommodate the isolation chamber and auditory equipment with temperature sensors attached to leads which ran outside the room and could be checked without opening the door. A thermostatically controlled space heater with a built in fan that directed heat out laterally and across the room was placed under the isolation test chamber but the room was so small that once it reached the desired temperature no further heating was required. It also served for the video taped open field testing.

6.3 Equipment.

Isolation Test Chamber. Two Brower Equipment chick brooders were assembled and modified to meet the requirements. The all-metal, sanitizable enclosures measured 33 in. x 42 in. x 11 in. (83.82 cm x 106.68 cm x 27.94 cm), with a heating assembly and thermostat. The thermostat (Model No. BRHTR20/At-10) had an arc of 3 ° C, the internal temperature was kept consistently at 29 ° C to 31 ° C and was monitored by four separate temperature gauges: Sper Scientific digital monitor, Model 800020 (with external leads); Ward's Natural Science Laboratory Thermometer, Catalog No. 15-1473; Ward's Electronic Indoor/Outdoor LCD Thermometer, Catalog No. 23-1620; Ward's Digital Probe Thermometer Model #15-1501; and humidity was measured by Radio Shack Indoor Humidity Gauge Thermometer, Catalog No. 63-1013. Leads were placed inside the Isolation chamber at various locations including inside an empty plastic pup cup, measuring 3 in. high x 9/16 in. in diameter (7.62 cm x 3.1025 cm); the leads ran outside of the room where they could be monitored without opening the door. The chamber walls and floor were snugly lined with 6.0 mm acoustic grade sound proofing

natural cork.. The top cover for the chamber consisted of a wooden frame covered by double thickness white acoustic grille cloth into which eight small windows were cut and covered by eight squares of non-outgasing clear heavy gauge plastic through which the pups and several of the other gauges could be monitored if necessary. The top-cover allowed auditory waves to enter but provided a heat and humidity outward barrier, also serving to filter the overhead ceiling light. Light from the ceiling fixture was measured, using a United Detector Tech, Model 40A Optometer light meter fitted with the standard filter on the meter, and without the light filtering chamber cover, and found to be at a level of 120 lux.. Light level measurements inside the individual small, white pup cups were no different from ambient levels inside of the chamber. Two small ventilation windows were cut in the end-walls of the chamber and one window was cut in the side that faced toward the interior of the room, and covered by the same grill cloth. This assembly was placed on two 30 in. high (76.2 cm) wooden sawhorses against one wall and directly in front of the auditory equipment. Light to the room was provided by an overhead ceiling fixture and light levels were taken both inside the chamber and inside the room.

6.4 Auditory Equipment.

For the purposes of utilization as an auditory mask, a white noise signal was recorded on a Merantz Professional Grade Tape Recorder, Model No. PMD331 using a high grade auditory tape and a Crown PZM-180 pressure zone microphone with a frequency response of 50 Hz to 18 kHz(+/- 3dB). One hour and 15 minutes of signal was taped and then transferred later to a CD. A sampling rate of 44.1 kHz and a word length of 16 bits was used, the "red book standard.", intentionally not filtering the signal in any manner or altering the frequencies contained on the tape in any way A two-tiered metal lab cart was placed directly across from the isolation chamber. The tiers were lined with 2 in. (5.08 cm) thick rubber mats to absorb any vibration from the equipment, and placed

Figure 5 : Position of experimental isolation chamber and speakers. See Methods Section for measurements of room.

under each speaker and receiver was an additional layer of insulating Styrofoam. The CD disk was played on a Marantz, Professional grade Compact Disk player, Model PMD330 through two Technics receivers, Model SA-DX750 (range 40 Hz to 20 kHz), Model SA-DX1050 (range 20 Hz to 20 kHz); one to handle the base signal and one to handle everything else. The signal was played through two Cambridge Ensemble III satellite two-way speakers: a 3-1/4 in. (8.255 cm) cone bass/mid-range driver, a 3/4 in. (1.905 cm) dome tweeter and a crossover. These speakers were mounted on the wall above the cart and angled down towards the top of the insolation chamber. A Cambridge 6-1/2 in. (16.51 cm) long-throw woofer (handling 45Hz to 18 KH, 80 hms, 200 wts), and a Sony, Model SA-WM40 subwoofer (range 20 Hz-170 Hz) and two RCA Model PRO-X88AV Tweeters (range 2000 Hz-25,000 KHz) wired with a Parts Express bass stopper (5.6 kHz/2.8 kHz, Model 266-225) was placed directly across from the chamber. The auditory cables were all professional grade RCA cables with a "monster cable" for the subwoofer. A figure of the placement is attached as Table 5.

Sound pressure levels were measured both inside the chamber and inside the room with a Bruel & Kjaer Precision Sound Level Meter, Type 2203, and were 84 +/- 4 dB inside the room and fluxuating at irregular time points; inside the chamber itself the read was from 74 +/- 4 dB (measured on C-weighting). A Radio Shack hand held sound pressure level meter, Model 332065A was calibrated, and checked against the Bruel & Kjaer meter and used every day to check SPL levels inside the chamber when setting up the equipment.

6.5 Weight Scales

A Ward's Scientific Portable Electronic Balance, Model 99g with readability of 0.001 grams, calibrated regularly, was used to weigh the pups on isolation test days.

6.6 Drug

d-Amphetamine sulphate was purchased from the Sigma-Aldrich Chemical Company, 3300 South Second Street, St. Louis, Mo 63118. Fresh aliquots were made on test days just prior to the beginning of testing. The drug was dissolved in 0.9% physiological saline and then placed into sterilized, individual containers which were placed on dry ice between trials. If tests were run in both the morning and afternoon, fresh aliquots were prepared at Noon time and stored in the same manner for the afternoon trials. Subjects were administered a dose of 2.0 mg/kg body weight, by I.P injection.

6.7 Open Field Test Chamber.

Adult subjects were tested in an open field box made of plexiglass, measuring 18.5 in. x 18.5 in. x 12.5 in. (46.99 cm x 46.99 cm x 31.75 cm) and covered with white paper on the outside surfaces. The floor of the box was divided into nine equal squares with black colored, plastic tape.

6.7a Video Equipment.

A Panasonic Professional/Industrial Video camera, Model AG-185U, was used to videotape all Open Field Trials and Post-baseline Trials. The camera was mounted on a tripod approximately 4 ft (121.92 cm) directly above the open field chamber (which was placed on the floor in the middle of the room) and angled downward. The single source of light was a 40 watt appliance bulb in a lamp fixture mounted on the wall (at a point which was approximately 75 in. (182.88 cm) above the floor, and test chamber and angled away from the chamber so as to diffuse the light.

6.8 PROCEDURES.

6.8a Isolation Procedure:

On PND 2 , pups were removed from the dam and placed into a round plastic container with no bedding in it; half the litter was removed at a time. They were placed in the plastic container under a warming lamp and temperature was measured inside the

pup huddle to see that it did not get warmer than 31 ° C. Each pup was removed one at a time weighed, and numbered with a fine-line permanent Sharpie marker, color coding each 6-pup group, and rotating the colors used each day between groups. (To further control for order effects, the order in which the 6-pup groups were taken from their litter were rotated each day.) The newly marked pup was then returned to its siblings' huddle until all pups had been weighed and marked (took less than 3-4 minutes). At this point, control pups were returned to the mother, and the remaining half of the litter was given the same procedure and returned to the mother.

Experimental pups experienced the same weighing and marking procedure, but instead of being returned to the dam and litter mates, they were then placed into individual round containers and carried all at once into the isolation chamber in the adjoining room, placed into the test chamber about ten inches apart, and the cover replaced and audio started. After one hour passed, they were gathered into one cup while still in the isolation chamber and then carried back to the colony room and returned to the dam. The second half of the testing litter was then given the same procedure. Cups were rinsed thoroughly between tests.

6.9 **Open Field Box Test:**

6.9a **Original Test**.

From the time the isolation procedure ended, until adulthood the animals were not handled except for weaning at PND 25, and changing cages twice a week. When they reached adulthood, subjects within a litter (whole litters were already previously, randomly assigned to either isolation or controls) were randomly assigned to either one of two open field test conditions: (1) those that would receive amphetamine injection prior to being placed in the open field box, and (2) those that would receive saline prior to being placed in the open field box. In double housed cages, one member would

receive drug and one member would receive saline. In cages where three were housed together, they also were randomly assigned to the drug/no drug condition.

Subjects receiving saline were the first of the homecage occupants tested so as to minimize any disruptive (stressor) influences which may occur when returning the amphetamine exposed subject to the homecage. Studies have shown that amphetamine does not clear (pharmacokinetic single dose t1/2: rat = 70 min) for approximately 140 minutes and replacing an animal back into the homecage after the 30 minute test had the potential of stressing the cage mate. After random assignment to cage, cage mates were matched to time of test, but were tested one day apart (at the same time as the cage mate had been tested).

On the day of testing, the animal was taken from the cage and weighed in the colony room; a mark was placed on its tail and it was put back into the home container while the drug dose was calculated and injection drawn from a fresh solution mixed at the start of the tests and distributed into sterilized, individual dose vials; the remaining vials were kept sealed and refrigerated on dry ice. The animal was then taken from its cage, quickly injected I.P and returned to the home cage again for 15 minutes. After 15 minutes, the animal was carried by hand to the testing room immediately next door and placed in the lower right corner of the open field box. The trials lasted 30 minutes and they were videotaped for scoring later. Between trials, the box was cleaned first with an enzymatic solution that removed any pheromes or traces of urine smells, and then cleaned with Equinox labware cleaner and wiped thoroughly dry with paper towels. Animals receiving saline followed the same procedure.

6.9b **Post-baseline Tests**.

The same procedure was followed as the original trials except that after the animal was weighed, a mark was placed on its tail, and it was placed back into its home cage. No drug or saline was administered. After 15 minutes, the animal was carried by

hand to the next room and placed in the open field box in the lower right hand corner. All trials were videotaped for 30 minutes, and scored at a later time. No person was present in the room when either the original or the post-baseline Open Field Tests were run.

7.0 Statistical Treatment - General

7.1 Procedures For Scoring Video Tape.

Other than previewing trial tapes on a daily basis to assure that trials had been recorded on the tape in total, the scoring of the videotapes was not begun until all of the experimental trials had ended and all behavior measures were complete. Attached as Table 6, is a copy of the scoring sheet which contains the rating scale from Daunais and McGinty (1995), which was used to score stereotypy, and also served as a tabulation sheet for both the rearing counts and bolus counts, time samples in bins of 5 minutes each, for 30 minutes. Attached as Table 7 is the scoring and tabulation sheet for Linecross counts, and attached to that sheet is a Stereotypy Assessment cover sheet, which served as a double check of the scoring on the Daunais and McGinty scale which was scored separately and at a later date. This check for reliability of assessment was done in addition to the following multiple-scoring-concordance-checks for accuracy.

A total of 96 subjects were scored on a total of four behavioral measurements each: (1) linecross; (2) stereotypy; (3) rearing; and (4) bolus. Each of these measures were scored in timed bins over the entire 30-minute trial(s). All linecrossing tapes were scored a second time for concordance, and approximately one-third of the total stereotypy measurements and one-third of the total of the rearing measurements were scored a second time to spot-check concordance. Tapes were scored by this researcher and although not meeting a systematic "blind" definition, this researcher can say that since the only visually identifiable marking was an assigned tape number, by the time the scoring was done there was no instant recall of which group that subject number

represented. The counts for linecrossing and the counts for rearing were added to provide a combined Locomotion score (combining horizontal and ventricle movement counts) for each subject.

7.0a. Statistical Analysis- General

The same basic procedures were applied to *all* of the data:

(1) A lengthy preliminary analysis was conducted to insure that it was reasonable to combine the data from the Main Inquiry Experimental Auditory Isolation Treatment Group (Litters No. 1, 3, 5) and to combine the data from the Main Inquiry No-Isolation Control Group (Control Litters No. 1, 2, 3) before running further statistical procedures on them. N.B.: For the sake of clarity, please note that from this section forward the Main Inquiry Auditory Isolation Treatment Experimental Group (Litters No. 1, 3, 5) are sometimes also referred to as EXP#1, EXP#3, and EXP#5 - thereby designating the individual Experimental Litters which comprised the Main Inquiry Auditory Isolation Treatment Experimental Group; also, from this section forward the Main Inquiry No-Isolation Control Group (Control Litters No. 1, 2, and 3) are sometimes also referred to as CG1, CG2 and CG3 - thereby designating the individual Control Litters which comprised the Main Inquiry No-Isolation Treatment Control Group. [7] After being screened in the lengthy screening procedures outlined in (2) below, planned aprori contrasts were run to ascertain if ExperimentalGroup Litters differed from each other, or if the Control Groups Litters differed from each other. Also, post hoc Newman-Keuls analysis of litters were run on results. No significant litter differences were found within either Experimental or Control Groups so the data was combined within each group for

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7. The group designated as Experimental Litter No. 2 was not used in any of the statistical analysis presented herein. At the time of weaning, which was after the Isolation procedure had already been run, it was found that all of the pups in that litter were male, and in view of the uniqueness of a same sex litter, none of the data produced by that litter were included in any of the analysis herein.

analysis. In preparation for this, separate screening was done litter by litter, and then on all combinations of experimental and control groups, and then holding individual conditions/treatments constant (isolation, no-isolation, drug, no-drug, and with and without sex as a variable). Graphs were plotted for each litter's scores. The only Experimental group litter that was found by apriori contrast and post hoc analysis to be significantly different from the other Experimental group litters on any of the measurements was EXP #4, which is the no-auditory condition group; the data from that group was analyzed separately as a separate experiment..

(2) Screening Procedures: Behavioral scores and subject measurements were entered into three data bases, Quatro Pro, Excel and SPSS. SPSS *Descriptives*, *Frequencies*, and *Explore* Procedures were used to screen all of the raw data for errors, and to check sample distributions by frequency plots, and normal distribution plots as well as residual distributions, scatter plots, Q-Q plots; also other graphs were run to aid in determining if the assumptions for the Analysis of Variance procedure would be met, as well as for the Repeated Measures Analysis of Variance. All statistical analysis was done by using SPSS Univariate ANOVA (version 11.0). Further, each of these screening procedures were run for all (a) experimental groups together, then all (b) control groups together, then (c) each of these groups holding each sex constant, and then each (d) treatment condition constant, screening for errors, normal frequency distributions, equality of variances and independence of samples. As was stated in the Methods Section, both random sampling and random assignment procedures for subjects were used throughout the study.

Furthermore, tests built into SPSS were included when running each SPSS Univariate ANOVA and Repeated Measures ANOVA to assess homogeneity of variance (Levene's and Box's Test), normal distribution, independence (Kolmogorov-Smirnov's Test) and homoscedasticity and linearity (in the Repeated Measures tests, if sphericity

TABLE 6 - Stereotypy Rating Scale; Scale for Rearing

SUBJECT No. ____
TRIAL/BASELINE DATE: ____

MODIFIED RATING SCALE FOR DAUNAIS AND MCGINTY(1995)

(1) Asleep, inactive (lying down w/eyes closed/open)
(2) Alert, actively grooming (NORMAL, grooming)
(3) Increased sniffing in one location.
(4) Intermittent rearing and sniffing (NORMAL, alert, active-sniffing and MOVING ABOUT CAGE, rearing intermittently)
(5) Increased locomotion and sniffing (increased locomotion, jerky movement)
(6) Intense sniffing in one location(NEARLY CONTINUOUS sniffing, biting gnawing or licking).
(7) Continuous pivoting and sniffing (with hyperactivity).
(8) Continuous rearing/sniffing OR CONTINUOUS SNIFFING, BITING OR LICKING-SAME PLACE.
(9) Maintained rearing and sniffing,>25 sec.
(10) Dyskinetic-Reactive(Backing up, jumping, abnormally maintained postures; dyskinetick movements-freezing-as described in open field review paper.

BIN 1	BIN 2	BIN 3	BIN 4	BIN 5	BIN 6

THE FOLLOWING ARE COUNTS FOR REARING, SCORED BIN BY BIN

BIN 1	BIN 2	BIN 3	BIN 4	BIN 5	BIN 6
1st 5 min.	2nd 5 min	3rd 5 min.	4th 5 min.	5th 5 min.	6th 5 min.
BOLUS=	BOLUS=	BOLUS=	BOLUS=	BOLUS=	BOLUS=
urine Y/N	urine Y/N	urine Y/N	urine Y/N	urine Y/N	urine Y/N

TABLE 7 - Behavior Scoring Sheet for Linecross

SUBJECT NUMBER:	
SEX:	
SUBJECT GROUP:	
SALINE/DRUG:	
TRIAL DATE:	
TRIAL TYPE:	Open Field
TIME BEGIN:	
TIME END:	
SCORING DATE:	
SCORE-TIME BEGIN:	
SCORE-TIME END:	
BOLUS:	
BEHAVIOR-TYPE SCORED:	Line Crossing=Movement of the animal that brought more than 3/4ths of its body over a line and into the next space or different compartment. BACK FEET HAVE TO CROSS LINE. Diagonal linecross=1.
NOTES ON OBSERVATION:	SCORED IN BLOCKS OF 10 MINUTES EACH. CATEGORY = EVENT Synopsis: First 10 min- Second 10 min- Third 10min-. Upon door opening=Startle, then 4-foot freeze and head lull in that position, avoidance behavior.

TABLE 7(a)-Behavior Scoring Sheet for Stereotypy

STEREOTYPY ASSESSMENT Animal Number:_____ IS THERE CLEAR STEREOTYPY:_____ More than one Type?_____ WHICH TYPES:_____ BEGINS IN WHICH BIN?_____ TRIAL Do Bin Scores increase or Decrease in Progression:_____ APPX. AMT OF FREEZING_____ WHAT BINS:_____ POSSIBILITY OF CORRELATION WITH LINECROSS NUMBERS?_____ INVERSE?_____ IS THERE GROOMING PRESENT:_____ which bins:_____ approx length of bouts:_____ WHEN DOES IT BECOME STEREOTYPIC IN NATURE:_____ VISIT CENTER SQUARE:_____ PORPHRIN MARKING PRESENT:_____ area and amount of marking:_____ PRESENCE OF BOLIS:_____ URINE:_____AMOUNT:_____	BASELINE

and compound symmetry assumptions were not met (Mauchly's Test), epsilon adjustments were made to increase the critical value of F -(Huynh-Feldt Adjustment).

(3) A very thorough inspection showed that although outliers were occasionally found, it did not seem reasonable to exclude any of the data on that basis, nor were any transformation made to any of the data. Reference is made to Table 8, which sets out the distribution of subjects included in the Main Inquiry-Experiment No. 1; and Table 8(a) sets out the subset of subjects used in Subinquiry No. 1; and Table 8(b), which sets out the distribution of subjects included in Experiment No. 2. As was said earlier, as part of the screening procedures frequency distributions, studentized residual plots, scatter plots and box plots were run on all data, which shows the distribution of values in each of the groups, identifies extreme scores and outliers. Since "extreme" is a relative term, SPSS does define the criteria as it would apply to any general set of scores screened by their Box-and-Wisker Plots: (1)"Outliers" are cases with values that are between 1.5 and 3 box lengths from either end of the box; and (2) extreme cases have values more than 3 box lengths from either end of the box. As stated before, box-and-wisker plots were run on all data sets as part of screening, and no extreme cases were found in any of the data. All else considered, it did not seem reasonable to exclude any of the outlier scores. Quoting from Glass and Hopkins (1996):

> "In a *normal distribution* less than 4 observations in 1000 meet the criterion for outliers(>2.70 S.D's from the mean). A further distinction as *extreme outlier* is made for Observations that deviate by as much as three times the Box length below Q1 or above Q3. In normal distributions, (>4.72 S.D.'s from the mean) an extreme outlier would be expected in only about one score in a million." (P 27)

TABLE 8 - Design of Main Inquiry -Experiment No. 1

2 x 2 x 2 ANOVA DESIGN

DRUG/NO-DRUG x ISOLATION/NO-ISOLATION x SEX

	ISOLATION		N=	NO-ISOLATION		N=
SEX	Male	Female		Male	Female	
DRUG	D x I x M (N=10)	D x I x F (N=9)	19	NI x D x M (N=10)	NI x D x F N=9)	19
NO-DRUG	ND x I x M (N=7)	ND x I x F (N=9)	16	ND x NI x M (N=8)	ND x NI x F (N=9)	17
Total	(N=17)	(N=18)		(N=18)	(N=18)	71

TABLE 8(a) - Design of Subinquiry No. 1 - Necessity of Drug Primer

2 x 2 ANOVA DESIGN

ISOLATION/NO-ISOLATION x SEX

	ISOLATION		N=	No-ISOLATION		N=
SEX	male	female		male	female	
NO-DRUG	ND x I x M (N=7)	ND x I x F (N=9)	16	ND x NI x M (N=8)	ND x NI x F (N=9)	17
Totals	(N=7)	(N=9)		(N=8)	(N=9)	33

TABLE 8(b) - Distribution of subjects in Experiment No. 2 and used for comparisons in Inquiry No. 2.

Treatment/Condition	EXP#4-No-Auditory	EXP#5-Auditory	CGX	CG#1
No-Isolation			13	11
Isolation	12	12		
Saline	6	5	6	6
Amphetamine	6	7	7	5
Male	7	9	9	3
Female	5	3	4	7
TOTAL "N"	12	12	13	11
Total Isol/No-Isol	24		24	

Glass and Hopkins further state that although data should be scrutinized to be sure that outliers do not represent "aberrant scores, resulting from data entry error", they also state that "Outliers should not be excluded just because they represent exceptions to a general trend".(p 113) Since none of the data was spurious (in either the Control or Experimental Conditions) none was excluded as an outlier score, nor were any transformations made to the data with the exception of using Studentized Residual plots as part of the screening procedures and assessment.

(4) A three-way ANOVA was run on the data from each of the five measurements, (Isolation/No-Isolation x Drug/No-Drug x Sex). In the experimental analysis there were no more than two groups in any one condition, therefore, post-hoc analysis could not be run on significant Main Effects, however apriori contrasts were run when appropriate. Means were plotted to interpret any interactions found.

(5) Repeated Measures were done on all of the time bins within each of the measures, and line graphs for estimated marginal means were produced.

7.1 Analysis of Experiment No. 1.

After preliminary screening, a Three-way Analysis of Variance was run on all independent and dependant variables (DV = behavioral measures total scores) (IV = Isolation/No-Isolation x Amphetamine/Saline x Male/Female) which is the same as (Treatment x Drug x Sex); with post-hoc Newman-Keuls (where possible), and simple contrasts on each. Further, a Repeated Measures Analysis of Variance was done on each of the behavioral measures over Time, using the individual time-bin scores for each Dependant Variable measure (Time x IV x DV bins). All significant interactions were further analyzed using simple ANOVA's. Significance levels on all ANOVA and post hoc comparisons were set to Alpha (.05).

TABLE 9 - Lists both Auditory and No-Auditory Isolation Treatment Groups v. Controls; Reports Significant Finding from Univariate ANOVA's; also reports for POSTBASELINE Univariate ANOVA results..

litter#	DRUG/SAL	LINECROSS Sig.Scores	STEREOTYPY Sig.Scores	REARING Sig.Scores	LOCOM. Sig.Scores
#4 vs.CGX	both(2x2x2)	MainEff: Drug..0001	MainEff: Drug .0001 Isol x Drug .018	MainEff: Drug .0001 Isol x Drug .012	MainEff: Drug .0001
#4 vs CGX	SALINE(only)	MainEff: Isol .0001 Sex .017	MainEff: Isol .009	MainEff: Isol .003	MainEff: Isol .0001 Sex .047
#4 vs CGX	AMPH(only)	no sig	no sig	no sig	no sig
EX135vCG1 23 all groups tog.	BOTH (2x2x2)	Main Eff Drug-.0001	Main Eff: Drug-.0001 Sex-.170 Interactions: Isol x Drug* .020	Main Eff: Sex .001 Drug .0001 Isolation .106	Main Eff: Drug .0001 Sex .051
EX135vCG1 23 all groups tog **POST-BASE** Line	BOTH (2x2x2)	Main Eff Sex .022	no sig	Main Eff: Sex .008 Drug .039	Main Eff Sex .013
#1 vs CG3	both(2x2x2)	MainEff: drug .0001	MainEff: drug .0001	MainEff: drug .0001	MainEff: drug .0001
#1 vs CG3	SALINE(only)	no sig	no sig	no sig	no sig
#1 vs CG3	AMPH(only)	no sig	no sig	no sig	no sig
#3 vs CGX	both(2x2x2)	MainEff: drug .019	MainEff: drug .0001 Isol x Drug .002	MainEff: drug .009	MainEff: drug .011
#3 vs CGX	SALINE(only)	Isol x Sex .002	MainEff: drug .002	Isol .008 Sex .047	Isol .001 Isol x Sex .022
#3 vs CGX	AMPH(only)	no sig	no sig	no sig	no sig
#5 vs CGX	both(2x2x2)	MainEff: drug .002	MainEff: drug .0001	MainEff: drug 0001 Sex .001	MainEff: drug 001 sex .043
#5 vs CGX	SALINE(only)	MainEff: Sex .010	no sig	MainEff: Sex .018	MainEff: Sex .010
#5 v. CGX	AMPH(only)	no sig	no sig	MainEff: Sex .007	no sig

Figure 1

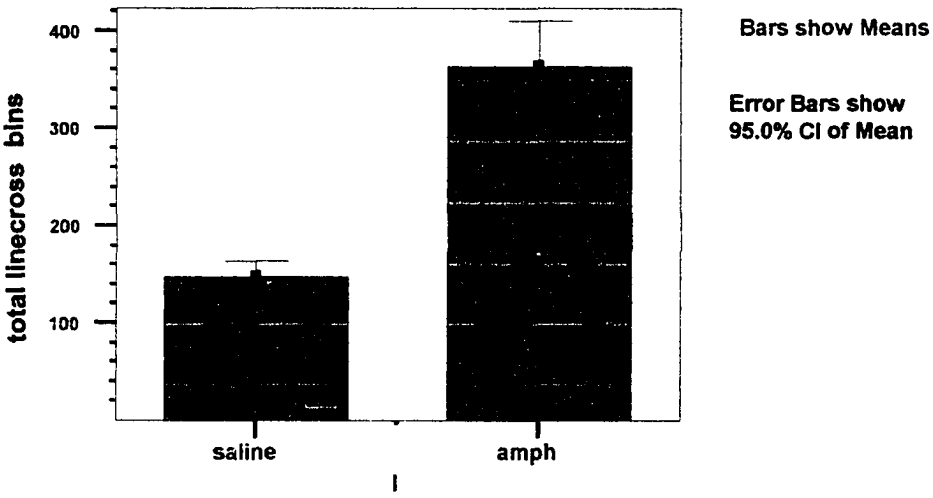


Figure 1(a)

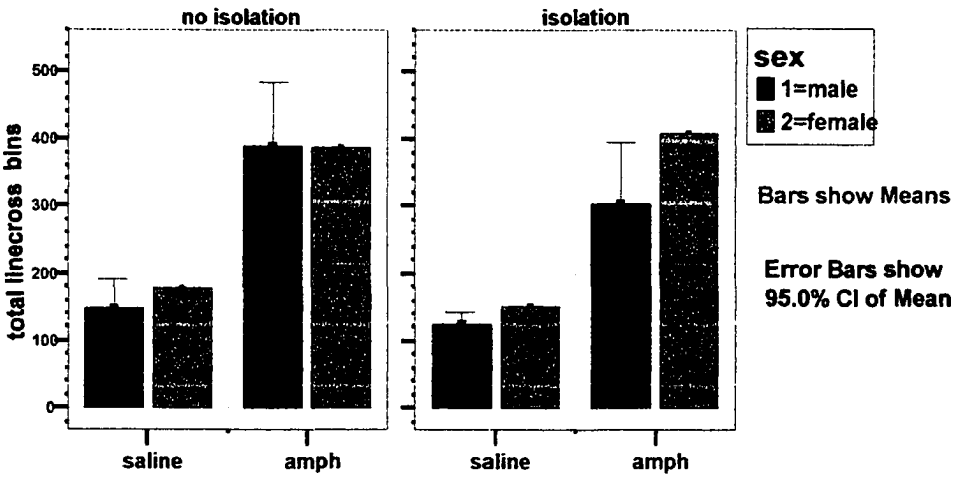


Figure 2

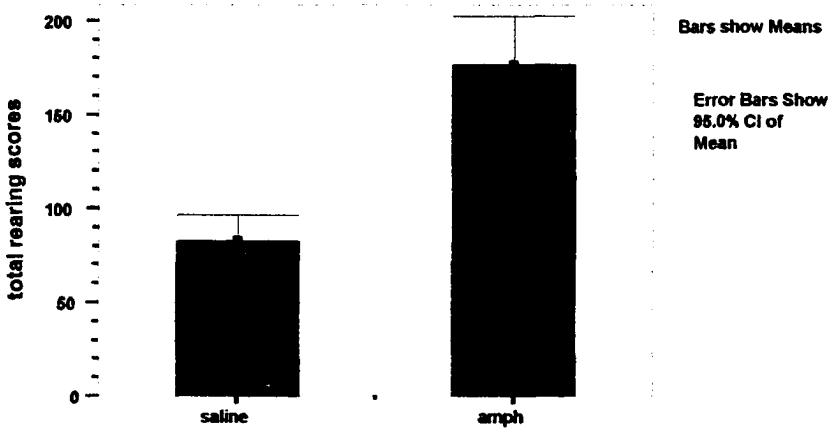


Figure 2(a)

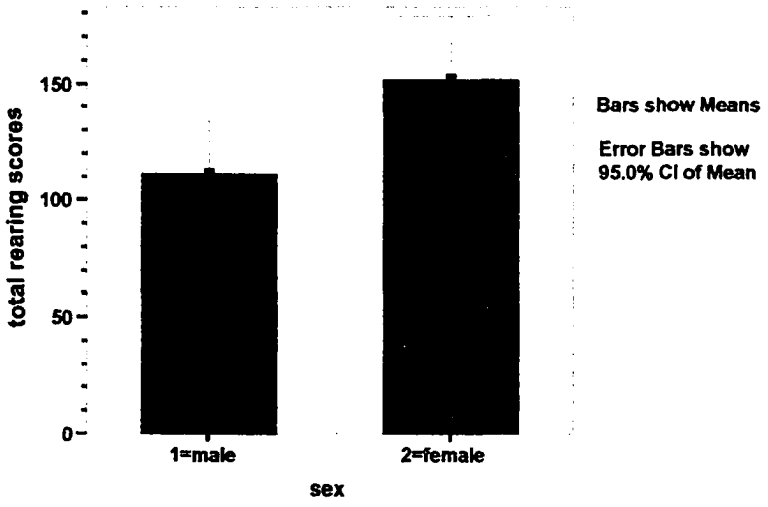


Figure 3

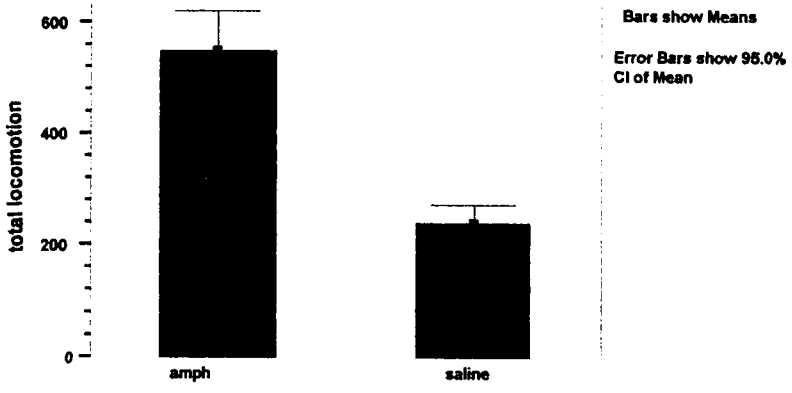


Figure 3(a)

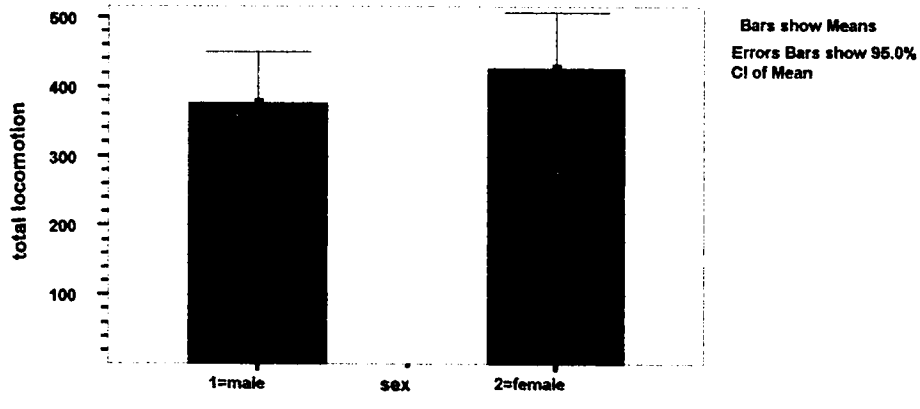
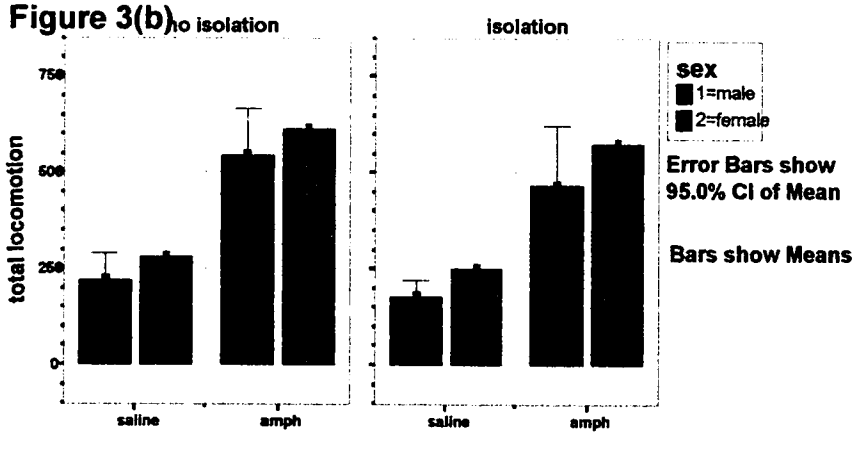


Figure 3(b)



7.2 RESULTS of Three-Way ANOVA'S:

Table 9 sets out all statistical findings under the heading "EXP 1, 3, 5 v. CG 1, 2, 3."

Effect of Drug Challenge on:

(1) Linecross:

There was a significant overall main effect of amphetamine challenge on Linecross scores, $F(1, 17) = 38.363$, $p < .0001$. However there were no other significant main effects, nor were there any significant interactions. A one-way ANOVA confirmed the direction of the drug effect. Amphetamine increased total linecross counts across each of the other two conditions, Isolation/No-Isolation and Male/Female, and is more or less constant across all levels of the other conditions, and without any significant interactions (Figure 1 and Figure 1a).

(2) Rearing and Locomotion:

There was a significant main effect of amphetamine challenge on rearing scores, $F(1, 63) = 52.904$, $p < .0001$ (Figure 2), and a main effect for sex, $F(1, 63) = 11.975$, $p < .001$ (Figure 2a). There were no other significant main effects or interactions. The same was true for *Locomotion*, a significant main effect for amphetamine on Locomotion, $F(1, 63) = 63.271$, $p < .0001$ (Figure 3), and a significant main effect for sex , $F(1, 63) = 3.953$, $p < .05$ (Figure 3a), with no other main effects or significant interactions. The direction of the main effect was that all Conditions receiving drug produced higher rearing and locomotion counts compared to those Conditions receiving saline; also females produced significantly greater rearing and locomotion scores than males across all levels of the other conditions (Figure 3b).

(3) *Stereotypy:*

There was a significant main effect for drug $F(1, 63) = 788.111$, $p < .0001$ (Figure 4), and a significant Isolation x Drug Interaction, $F(1, 63) = 5.715$, $p < .020$ (Figure 4a). As can be seen in Figure 4a, the effect of amphetamine or saline on stereotypy scores

depended on inclusion in the Isolation or No-Isolation Condition. Higher stereotypy scores were produced by the No-Isolation Conditions receiving amphetamine when compared to the No-Isolation Conditions receiving saline; and the Isolation Treatment Conditions receiving amphetamine had higher stereotypy scores than Isolation Treatment Conditions receiving saline. Thus, even though, overall, the amphetamine condition produced higher stereotypy scores than the saline condition, the level of the scores produced depended on whether or not the subject was in the Isolation or No-isolation Condition.

(4) *Bolus:*

There was a significant main effect for the drug/saline condition $F(1, 63) = 5.491$, $p < .022$ (Figure 5), and two significant interaction effects: Isolation x Sex , $F(1, 63) = 5.218$, $p < .026$ (Figure 5a), and Isolation x Drug x Sex , $F(1, 63) = 7.170$, $p < .009$ (Figure 5b). As can be seen in Figures 5, 5a, and 5b, the saline condition produced the highest number of bolus depending on the particular combination of sex and treatment: Males in the Isolation Treatment condition given saline before trials had higher scores when compared with (1) males in the Saline/No-isolation treatment condition; and (2) females in the Saline/Isolation Condition. However males in the Saline/No-Isolation condition had lower scores than females in the Saline/No-Isolation Condition. Males in either of the amphetamine Isolation/No-Isolation Treatment conditions had higher scores than females in either of the amphetamine Isolation/No-Isolation Treatment conditions (Figure 5c).

Figure 4

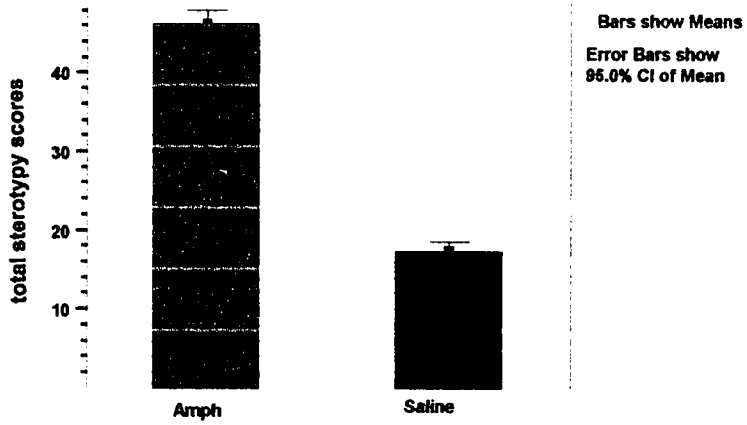


Figure 4(a)

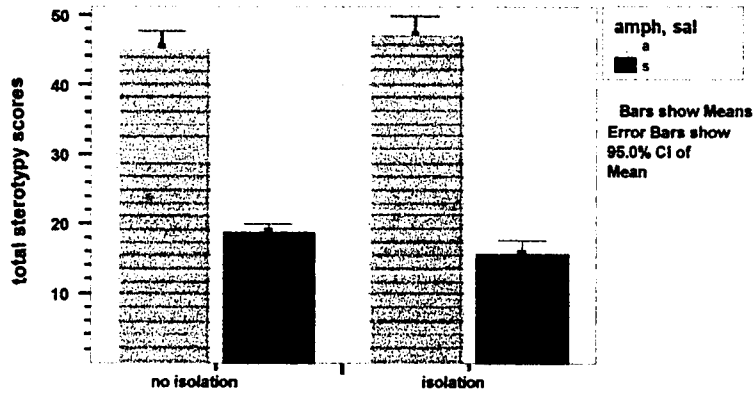


Figure 5

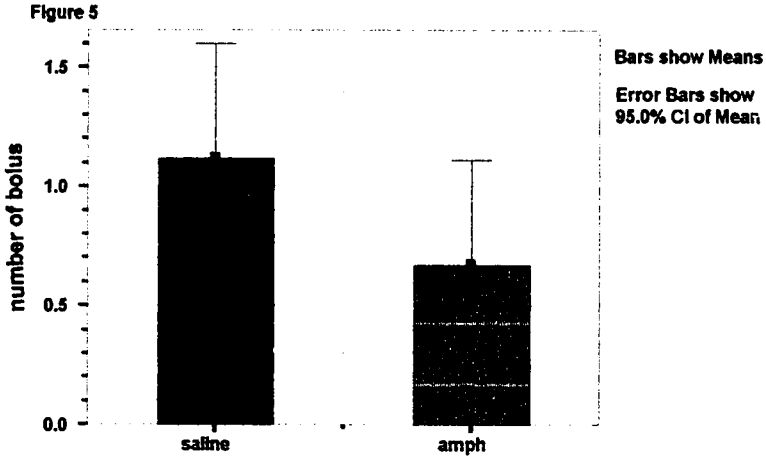


Figure 5(a)

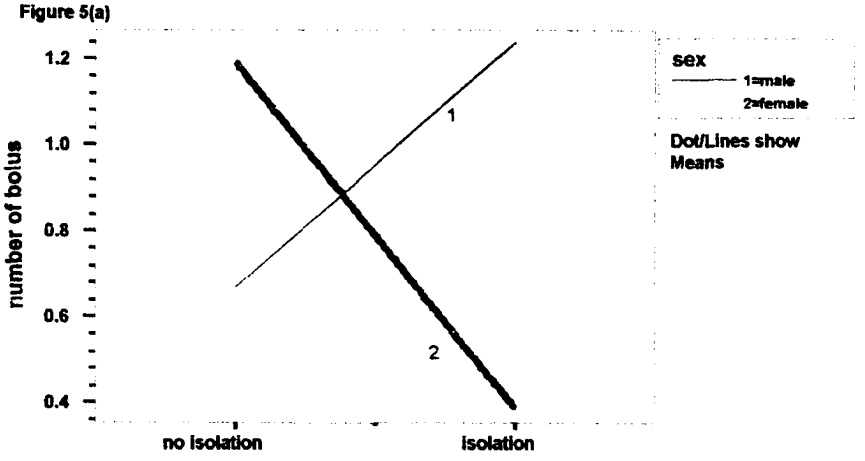


Figure 5(b)

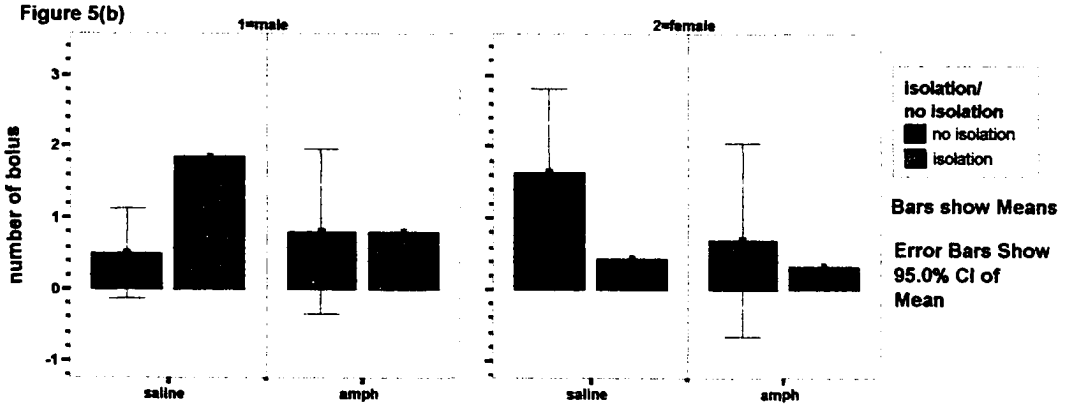
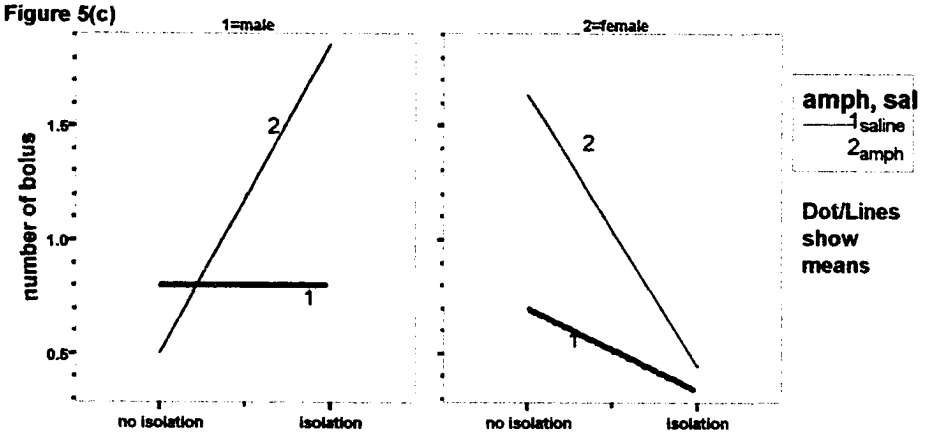


Figure 5(c)



7.3 Repeated Measures ANOVA's:

Table 10 sets out the statistical results of the 2 x 2 x 2 analysis of scores over Time, under the heading "Main Inquiry Auditory Isolation Treatment Group (Litters No. EXP 1,3,5) v. No-Isolation Control Group (Litters No. CG 1,2,3)."

Four figures, Figures 6, 7, 8, and 9 which are plots of the estimated marginal means for (1) Isolation with Saline conditions; (2) Isolation with Amphetamine conditions; (3) No-Isolation with Saline conditions; and (4) No-Isolation with amphetamine conditions. The measure scores are plotted across the separate time bins for the following behaviors: *(1)Linecross, (2)Rearing, (3)Stereotypy and (4)Locomotion. The results are as follows:*

(1A.) For Linecross, there was a significant Within subject effect for Time, $F(1.369, 86.267) = 25.405$, $p < .0001$, and a significant Time x Drug Interaction, $F(1.369, 86.267) = 33.446$, $p < .0001$. As can be seen in Figure 6, both Isolation and No-Isolation Conditions receiving amphetamine had an increase in linecross counts in bin 3 from bin 2, and amphetamine groups had significantly greater scores for all three bins (total 30 minutes) when compared to both Isolation and No-Isolation Conditions receiving saline (Between subject effect for Drug, $F(1, 63) = 53.510$, $p < .0001$). The results for Rearing, Locomotion and Stereotypy measures were similar to Linecross.

Looking at Figures 6, 7, 8, and 9 - scores for behavior measures Linecross, Rearing, Stereotypy and Locomotion, you will note a pattern of behavior with significant findings that are the similar across all four measures:

Across all time bins for all conditions, there was a significant Within-Subjects Time x Drug interaction ($p < .0001$), and a significant Between-Subjects Drug Effect ($p < .0001$), with amphetamine producing higher scores in both the Isolation and No-Isolation conditions when compared with their Saline Controls. However, after

TABLE 10- Repeated Measures ANOVA for all groups.

litter#	DRUG	LINECROSS		STEREOTYPY		REARING		LOCOM.	
		Within	Between	Within	Between	Within	Between	Within	Between
EXP v C123	Both	Time .0001 TxDrug .0001	Drug .0001	Time .0001 Time x Isol .012 Time x Drug .0001	Drug .0001 Isol x Drug .020	Time .0001 Time x Isol .023 Time x Drug.001 TxDxSex .033	Drug .000* Sex .001	Time .0001 Time x Drug .0001 Time x Isol .043	Drug .0001 Sex .050
	SALINE	Time .000*	no sig.	Time .0001 Time x Isol .011	Isol .019	Time .000*	Sex. 015	Time .000*	no sig.
	AMPH	no sig	no sig	no sig.	no. sig.	Time .005 Time x Sex .048*	Isol x Drug .009	Time .006 Time x Drug .0001 Time x Isol x Drug .009 Time x Drug x Sex .0001 Time x Isol x D x Sex .0001	no sig.
EXP#4 vCG3	BOTH	Time.007	Drug .0001	Time .005 Time x Drug .0001	Drug .000* Isol x Drug .018	Time .0001	Drug .0001 Isol x Drug .012	Time .017 Time x Drug .020	Drug .0001
EXP4 v CG3	SALINE	Time 0001 Time x Isol .032 Time x Sex .019	Isol. .0001 Isol x Drug .017	Time .0001	Isol. .009	Time .0001	Isol. .003	Time .0001	Isol. .0001 Sex .047
	AMPH	no sig.	no sig.	no sig.	no sig.	no sig.	no sig.	no sig.	no sig.
EXP5 v.CG3	BOTH	Time x Drug .001	Drug .002	Time .012; Time x Drug .012	Drug .0001	Time .0001	Sex .001; Drug .0001	Time .027; Time x Drug .002	Sex .043; Drug .001
	SALINE	Time. 0001	no sig.	Time .0001	no sig.	Time .0001	no sig.	Time .0001	no sig.
	AMPH	no sig.	no sig.	no sig.	No sig.	no sig.	no sig.	no sig.	no sig.

the first 15-20 minute period, all of the combined conditions in this Main Inquiry study produced the following ordered placement of scores which was consistent across all four behavioral measures:

(1) In the No-Isolation Control Condition those subjects receiving Amphetamine scored highest*;

(2) In the Auditory Isolation Treatment Condition those subjects receiving Amphetamine scored second highest;

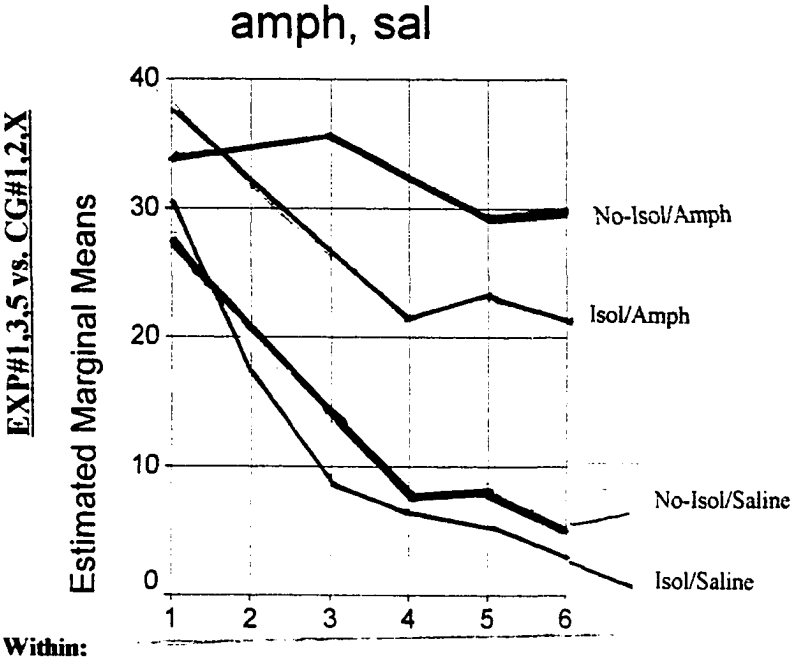
(3) In the No-Isolation Treatment Condition those subjects receiving Saline scored second lowest;

(4) In the Auditory Isolation Treatment Condition those subjects receiving Saline scored lowest.

*the one exception to this ordering was for stereotypy where the scores for the No-Isolation/amphetamine Condition did not rise above the Isolation/amphetamine Condition until the last time bin (Figure 8). A separate ANOVA was run on the Stereotypy Isolation/Amphetamine and No-Isolation/Amphetamine groups comparing the scores between just these Conditions at each separate time bin. No significant differences (at the p 05 level) were found between these two Conditions at any of the individual time bins.

The first 10 minutes of any initial Open Field Box exposure is generally considered to be a baseline period of activity which reflects the effects of the novelty of being introduced into the box for the first time. Patterns of behavior after the first 15-20 minutes are considered to be the behavior you are intending to measure (i.e. drug effects, anxiety behavior, exploratory behavior). (Kelly, A. E., 1998; Bardo, M. T. & Bevins, R. A., 2000; Bardo, M. T., Rowlett, J. K. & Harris, M. J., 1993; Walsh, R. N. & Cummins, 1976; Valle, F. P , 1969; and Tzschentke, T. M.,, 1998).

(2.) The individual statistical results of the Repeated Measures ANOVA for



REARING

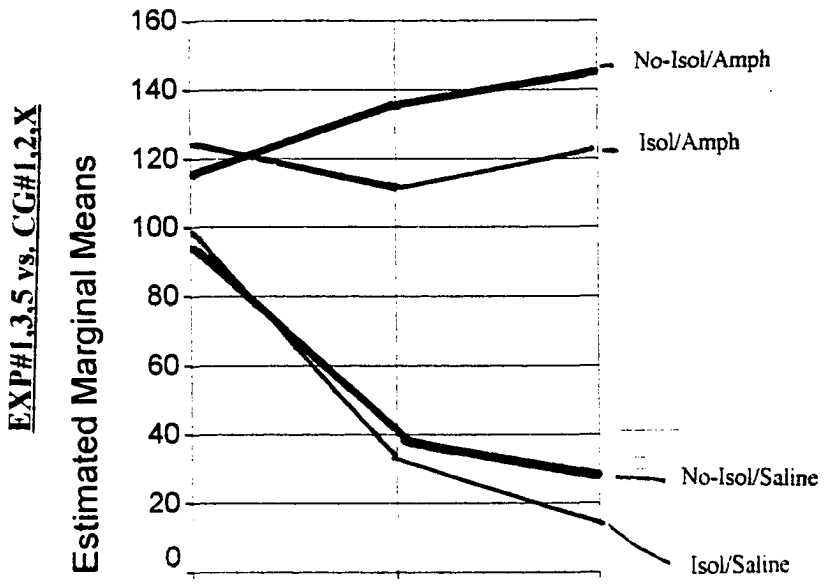
Within:

Time, F(2.646, 166.725) = 38.906, p<.0001;
Time x Isol., F(2.646, 166.725) = 3.437, p< 023;
Time x Drug, F(2.646, 166.725) = 6.210, p<.0001
Time x Drug x Sex, F(2.646, 166.725) = 3.126, p<.033;

Between:

Drug, F(1, 63) = 52.904), p<.0001;
Sex, F(1, 63) = 11.975, p<.001

FIGURE 7



LINECROSS

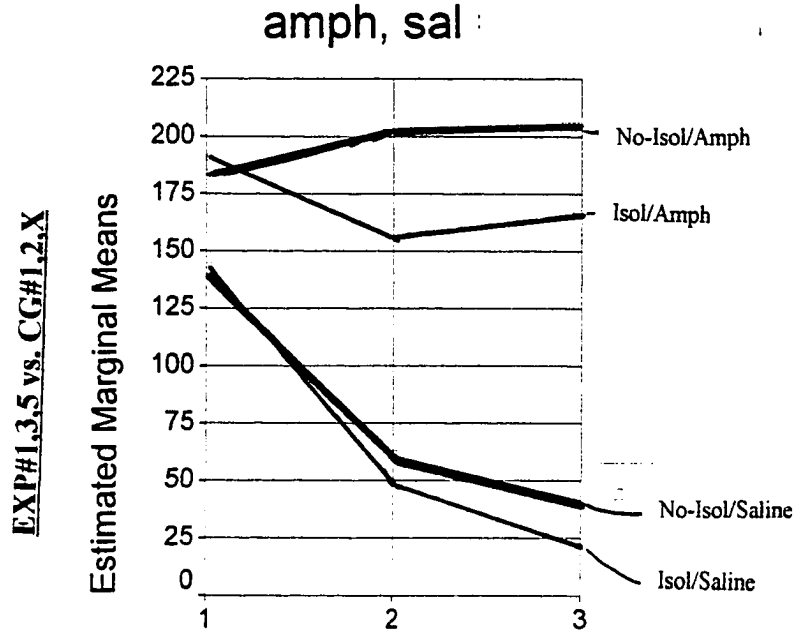
Within :

Time, F(1.369, 86.267) = 25.405, p<.0001
Time x Drug, F(1.369, 86.267) = 33.446, p<.0001

Between:

F(1, 63) = 53.510, p<.0001

FIGURE 6



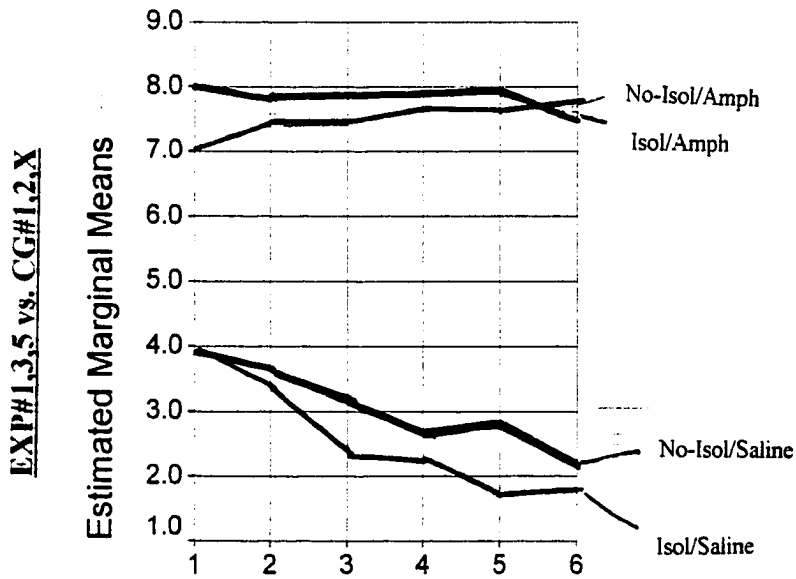
LOCOMOTION **Within:**

Time, F(1.436, 90.449) = 35.031, p<.0001;
Time x Isol., F(1.436, 90.449) = 3.691, p<.043;
Time x Drug, F(1.436, 90.449) = 29.148), p<.0001;

FIGURE 9

Between:

Drug, F(1, 63) = 63.279), p<.0001;
Sex, F(1, 63) = 3.957, p<.051.



STEREOTYPY **Within:**

Time, F(4.675, 294.509) = 13.028), p<.0001;
Time x Isol., F(4.675, 294.509) = 3.073, p<.012;
Time x Drug, F(4.675, 294.509) = 19.411, p<.0001;

FIGURE 8

Between:

Drug, F(1, 63) = 7.88.11, p<.0001;
Isol. x Drug, F(1, 63) = 5.715, p<.020.

Linecross, Rearing, Stereotypy and Locomotion are listed under Figures 6, 7, 8, 9, including the Within-Subjects and Between-Subjects F-tests for each.

7.4 Discussion of Experiment No. 1.

The results of the Univariate, and the Repeated Measures ANOVA support the hypothesis that subjects receiving perinatal Early Deprivation Isolation Treatment (ED) produce significantly different scores on each of the several behavioral measures. However a portion of the predicted direction of the difference between conditions was opposite to the hypothesis, that is, the statistical findings from the Repeated Measures ANOVA show that, in response to a 2.0 mg/kg amphetamine pre-stressor event, after the first 15-20 minutes of the test, subjects in the Isolation/amphetamine Experimental Treatment Condition produced scores on all four behavioral measures that were significantly *lower* than the scores produced by the No-Isolation/amphetamine Condition. However, the prediction that subjects in this Isolation/Amphetamine Condition would produce scores significantly *higher* than the scores produced by the No-Isolation Treatment Control Condition subjects, and the Isolation Treatment Condition subjects receiving saline at the time of trials, was upheld by these results. Also the prediction that the Isolation Treatment subjects receiving *saline* would produce lower scores when compared to the No-Isolation subjects receiving saline was upheld by these results.

The consistency in the pattern across all four measures, taken together, could be interpreted as lending support to a particular isolation paradigm model which states that subjects experiencing Early Deprivation will produce decreased behavior scores relative to No-Isolation Control subjects in response to any one of several types of pre-stressor events. The purported definitive difference is the type and *strength* of the adult test pre-stress event, and the exact Isolation Treatment experienced perinatally. The discussion of this idea is reserved until the final Discussion section.

8.0 Experiment No. 2: Investigation of Effects of Auditory Isolation Treatment in this Particular Experimental Environment.

In several reviews of the Early Deprivation literature covering this field of inquiry, such as that published by Lehrmann and Feldon (2000)(see Table 1), and others, a call is made to standardize methodological issues to reduce the number and sources of inconsistent findings. The use of an auditory mask is fairly standard in Early Deprivation paradigms as well as in many other types of behavioral studies. The mask is usually some form of white noise. "White Noise" is defined as "...an auditory stimulus that has all frequencies represented in random fluctuation." (Penguine Dictionary of Psychology).

Although pups' outer ear canals (pinna) are not open and "audition" is not considered to be fully functional until day PND 12-14, there is always the possibility of producing a stimulatory response in individual nuclei within the auditory system which are on-line and are responsive to sound pressure levels (i.e., various functioning mechanico-receptors in the auditory pathway), to ultrasonic vocalizations (USV) or to physical sound wave pressure existing in the test environment. The function of an auditory mask is to eliminate extraneous noise and possible confounding variables in the environment, such as USV calls from siblings when simultaneously tested nearby since pups emit USV when separated from their mothers. When tested together in the same location the pups' perception of being isolated might be voided if they picked up the vocalizations of their siblings. If the mask is played as a continuous, non-fluxuating noise the mask is not usually beyond 80 dB (more often limited to the lower 70's dB range depending on the acoustics in the test environment); a continuous, non-fluxuating 100 dB white noise mask is aversive and can be destructive if the subject is exposed to more than 6-10 minutes (depending on age of subject) (Freeman, S., Khvoles, R., Cherny, L. & Sohmer, H., 1999).

Studies done of intracellular recording of auditory nuclei show excitatory effects in neurons at 100 dB, but inhibitory effects are produced in the same nuclei at 110-120 dB (Ryan, A. F., Axelsson, G. A. & Woolf, N. K., 1992). It is also interesting to note a few facts in conjunction with this: (1) Limited to a very specific developmental time period of PND 1-14 only, acetylcholine is present in the developing auditory system, and then disappears; (2) Although so-called "promiscuous binding" is ubiquitous in the brain, none so great as the "...cholinergic and noradrenergic nerve terminals which respond not only to acetylcholine and norepinephrine, ...but also to other substances that may be present in tissues, including prostaglandins, ...dopamine, 5-hydroxytryptamine, GABA, opioids peptides and many other substances." (Rang, 1995. p 109), and, also, (3)"Sympathetic neurons in the brain during development switch from being cholinergic to being noradrenergic and release more than one transmitter at a time."(Rang.); (4) Endocrine tissue, including the posterior pituitary, arises from mesoderm - not ectoderm- early in development; inner ear anatomical regions arise from surface epithelium and have cholinergic receptors that detect pressure and movement, as well as sound pressure level receptors in the auditory pathway. One of the primary neurotransmitters functioning in mesoderm and endoderm is acetylcholine.

Experiment No. 2 was designed to test a subgroup of animals using the identical isolation paradigm but without any masking noise present in the environment; this subgroup is designated the Without-Auditory Isolation Treatment Group, ***EXP#4***.

Motivation to test the exclusion of the white noise mask came from the consideration of information obtained from five areas:

(1) The work of Turkewitz, et al (1993), along with several of his graduate students over a span of several years, where the focus of investigation was the role of developmental limitations of sensory input on sensory and

perceptual organization of the brain as it applies to various characteristics of Neonatal Intensive-Care Units (NICU).

(2) The published work of Philbin, and that of Glass, both of whom have investigated neonatal vulnerability while in NICU units (Philbin, Licklieter & Graven, 2000; Philbin, Ballweg & Gray, 1996; Glass, 1999).

(3) The work of Lewkowicz, his investigation of sensory processing by human infants during their first six months, and his data on the *equivalency* of sensory processing of multiple types of input up to age four months, that is, before four months of age human infants process all sensory inputs as quantitatively additive, including auditory, visual, touch, smell and taste, and as neurologically excitatory stimulus (Lewkowicz & Turkewitz, 1980).

(4) Approximately a year's time spent in observation of high risk neonates at the NICU Unit of Staten Island Hospital, and the publications of Gardner, on the development of high risk, drug-exposed infants, and Karmel, on the use of Brainstem Auditory Evoked Potential Response (BAER) in diagnosing developmentally at risk infants (Gardner, Karmel, Norton, Mangano & Brown, 1990). Also, the work of their associate, Mangano , who has measured changes in cortisol levels in High Risk infants in neonatal intensive care units after being exposed to various levels of invasive and non-invasive stressors (Mangano, Gardner & Karmel, 1993).

(5) The personal observation that particularly during the first five days of isolation treatment exposure, the rat pups appeared (within minutes after being placed into the Isolation chamber with the auditory mask activated) to promptly fall asleep One possible explanation for this is developmental "defensive inhibition", which is defined by Walsh and

Cummins (1976) as: "...an apparent sleep due to intense arousal, which has a gradual development, closed eyes and relaxed posture."(p 488.) Defensive inhibition is seen in various altricial species, including human premature and full-term infants and in rats (Llynn, 1966; Hofer, 1970; Walsh & Cummins, 1976; and Blumberg, Efimova & Alberts, 1992).

The goal of this experiment was to test whether the manipulation of the auditory mask as an Independent Variable in this particular experimental setting would disclose significant differences in the behavioral measure test scores (of the perinatal subjects which were later tested in adulthood, when compared to the scores of subjects receiving the Isolation Treatment Without Auditory Mask (i.e., Auditory/No-Auditory x Isol/NoIsol x Drug/NoDrug x Sex). It was hypothesized that if a difference in behavior resulted from the change in the Isolation Treatment methodology it would result in:

(1) Subjects in the EXP#4 (No auditory mask) combined-conditions producing lower scores on all test measures when compared with the scores produced by subjects in the Main Inquiry Auditory Isolation Treatment Group.

This result was predicted based on the presumption that the EXP#4 subjects would be exposed to lower levels of environmentally generated excitatory stimulation during the daily one-hour treatment procedure than the Main Inquiry Auditory Isolation Treatment Group as the presence of the auditory mask could be a source of increased environmental stress during the Isolation Treatment.

8.1 METHODS

8.2 Subjects:

All animals were maintained in accordance with the NIH Guide for Care and Use of Animals. One additional time-pregnant Sprague Dawley dam was obtained from Charles River Laboratories, timed to arrive at the Hunter College Animal Facility on E15, allowing five to seven days for acclimation to the Facility before parturition. The dam was inspected and weighed upon arrival at the facility, and then housed in the isolation colony room. The dam gave birth to 12 pups, which is the N for this

experimental group This experimental group was designated the "No-Auditory Isolation Treatment Group (Litter No. EXP #4)."

8.2a Procedures.

The same procedures were followed as in Experiment No. 1, the dam was housed in the same isolation colony, during the same time periods as the other experimental and control groups, experiencing the same environment and treatment as the rest of the animals in the colony. The only difference this group experienced was in the Isolation Treatment test situation when during the 60 min isolation period the auditory equipment was not turned on. See the methods section in Experiment No. 1 for further general details. It should also be noted that USV vocalizations within the chamber were measured using a small battery powered monitor with headphones and leads outside the room that converted USV frequencies into auditory clicks able to be heard by human ears. When this monitor was placed between the individual cups within the Isolation Chamber itself in the first minutes of the test *without* the Auditory Mask present, pup calls could be faintly detected but all USV calls reliably ceased after 5-6 minutes of being placed in the chamber. When the Auditory Mask was present (only in the Main Inquiry experiment), USV calls could *not* be detected at any time during the Isolation Treatment.

8.3 Statistical Treatment.

All of the same procedures listed in Paragraph 7.0a (1) through (5) above were also applied to this analysis. A three-way Analysis of Variance (Treatment x Drug x Sex) was done on all behavioral measures, followed by a One-Way ANOVA on all main effects and post-hoc Newman-Keuls. The results of the three-way ANOVA's are set out in Table 9. Of more relevance to this particular study, and a much more interesting result is the Repeated Measures Analysis of Variance which were conducted on the time-bins for each behavior, for each of the combined-Treatment (or no-Treatment)

conditions. And it is the Repeated Measures ANOVA(s) results that are set out and discussed below in detail.

8.4 Results of Repeated Measures Analysis of Variance.

In an attempt to make an organized presentation of this experimental data and to make the necessary comparisons required to describe the behavioral differences between these No-Auditory Isolation Treatment (EXP#4) animals and those animals experiencing the Isolation Treatment WITH the Auditory mask included (i.e., Main Inquiry, EXP#1, 3, 5), three separate Tables (labeled A, C, & E) are set forth which contain a treatment imposed hierarchical ordering of the individual time-bin marginal means for each combined-condition; these tables also serve as a flow chart mapping the direction of score changes within the conditions across time bins. Two Tables (A & C), have attached to them four graphed figures each which :

(1) Plot the lines representing all four combined-conditions (Isolation treatment x Drug-[or not]) and their respective behavior scores across time, including their ordered placement as individual combined-condition groups, at the end of Time 1, and at the end of Time 2 (for Linecross and Locomotion T-2 is 20 minutes into the trial; for Rearing and Stereotypy this is 15 minutes into the trial); and for each successive time-bin-interval throughout the testing (a total of 30 minutes for each behavior in the open field box).

(2) Each Table also shows the ordered placement of each of the combined-conditions at T-3 (or T-6), which is the end of the last time bin for each individual measured behavior.

(3) These three Tables summarize the data obtained from the original Repeated Measures ANOVA(s) run on three separate comparisons of the following groups:

(a) EXP#1,3,5 vs. CG#1,2,3 , (the Main Inquiry Auditory Group) (i.e, Table A);

(b) EXP#4 v. CG3,(the No-Auditory Isolation Treatment Group)(i.e., Table C);

(c) EXP#5 v. CG3 (cohort Auditory Isolation Treatment Group)(i.e.,Table E).

BEHAVIOR MEASURED	TIME 1 end 10 min	Hierarchy of Group		Hierarchy of Group	TIME 2 end 20 min	T-3 end 30 min	T-4	T-5	TIME 6	Hierachy of Group
LINECROSS	(10.658) 124.128	Isol/Amph		No-Isol/Amph	136.172 (11.462)	➡	➡	➡	➡	No-Isol/Amph
	(10.658) 116.522	No-Isol/Amph		Isol/Amph	111.261 (11.402)	➡	➡	➡	➡	Isol/Amph
	(11.689) 96.770	Isol/Saline		No-Isol/Saline	38.875 (12.122)	➡	➡	➡	➡	No-Isol/Saline
	(11.271) 94.639	No-Isol/Saline		Isol/Saline	31.373 (12.572)	➡	➡	➡	➡	Isol/Saline
	end 5 min				**end 10 min**	**end 15 min**	**end 20 min**	**end 25 min**	**end 30 min**	
REARING	(4.002) 38.633	Isol/Amph		No-Isol/Amph	34.811 (3.113)	➡	➡	➡	➡	No-Isol/Amph
	(4.002) 33.978	No-Isol/Amph		Isol/Amph	31.539 (3.113)	➡	➡	➡	➡	Isol/Amph
	(4.389) 30.627	Isol/Saline		No-Isol/Saline	20.243 (3.292)	➡	➡	➡	➡	No-Isol/Saline
	(4.232) 27.667	No-Isol/Saline		Isol/Saline	17.643 (3.415)	➡	➡	➡	➡	Isol/Saline
	end 10 min				**end 20 min**	**end 30 min**				
LOCOMO.	(16.323) 194.300	Isol/Amph		No-Isol/Amph	204.417 (15.178)	➡	➡	➡	➡	No-Isol/Amph
	(16.323) 185.311	No-Isol/Amph		Isol/Amph	158.483 (15.178)	➡	➡	➡	➡	Isol/Amph
	(17.903) 145.040	Isol/Saline		No-Isol/Saline	60.778 (16.051)	➡	➡	➡	➡	No-Isol/Saline
	(17.262) 142.549	No-Isol/Saline		Isol/Saline	47.000 (16.647)	➡	➡	➡	➡	Isol/Saline
	end 5 min				**end 10 min**	**end 15 min**	**end 20 min**	**end 25 min**	**end 30 min**	
STEREO.	(.224) 7.989	Isol/Amph		Isol/Amph	7.844 (.242)	➡	➡	7.889(.245)	7.694(.224)	No-Isol/Amph
	(.224) 7.7044	No-Isol/Amph		Isol/Amph	7.500 (.242)	➡	➡	7.650(.245)	7.683(.224)	Isol/Amph
	(.246) 3.944	Isol/Saline		No-Isol/Saline	3.646 (.256)	➡	➡	➡	➡	No-Isol/Saline
	(.237) 3.875	No-Isol/Saline		Isol/Saline	3.381 (.266)	➡	➡	➡	➡	Isol/Saline
➡	Arrow indicates no further change in ordering of hierarchy			**TABLE (FLOW CHART) "A."** **Main Inquiry Auditory Isolation Treatment** EXP#1,3,5 vs.CG#1,2,3						

(1) Each of the four graphs which are attached to each of the individual two Tables enumerate the "F" statistic and significant data for each of the groups of combined-conditions' subjects, whose behavior is represented by the lines plotted in each graph. These "F" statistics are from the individual Repeated Measures ANOVA analyses carried out on that particular Isolation Treatment Group.

(2) A post hoc, Pair-wise t-test, as well as a 2 x 2 x 2 (Drug/No-Drug x Sex x Litter) across Time, Repeated Measures ANOVA was carried out on EXP#4 (Auditory Isolation Treatment) vs. EXP#5 (Auditory Isolation Treatment Cohort) raw data for each of the four measured behaviors. No Table was produced, but the F-Tests and graphs will be reported as part of the discussion of the Repeated Measures ANOVA for the No-Auditory group, EXP#4.

Table A–EXP#1, 3, 5 v. CG#1, 2, 3 -Main Inquiry(Auditory Isolation Treatment Groups):

First, reference is made to Paragraph(s) "7.2", "7.3", and "7.4" above as these also discuss the results of the Auditory-Isolation Treatment Main Inquiry experimental groups contained in Table A, but focuses on a general overview whereas this study's main concern is comparing Auditory vs. No-Auditory group differences.

Second, as can be seen in Table A, in these Auditory-Isolation Treatment experimental groups, there is a quantitative and qualitative Time-dependant behavioral shift in the level of the scores of behavior obtained by these behavioral measures. The constancy of pattern in this behavioral data across all of the combined-condition groups, and across all of the four different behavioral measures is notable in its regularity, fand constancy. When placed in the open field box, in the same corner, during the first 15-20 minutes the animals in these individual, combined-condition groups achieve scores that place them in a fixed order of performance between the groups----a "post-starting-position" whose order is fixed across measures. Between T-1(end of first 10 minutes)

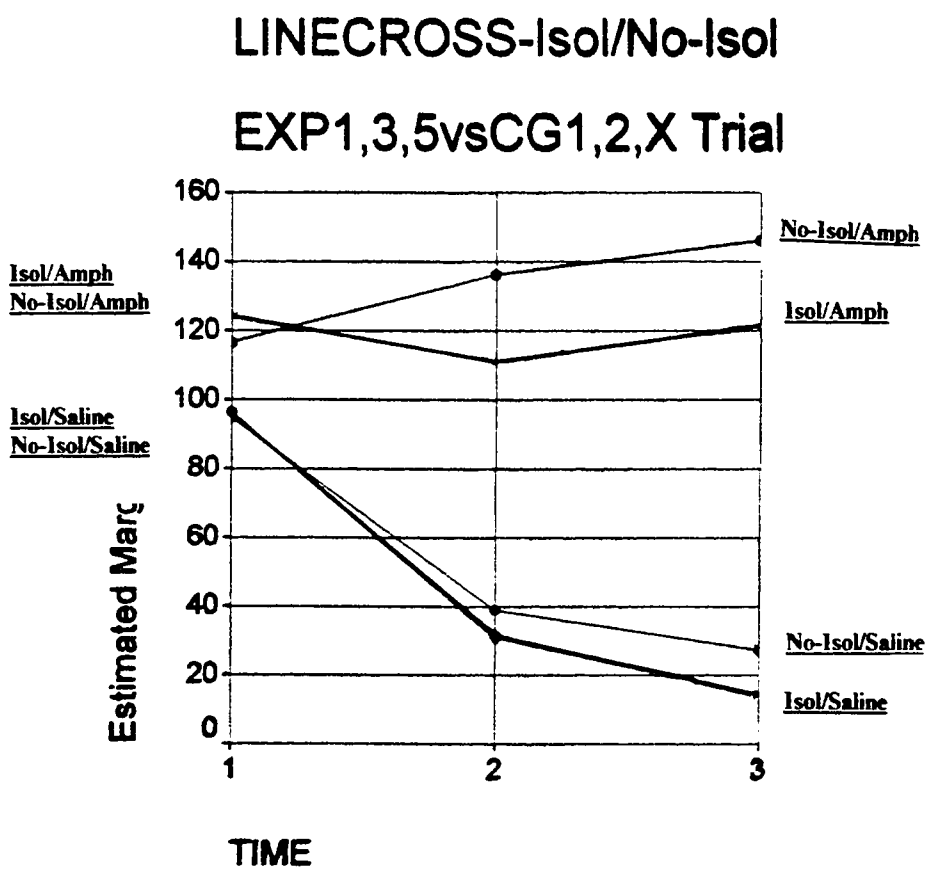
and before the T-2 (end of next 10 minute period), each combined-condition within the group has "switched" its level and direction of behavior, individually and as a combined-condition Group, to shift into a new, fixed order between conditions that finishes at T-3 in the same order as is fixed at T-2. This "hierarchy-prime" is still in place at the finish of the trial at 30 minutes, regardless of whether the level of behavior within the individual combined-condition groups' scores either rise or fall. The ordering of between-combined-condition group performance is maintained from the beginning of the T-2 Time-point forward (i.e., again, it should be noted that for Linecross and Locomotion, this point is at end of T-1 and beginning of T-2; for Rearing and Stereotypy, this is at the end of T-2 and beginning of T-3 because Rearing and Stereotypy are scored in 5 minute bins).

Third, as can be readily observed in Figure 10; 10a; 10b and 10c, as time progresses, two noticeable effects occur in the animal's behavior, and which is reflected in the data:

(1) Specific combined-condition subjects' behavior "shift-out". That is, behavior scores either increase or decrease together, as a combined-condition, at or before T2.

(2) At the end of the 15-20 minute-time-point, and after the behavioral shift happens, specific combined-condition's subjects' behavior either increase together or decrease together. More specifically, the No-Isolation/Amphetamine combined-condition subject's behavior scores increases in coordinately with the Isolation/Amphetamine combine-condition subjects behavior scores. Contrasted with this, the No-Isolation/Saline combined-condition subjects' behavior decreases concomitantly with the Isolation/Saline combined-condition subjects'

The shifting upward of levels of behavior for *both* the No-Isolation/Amphetamine condition and the Isolation/Amphetamine condition when compared to the other two combined-conditions, can reliably be shown to result from the administration of the drug;



Within :

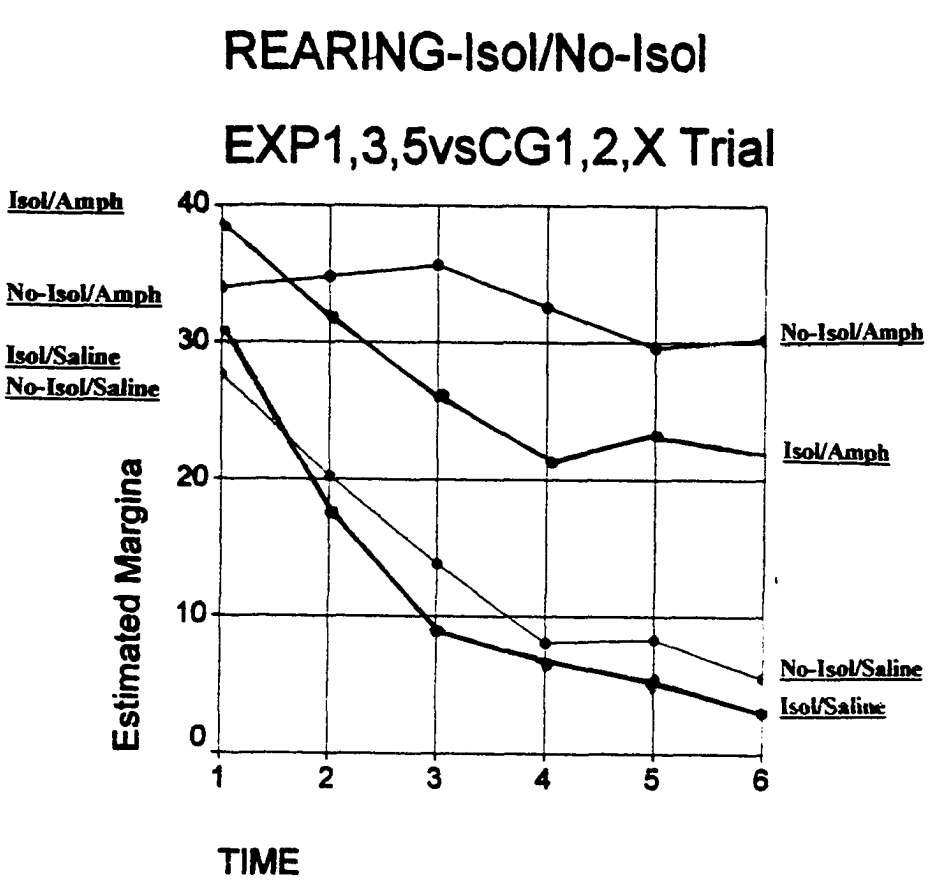
Time, F(1.369, 86.267) = 25.405, p<.0001

Time x Drug, F(1.369, 86.267) = 33.446, p<.0001

Between:

F(1, 63) = 53.510, p<.0001

FIGURE 10



Within:

Time, F(2.646, 166.725) = 38.906, p<.0001;
Time x Isol., F(2.646, 166.725) = 3.437, p<. 023;
Time x Drug, F(2.646, 166.725) = 6.210, p<.0001
Time x Drug x Sex, F(2.646, 166.725) = 3.126, p<.033;

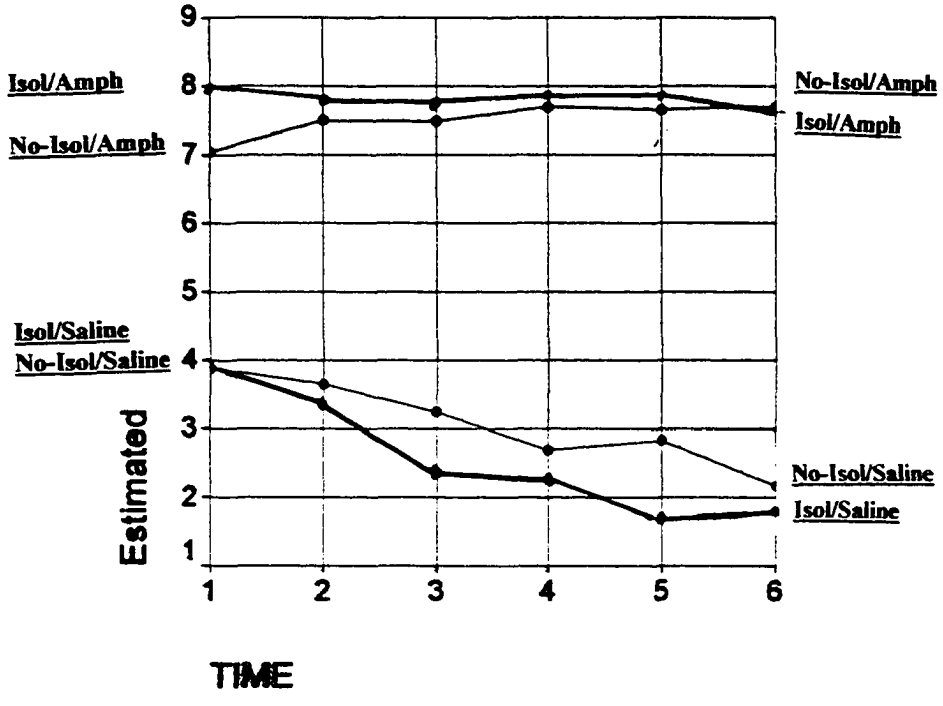
Between:

Drug, F(1, 63) = 52.904), p<.0001;
Sex, F(1, 63) = 11.975, p<.001

FIGURE 10(a)

STEREOTYPY-Isol/No-Isol

EXP1,3,5vsCGX1,2,X Trial



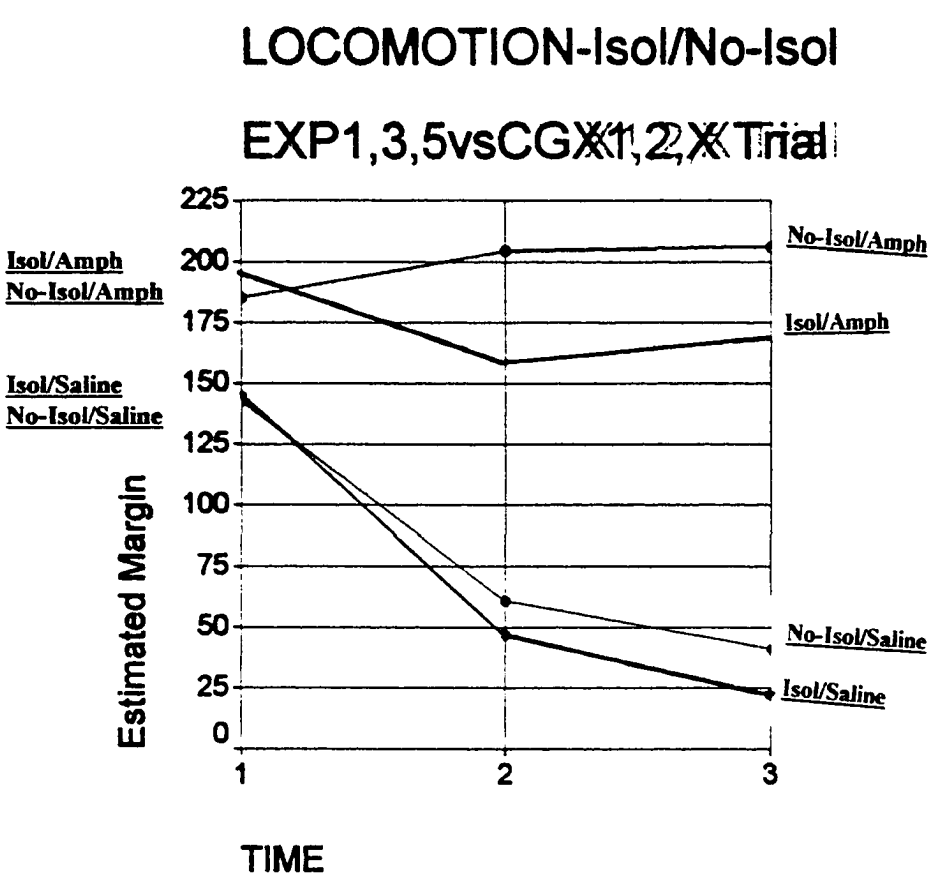
Within:

Time, F(4.675, 294.509) = 13.028), p<.0001;
Time x Isol., F(4.675, 294.509) = 3.073, p<.012;
Time x Drug, F(4.675, 294.509) = 19.411, p<.0001;

Between:

Drug, F(1, 63) = 7.88.11, p<.0001;
Isol. x Drug, F(1, 63) = 5.715, p<.020.

FIGURE 10(b)



Within:

Time, F(1.436, 90.449) = 35.031, p<.0001;
Time x Isol., F(1.436, 90.449) = 3.691, p<.043;
Time x Drug, F(1.436, 90.449) = 29.148), p<.0001;

Between:

Drug, F(1, 63) = 63.279), p<.0001;
Sex, F(1, 63) = 3.957, p<.051.

FIGURE 10(c)

but the difference in the order of the levels of behavior obtained between these two combined-condition groups (No-Isolation Condition scores higher than Isolation Condition scores) is consistent and maintained across behavioral measures. This result is interesting because this behavioral outcome differs from that of some studies which are currently in publication, and, also, because some of the current Isolation Paradigm models would not predict this outcome.. This result addresses the effects of the Isolation Treatment on the effects of the psychostimulant drug (used as the pre-stressor stimulus), and the resulting interaction (or synergism) between Isolation Treatment and drug.

What perhaps may be more indicative of pure Isolation Treatment effects is the consistent, ordered placement of those combined-condition subjects receiving just Saline at trial, (especially the Auditory Isolation Treatment subjects receiving Saline at trial), and the steady rate of decrease of behavior scores for these groups. It can be noted that those animals that received Auditory Isolation Treatment as pups, even if they had higher scores and placed higher in the combined condition order before the end of T-1, after the 20-minute-change point they produced the lowest behavior scores of any other combined-condition in the Main Inquiry. At the same time, in these combined-condition groups included in the Main Inquiry, (and which did experience the auditory mask during the isolation exposure as pups), when they are tested as adults, produce scores on the Stereotypy measure which (when compared to the No-Isolation/amphetamine Condition subjects) were not significantly different from them, but which were significantly higher than the No-Isol/Saline and Isol/Saline Condition subjects.

On the other three measures, Linecrossing, Rearing and Stereotypy, these Isolation/Amphetamine Condition animals consistently had lower scores than the No-Isolation/Amphetamine Condition Both amphetamine conditions, of course, produced scores significantly higher than the No-Isolation/Saline and Isolation/Saline Conditions.

Table C; No-Auditory Isolation Treatment Group (EXP#4) vs. No-Isolation Control Group (CG3).

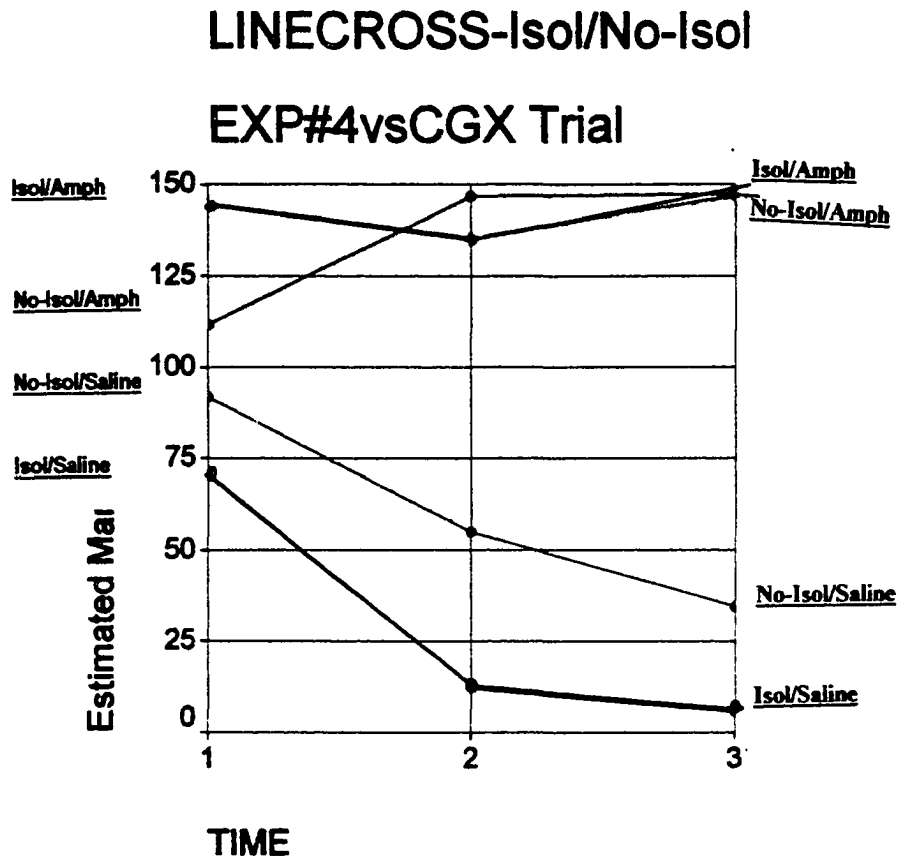
Attached to Table C are four line graphed-figures, No. 11, 11a, 11b and 11c. One of the obvious differences in the graphs of the data produced by this No-Auditory Isolation Treatment Group EXP#4, when compared to the Main Inquiry Auditory Isolation Treatment Group (EXP#1,3,5 v. CG1,2,3) graphed data is the *absence* of a 20-minute Time-point "behavioral shift" for the No-Auditory EXP#4 group EXP#4's level of behavior relative to the other combined-condition groups' level of performance (as reflected by the group Mean Scores shown in Table D) does not change from the ordering established within the first 15-20-minutes of the open field test (fixed by end of T-1{ or T-2}) and such order is preserved through to the end of T-3 (or T-6) which is the end of the trial. The only exception is the two groups that received drug in the Linecross measure, and this order between these two combined-condition groups re-establishes itself to the T-1 order before the end of the last time bin. This re-established hierarchy then matches the between combined-condition ordering seen in the other three measures, i.e. Rearing, Stereotypy and Locomotion at the end of the last time bin.

Second, contrary to the pattern set in the Main Inquiry Auditory-Isolation Treatment Group (i.e, EXP#1,3,5), this EXP#4 No-Auditory Isolation Treatment Group, when given the amphetamine stress-primer, produced consistently higher behavior scores when compared to it's control comparison group, CG3. Whereas, after receiving the drug primer, the Main Inquiry Auditory Isolation Group (Litters No. 1, 3 and 5), (when compared to their control group (Control Litters No.1,2,3) consistently scored lower than the No-Isolation/Amphetamine control/comparison groups.

Third, planned apriori contrasts were carried out comparing the scores on each of the apriori behavioral measures between the Main Inquiry Auditory Isolation Groups,

BEHAVIOR MEASURED	TIME 1	Hierarchy of Group	10min	Hierarchy of Group	TIME 2	T-3 (score-end 30min)	T-4	T-5	TME 6	Hierachy of Group
LINECROSS	(13.552) 144.167	Isol/Amph		No-Isol/Amph	146.300 (27.424)	147.300 (27.424)*	→	→	→	No-Isol/Amph
	(13.886) 111.700	No-Isol/Amph		Isol/Amph	144.167 (13.552)	147.000 (26.763)*	→	→	→	Isol/Amph
	(14.374) 91.750	No-Isol/Saline		No-Isol/Saline	54.875 (23.132)	→	→	→	→	No-Isol/Saline
	(14.374) 70.875	Isol/Saline		Isol/Saline	12.750 (23.132)	→	→	→	→	Isol/Saline
						(Score-end 15min)				
REARING	(4.408) 53.500	Isol/Amph		Isol/Amph	46.167 (5.572)	→	→	→	→	Isol/Amph
	(4.516) 28.850	No-Isol/Amph		No-Isol/Amph	35.300 (5.710)	→	→	→	→	No-Isol/Amph
	(4.675) 23.875	No-Isol/Saline		No-Isol/Saline	24.125 (5.910)	→	→	→	→	No-Isol/Saline
	(4.675) 13.000	Isol/Saline		Isol/Saline	9.625 (5.910)	→	→	→	→	Isol/Saline
						(Score-end 30min)				
LOCOMO.	(20.864) 243.833	Isol/Amph		Isol/Amph	208.167 (29.036)	→	→	→	→	Isol/Amph
	(21.379) 172.850	No-Isol/Amph		No-Isol/Amph	207.200 (29.754)	→	→	→	→	No-Isol/Amph
	(22.129) 139.750	No-Isol/Saline		No-Isol/Saline	86.500 (30.798)	→	→	→	→	No-Isol/Saline
	(22.129) 93.500	Isol/Saline		Isol/Saline	18.875 (30.748)	→	→	→	→	Isol/Saline
						(-End-15 min)				
STEREOTYPY	(.441) 8.000	Isol/Amph		No-Isol/Amph	8.150 (.268)	8.000 (.354) IA	→	→	→	Isol/Amph
	(.451) 7.100	No-Isol/Amph		Isol/Amph	7.833 (.261)	7.700 (.446) NIA	→	→	→	No-Isol/Amph
	(.467) 4.000	No-Isol/Saline		No-Isol/Saline	4.000 (.277)	→	→	→	→	No-Isol/Saline
	(.467) 3.1750	Isol/Saline		Isol/Saline	2.250 (.277)	→	→	→	→	Isol/Saline
				*These two groups are not significantly different from each other, they are overlapping at end of T3. Arrows indicate no further change in hierarchy of scores						

TABLE (FLOW CHART) "C." - No-auditory Isolation Treatment EXP#4 v. CG3



Within:

Time x Drug, $F(1.951, 33.162) = 5.837$, $p<.001$;

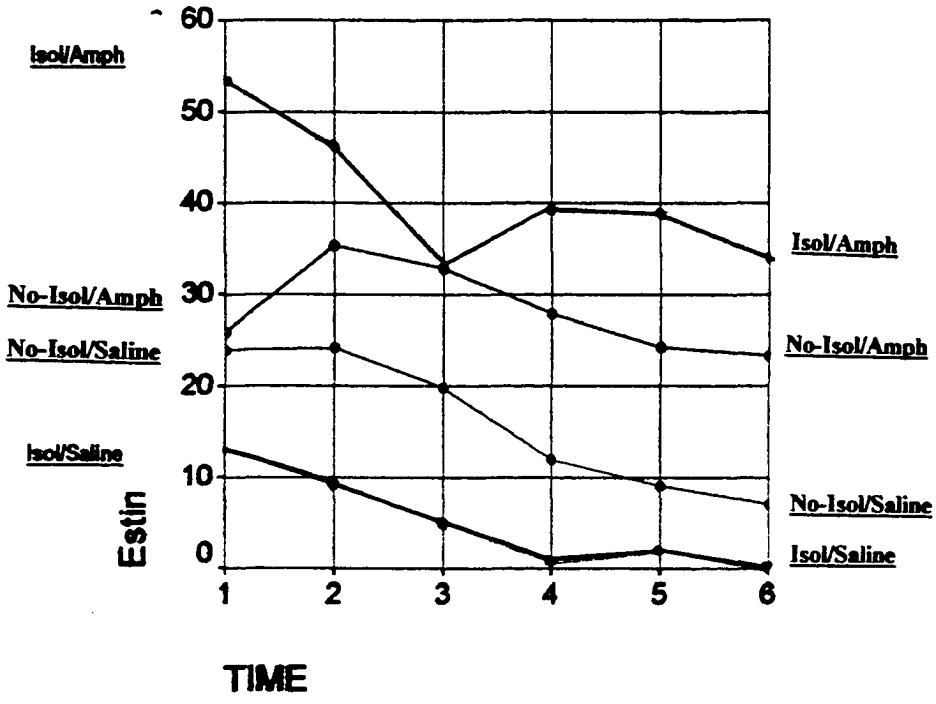
Between:

Drug, $F(1, 17) = 29.810$), $p<.001$.

FIGURE 11

REARING-Isol/No-Isol

EXP#4vsCGX Trial



Within:

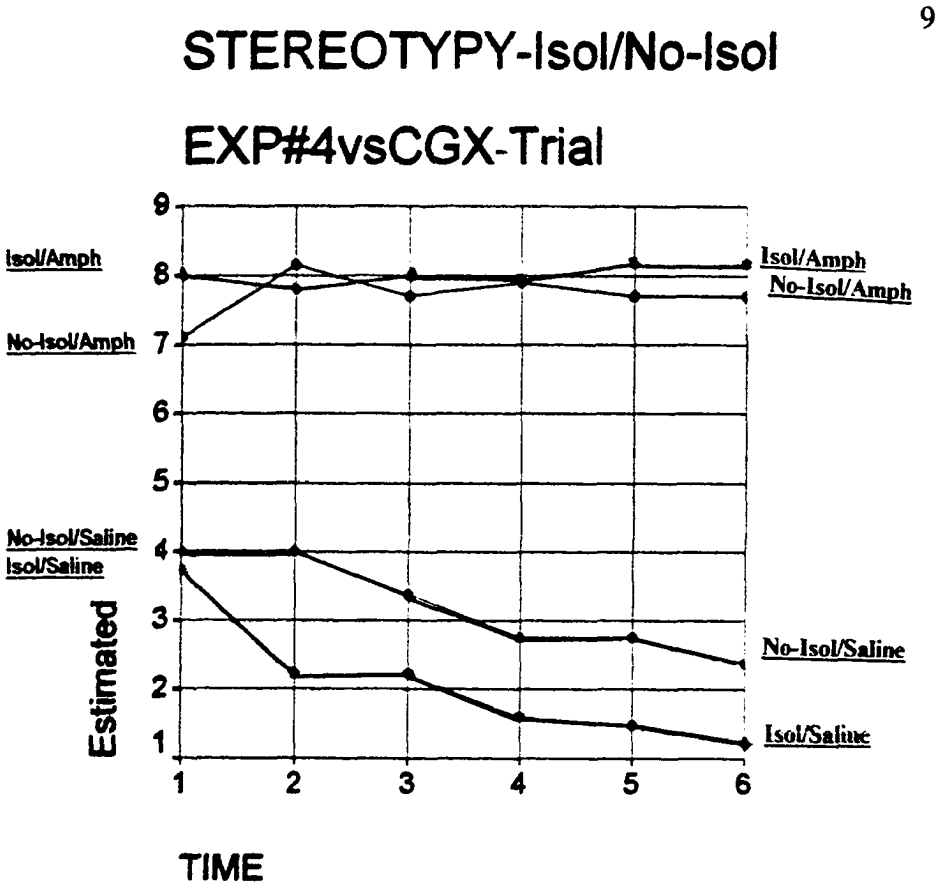
Time, $F(4.276, 72.700) = 8.889$, $p<.0001$;

Between:

Drug, $F(1, 17) = 33.126$, $p<.0001$;

Isol.x Drug, $F(1, 17) = 7.925$, $p<.012$.

FIGURE 11(a)



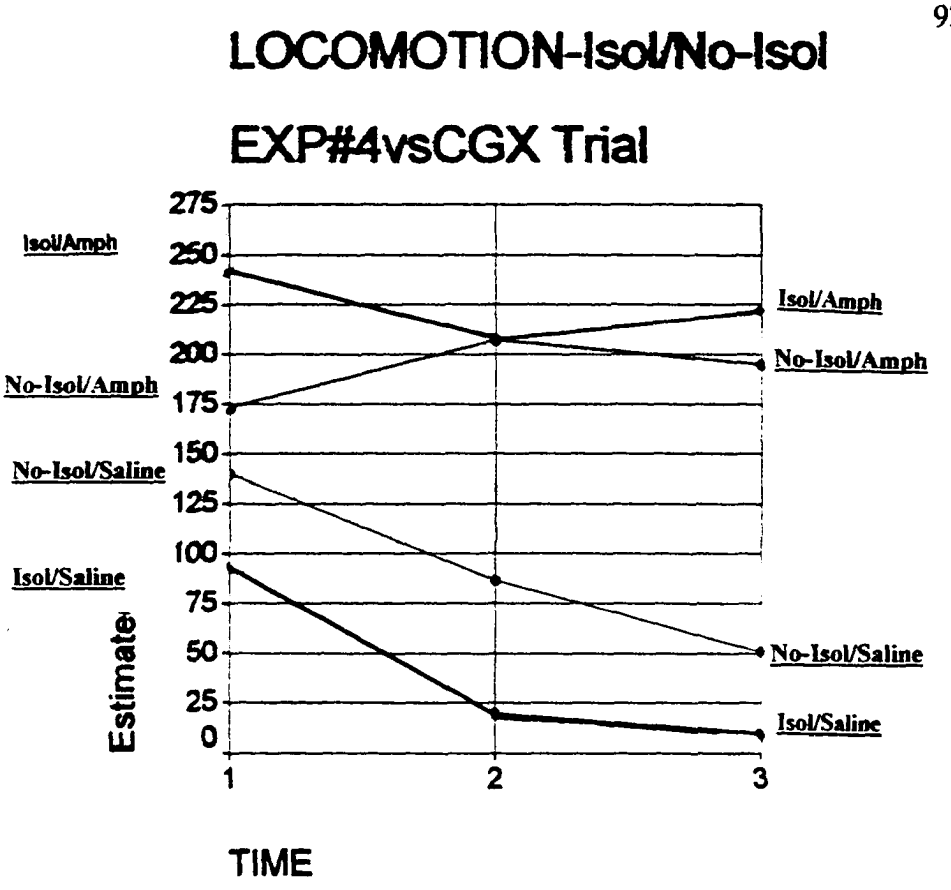
Within:

Time, F(4.540, 77.174) = 3.813, p<.005;
Time x Drug, F(4.540, 77.174) = 7.075), p<.0001;

Between:

Drug, F(1, 17) = 363.916, p<.0001;
Isol.x Drug, F(1, 17) = 6.789, p<.018.

FIGURE 11(b)



Within:

Time, F(1.929, 32.787) = 4.688, p<.017;

Time x Drug, F(1.929, 32.787) = 4.505, p<.020;

Between:

Drug, F(1, 17) = 38.363, p<.0001

FIGURE 11(c)

Litters No. EXP# 1, 3, 4, and 5.) When the Amphetamine Condition is included in the apriori analysis, no significant differences are found between the experimental groups (Figure 12). However, when the drug condition is excluded and the contrast analysis is carried out only on subjects receiving saline (total N = 27), the ONLY Conditions found to be significantly different from the other experimental Conditions at the $p<.05$ significance level on *ANY* of the behavior measures is the No-Auditory Isolation Treatment Group, (Litter No. EXP#4). (Figure 12a). Levene's Test of Homogeneity of Variances was **not** found to be significant (and, therefore, equal variances could be assumed), and the Planned Apriori Contrast Test t-statistic for GROUP EXP#4, are as follows:

For Total Linecross, $t(22) = 1.797$, $p < .05$ (1-tailed); For Total Stereotypy, $t(22) = 1.782$, $p < .05$ (1-tailed); For Total Rearing, $t(22) = 3.152$, $p < .01$ (1-tailed); For Total Locomotion, $t(22) = 2.495$, $p < .05$ (1-tailed). This was the first statistical indication that the statistical alternative hypothesis in this experiment, i.e., that the scores on the behavioral measures for the No-Auditory Isolation Treatment Group EXP#4 would be significantly different from those scores produced by the Main Inquiry Auditory Isolation Treatment Group (EXP# 1, 3, and 5), would be upheld. A proposed answer as to why this difference *cannot be detected* when the analysis includes those animals that received the psychostimulant drug prior to testing could be that the physiological effects of the drug ameliorate or "mask" the long-term behavioral effects produced by experiencing the perinatal isolation treatment. In fact, it could be argued that administration of the psychostimulant drug ameliorates or "masks" not only the differences in behavior resulting from experiencing either one or the other of the Isolation Treatment Methodologies (with or without auditory mask), but that the *degree or magnitude of such amelioration is dependant on which type of Isolation Treatment the subject was exposed.* That is the question or inquiry addressed in Section 9.0 below.

TABLE E; EXP#5--Auditory Isolation Treatment Group v. No-Isolation Control Group CG3.

The obvious feature of Table E is not surprising and it would be expected since this is an Auditory Isolation Treatment Group, that is, the presence of the 20-minute Time-point behavioral shift in the level of behavioral performance (as reflected by the flow chart aspect of the Mean Scores shown in Table E). Again, this reflects changes from the order established within the first T-1 to the beginning of T-2 of the open field test. At T-2 there is a further "cross-over" in Linecross and Stereotypy, which then establishes the following order at the end of the trial for all of the groups: Isol/Amph-->No-Isol/Amph->No-Isol/Saline->Isol/Saline. Table E is included here so that when EXP#4 is compared to EXP#5, the salient data is present.

As was explained earlier, this particular No-Isolation Control Group, CG3, is appropriate for use as the choice of control group for these comparisons because it is the "cohort" of each of these two experimental groups, i.e. all three were delivered from same vendor the same day; pups were born on the same day and testing as pups and behavioral tests in adulthood were conducted either simultaneously or within a week of the particular round of testing beginning.

This cohort relationship also provides a rationale for comparing the EXP#4-No-Auditory Isolation Treatment conditions with the EXP#5-Auditory Isolation Treatment conditions directly. As can be seen in Table 8(a), the subject numbers and other subject variables are also similar.

What can be said when a comparison is made between two previously made comparisons? In this case, EXP#4 (no-auditory) and EXP#5(auditory) were first subjected to a R-Measures ANOVA comparing each of them separately to the same

BEHAVIOR MEASURED	TIME 1 end 10min	Hierarchy of Group		Hierarchy of Group	TIME 2 end 20 min	T-3 end 30min	T-4	T-5	TIME 6	Hierachy of Group
LINECROSS	(25.716) 120.250	Isol/Amph		No-Isol/Amph	146.650 (24.286)	158.250 (37.503)	→→→	→→→	→→→	Isol/Amph
	(19.919) 111.700	No-Isol/Amph		Isol/Amph	145.667 (31.353)	147.300 (29.049)	→→→	→→→	→→→	No-Isol/Amph
	(21.734) 93.833	Isol/Saline		No-Isol/Saline	54.875 (26.139)	34.500 (30.069)	→→→	→→→	→→→	No-Isol/Saline
	(20.618) 91.750	No-Isol/Saline		Isol/Saline	39.167 (26.498)	15.917 (31.695)	→→→	→→→	→→→	Isol/Saline
	end 5 min				**end 10 min**	**end 15 min**			**30 min**	
REARING	(7341) 47.333	Isol/Amph		Isol/Amph	42.333 (6.321)	→→→	→→→	→→→	→→→	Isol/Amph
	(6.265) 34.583	Isol/Saline		No-Isol/Amph	35.300 (4.896)	→→→	→→→	→→→	→→→	No-Isol/Amph
	(13.742) 25.850	No-Isol/Amph		No-Isol/Saline	24.125 (5.068)	→→→	→→→	→→→	→→→	No-Isol/Saline
	(5.943) 23.875	No-Isol/Saline		Isol/Saline	23.585 (5.342)	→→→	→→→	→→→	→→→	Isol/Saline
	end 10 min				**end 20 min**	**end 30 min**				
LOCOMOTION	(35.701) 209.917	Isol/Amph		Isol/Amph	228.083(39.705)	→→→	→→→	→→→	→→→	Isol/Amph
	(27.654) 72.850	No-Isol/Amph		No-Isol/Amph	207.200 (30.765)	→→→	→→→	→→→	→→→	No-Isol/Amph
	(30.173) 152.000	Isol/Saline		No-Isol/Saline	86.500 (31.834)	→→→	→→→	→→→	→→→	No-Isol/Saline
	(28.625) 139.750	No-Isol/Saline		Isol/Saline	59.750 (33.556)	→→→	→→→	→→→	→→→	Isol/Saline
	end 5 min				**end 10 min**	**end 15 min**			**30 min**	
STEREOTYPY	(.689) 8.083	Isol/Amph		No-Isol/Amph	8.150 (.323)	8.000(.474)	→→→	→→→	→→→	Isol/Amph
	(.533) 7.100	No-Isol/Amph		Isol/Amph	7.917 (.417)	7.700 (.367)	→→→	→→→	→→→	No-Isol/Amph
	(.552) 4.000	No-Isol/Saline		No-Isol/Saline	4.000 (.334)	3.375 (.380)	→→→	→→→	→→→	No-Isol/Saline
	(.582) 4.00	Isol/Saline		Isol/Saline	3.500 (.352)	2.833 (.401)	→→→	→→→	→→→	Isol/Saline
	ARROW indicates there are no further changes in hierarchy				**TABLE (FLOW CHART) "E."–Auditory Isolation Treatment EXP#5 v. CG3**					

Control Group, i.e. EXP#4 vs. CG3; and then EXP#5 vs. CG3. What can be observed about the ANOVA analysis of the individual comparisons is this:

(1) For Linecross, the within subjects variable "Time" was significant for each at the $p < .001$.

(2) For Rearing, the within subjects variable Time was significant for each at the $p < .0001$ level and both had a between subjects significant F at the $p < .0001$ level, EXP#4 $F(1, 17) = 33.126$; and for EXP#5 $F(1, 17) = 1\ 0.162$. However, EXP#4 had an additional between subject effect for Drug x Isolation, $F(1, 17) = 7.925$, $p < .012$, which was not present at all for EXP#5.

(3) For Locomotion, both EXP#4 and EXP#5 had within subject effects for Time, and for Time x Drug; and between subject effects for Drug, but only EXP#4 had an Isolation x Sex, $F(1, 17) = 6.754$, $p < .019$ interaction.

(4) For Stereotypy, the Within subject effects in both groups had significant F's for Time, with EXP#5 having an additional Time x Drug interaction, significance $p < .002$; however the between subject effect for EXP#4 had not only Drug (both groups significant at $p < .0001$), but EXP#4 had an additional Between subject effects for Isolation, $F(1, 17) = 12.345$, $p < .003$ and Isolation x Drug, $F(1, 17) = 5.786$, $p < .028$, neither of which were seen in the EXP#5 group (Note: the entire list of significant findings can be found listed in the Tables and attached Figures).

It may be noted that, as will be discussed in Section 9.0, the statistical main effects for "Drug" usually flatten or override any main effects produced by Isolation Treatment. However, when saline is held constant, the EXP#4 No-Auditory Isolation Treatment group is the only treatment group that has a between subject main effects for Isolation across all of the measures.

Comparison of EXP#4 with EXP#5-Comparison Without a "Control Group"

Figure 12a shows the statistically significant difference when comparing EXP#4 in the saline condition to all of the other Main Inquiry experimental groups (which are auditory Isolation Treatment groups). ANOVA run on all experimental groups shows significant differences: Linecross, $F(4, 22) = 3.936$, $p<.015$; Stereotypy, $F(4, 22) = 3.226$, $p<.032$; Rearing, $F(4, 22) = 5.322$, $p <.004$; and Locomotion, $F(4, 22) = 5.081$, $p <.005$, and apriori contrasts show that the only litter which is significantly different is Litter #4, at p 05, for all measures. Figures 13 and 13(a)(b)(c)(d), which visually depict the relationship between Litters EXP#4 and EXP#5 only, exhibit the same relationship across all measures: EXP#4 subjects produced higher scores than EXP#5 subjects in the amphetamine condition, and EXP#4 subjects produced lower scores than EXP#5 subjects in the saline condition.

8.5 Discussion of Experiment No. 2:

The inquiry in its simplest terms is answered. As can be seen in Figures 14, 15, 16, and 17, which visualize the behavioral performance of the No-Auditory Isolation Treatment Group EXP#4 v. No-Auditory Control Group CG#3 (indicated by the blue dotted lines), within the performance of the Main Inquiry Auditory Isolation Treatment Group Litters No. EXP#1,3,5 v. No-Isolation Control Group Litter No. CG#1,2,3, the inclusion or exclusion of an auditory mask makes a very clear and statistically significant difference.

The No-Auditory Isolation Treatment Group (EXP#4) had what could be termed a stronger (including quicker onset) and more exaggerated behavioral response to the psychostimulant drug. On these Figures the two Control conditions of the No-Auditory

FIGURE 12 - Auditory Isol. EXP1,3,5

v. No-Auditory Isol. EXP#4

AMPHETAMINE only

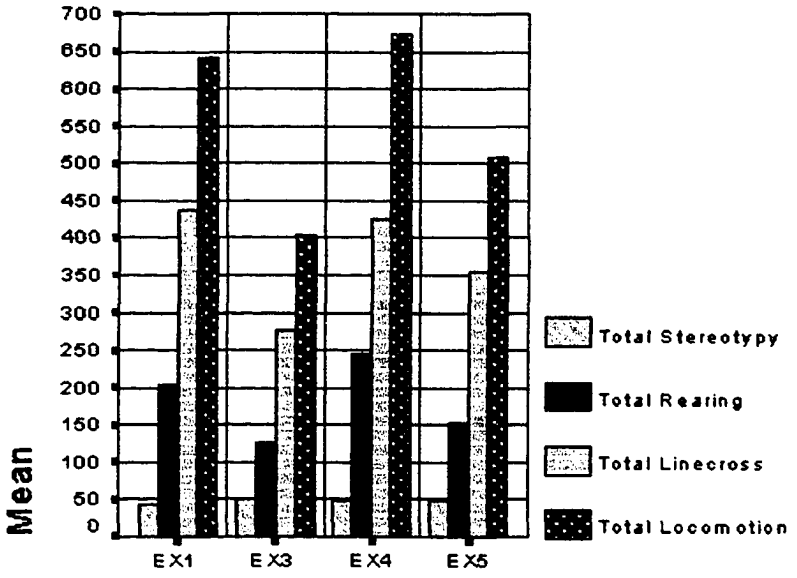
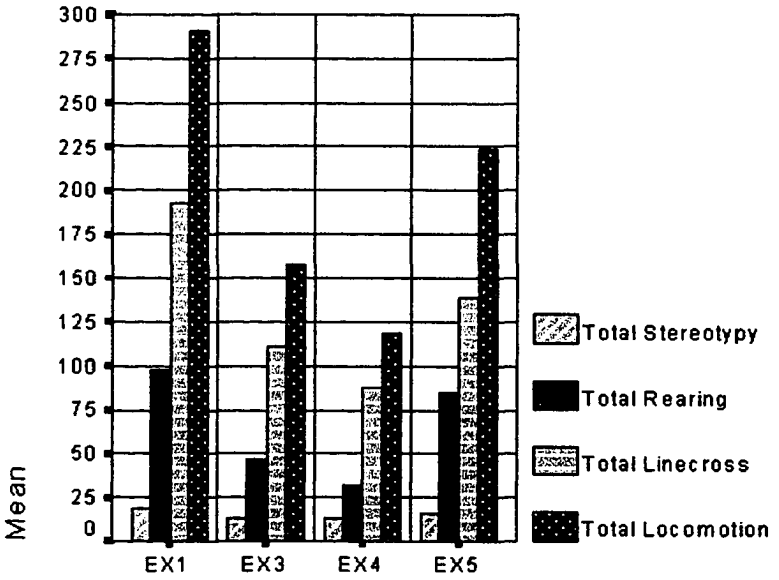


FIGURE 12(a) Auditory Isol. EXP1,3,5

v. No-Auditory Isol. EXP#4

SALINE Condition Only



FIGURES 13(a)(b)(c)(d)

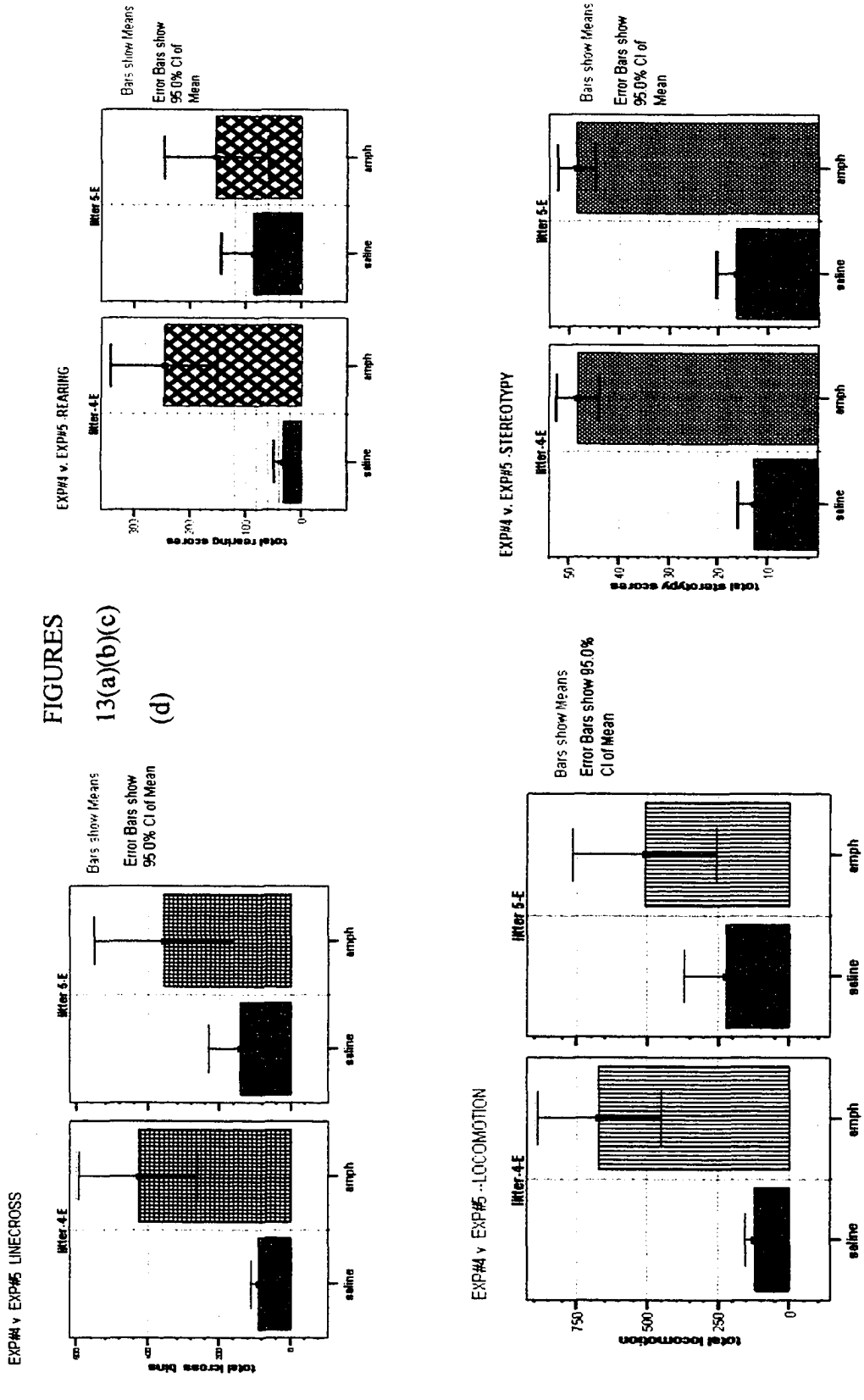


TABLE 11 - POSTBASELINE REPEATED MEASURES for all groups.

Litter #	GROUP	LINECROSS Sig.Scores		STEREOTYPY Sig.Scores		REARING Sig.Scores		LOCOMOTION Sig.Scores	
		Within	Between	Within	Between	Within	Between	Within	Between
EXP 135v C123	Both	Time .0001 TxSex .038 TxDrug .043 TxDrug xSex .012	Sex .045	Time .0001	no sig	Time .0001 Time x Sex .005	Sex .045	Time 0001 Time x Drug 054 Time x Sex .009	no sig
	SALINE	Time .0001	no sig.	Time .0001	no sig	Time .0001	no sig.	Time .0001	no sig.
	AMPH	Time .0001 Time x Sex .001*	Sex.010	Time .0001	no. sig.	Time .0001	Sex .029	Time .0001 Time x Sex .003	Sex. 012
EX#4 vCGX	Both	Time .0001 Time x Drug .004 Time x Sex .018	Isol .055 Sex .048 Isol x Sex.028	Time .0001	Isol .003 Drug .017	Time .0001 Time x Drug .007	Drug .028 Isol x Sex .016	Time .0001 Time x Drug 004 Time x Sex. 053	Drug 0001 Isol x Sex .019
	SALINE	Time .0001	Isol..059	Time .001	Isol .031	Time .0001	no sig.	Time .0001	IxSex.056
	AMPH	Time .0001	Sex .037	Time .001	Isol .045	Time .0001	no sig.	Time .0001	no sig.
EX#5 v CG3	Both	Time .0001 Time x Sex.037	Sex .010	Time .0001	no sig.	Time .0001 Time x Isol .058· Time x Sex .014· Time x Drug 024· Time x Isol X Sex .012· Time x Isol. x Drug .052	Sex .003	Time .0001; Time x Isol .051· Time x Drug .029· Time x Sex .012	Sex .004
	SALINE	Time .0001	no sig.	Time .0001	no sig.	Time 0001·Time x Sex .041	no sig.	Time .0001	no sig.
	AMPH	Time .0001	Sex .0001	Time .001	Isol..040	Time 0001; Time x Isol.010	Sex .015	Time.0001	Sex .008
	.								

FIGURE 14

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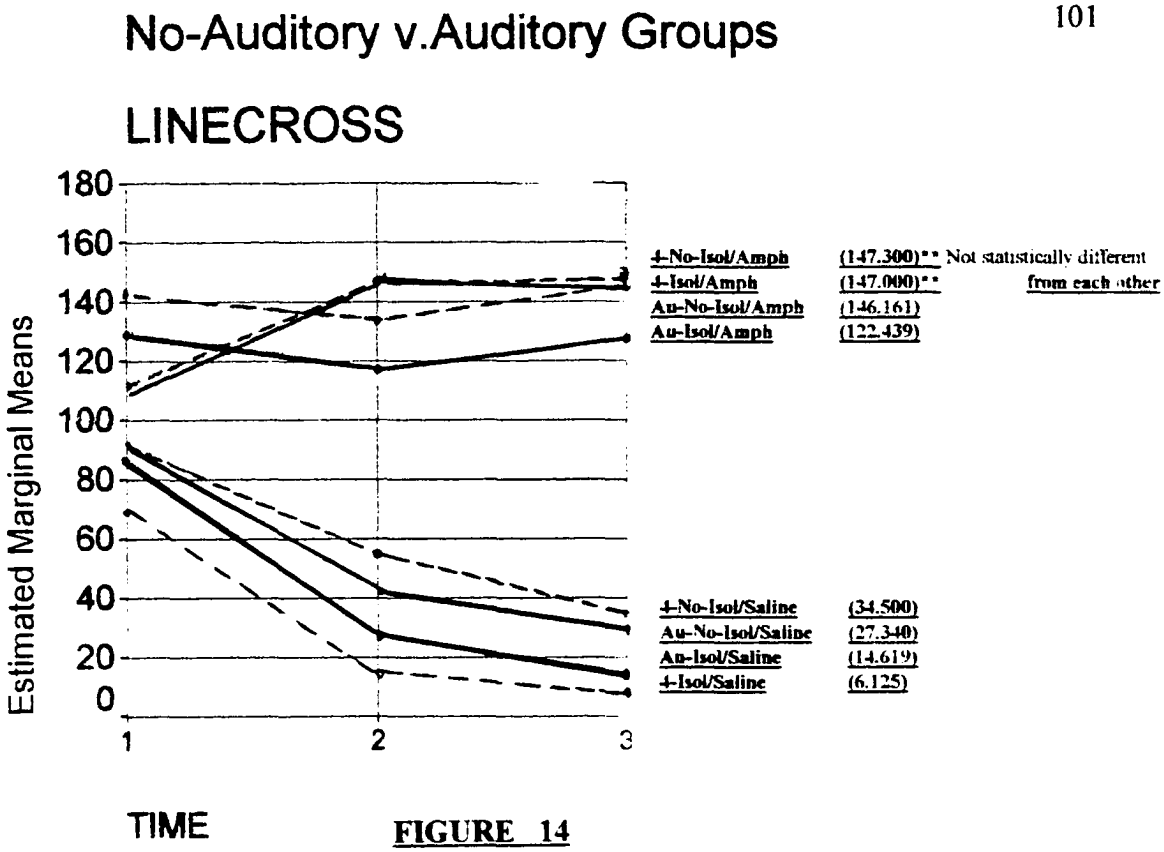

FIGURE 14

FIGURE 15

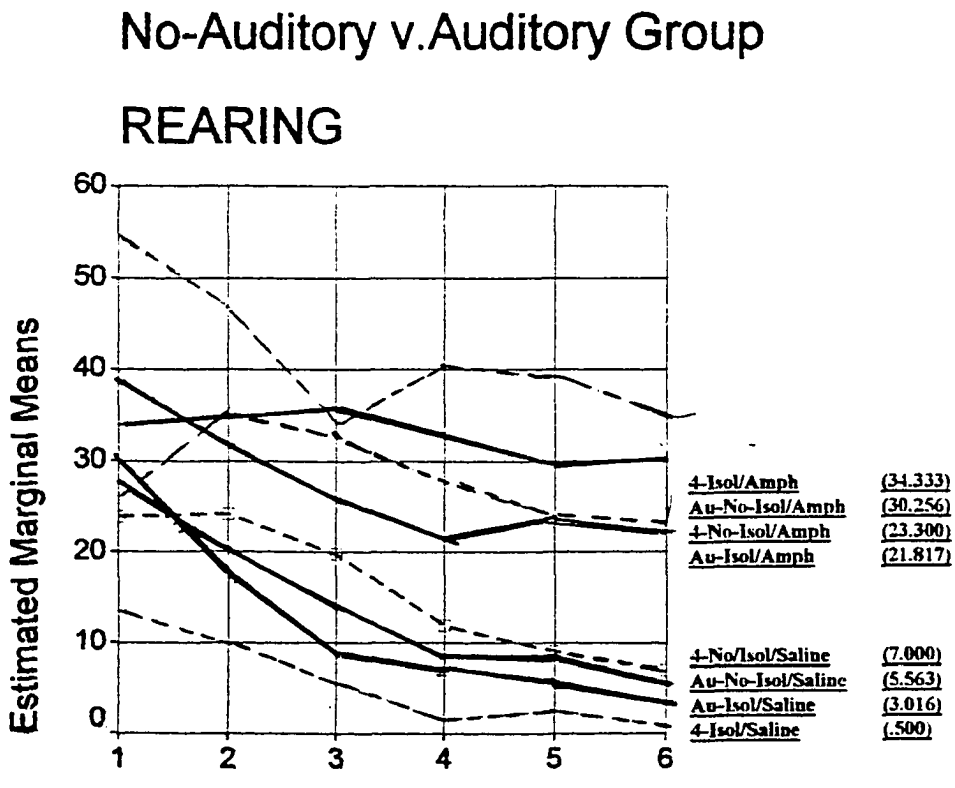

FIGURE 15

FIGURE 16

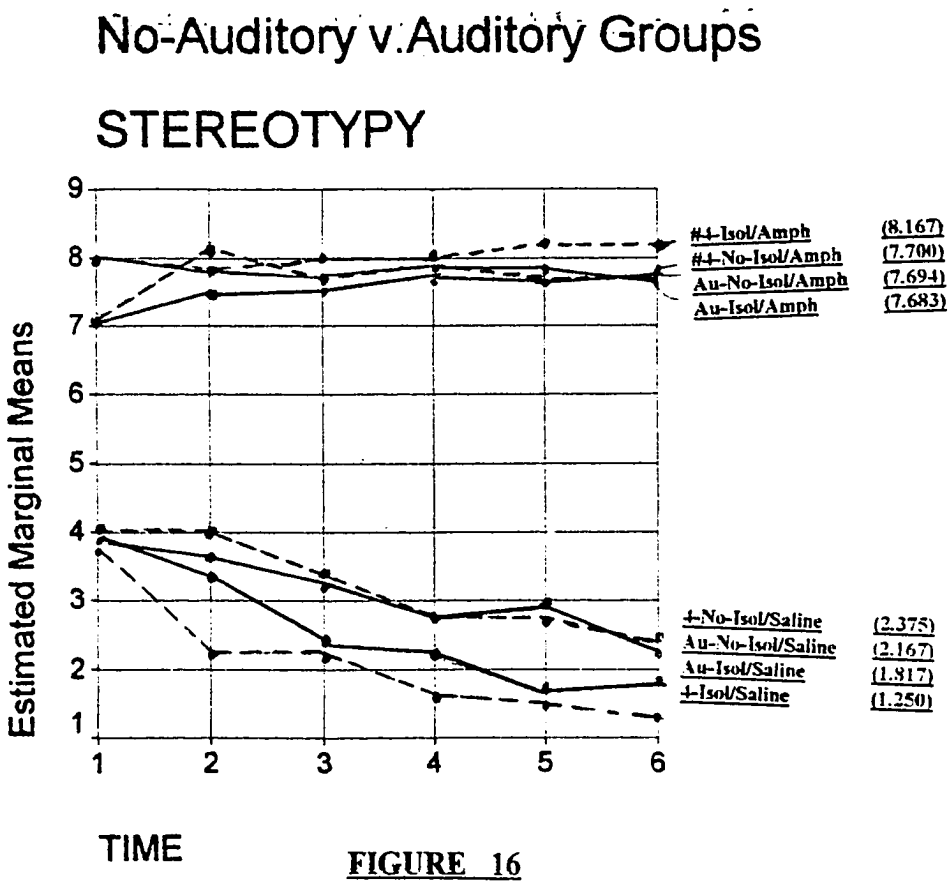

FIGURE 16

FIGURE 17

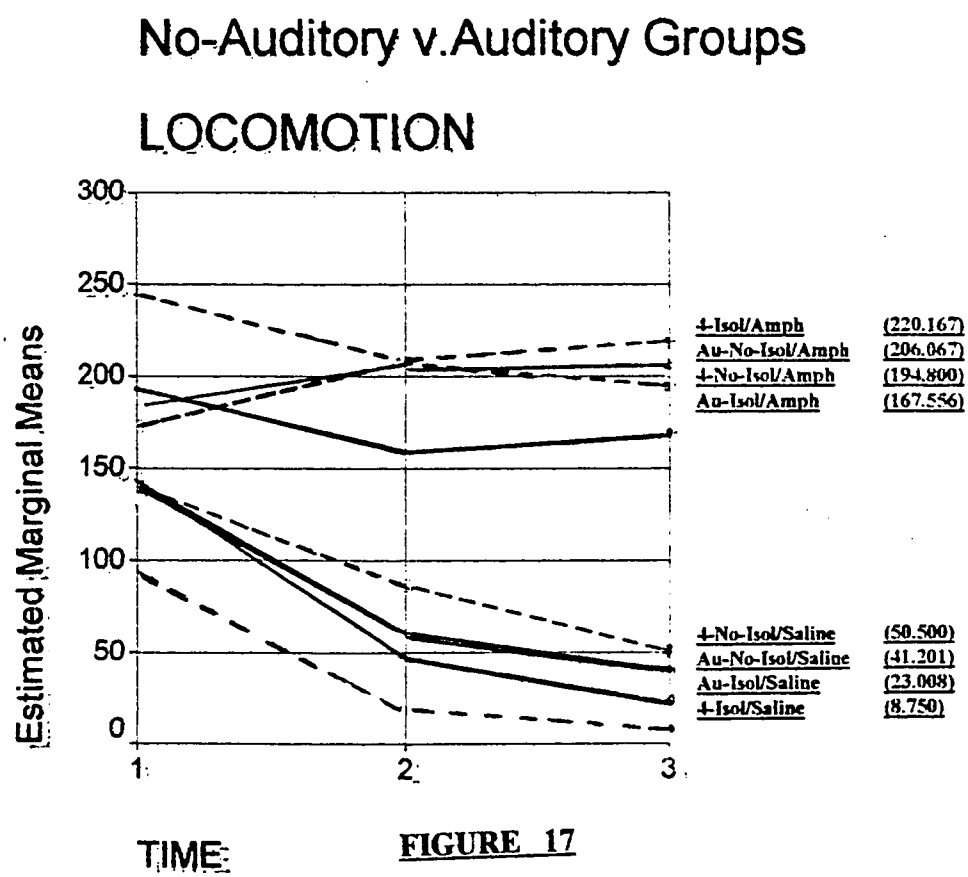

FIGURE 17

Isolation Treatment Experiment are labeled "4-No-Isolation/Saline" and "4-No-Isolation/Amphetamine" to distinguish them from the Main Inquiry (Auditory included) Control Group Litters No. CG#1, 2, 3, and the statistical results from the Main Inquiry's Repeated Measures ANOVA, and the overall mean scores for the individual combined-conditions are in brackets next to the labels which are also shown on the graph.

The two Experimental conditions of the No-Auditory Experiment are labeled "4-Isolation/Saline" and "4-Isolation/Amphetamine" to distinguish them from the Auditory Isolation Treatment Main Inquiry Experimental Group (Litters EXP#1, 3, and 5), and the statistical results from the Main Inquiry's Repeated Measures ANOVA .which are also shown on the graph.

Examining the performance of experimental subjects in the No-Auditory Isolation Treatment (EXP#4) group, for the condition that received only Saline at Trial, these subjects produced *lower* scores across *all* behavioral measures when compared to all other combined-conditions of the Main Inquiry Auditory Treatment Group, and these findings support the original hypothesis for Experiment No. 2.

However, examining the performance of experimental subjects in the No-Auditory Isolation Treatment Group(EXP#4) for the condition that received Amphetamine at Trial, subjects in this Group produced *higher* scores across *all* behavioral measures when compared to all other combined-condition groups in Experiment No. 2 -(No-Auditory Isolation Treatment,) and *higher* scores across *all* behavioral measures when compared to all other combined-condition groups in the Main Inquiry Auditory Isolation Treatment Group This finding was unexpected and therefore not predicted in the hypothesis. It was not expected that these subjects would have an even greater response to the psychostimulant drug (stress primer) than the Main Inquiry Auditory Isolation Treatment Group

One might note that the EXP#4–No-Auditory Isolation Treatment subjects'

behavioral profile is very similar to that of the Lewis Rat Strain and not the typical Sprague Dawley profile. This idea comes from personal observation and working with both Lewis Rats and Fisher Rats during various drug exposure experimental trials prior to and unrelated to this dissertation work. In the literature, Lewis Rats, in general, have been shown to "run low on dopamine" in several of the brain areas that have a synergistic interaction with the HPA and also with the Hypothalamic Pituitary-Steriodial Axis ("HPSA") (Gattaz & Brunner, 1996). Although the research literature is by no means definitively clear on the subject, it is generally accepted that Lewis Rats (the strain was derived from Sprague Dawleys several decades ago), have a distinct hormone profile from Sprague Dawleys and, also, from Fisher Rats. Fisher Rats are often characterized as the behavioral antipathies of Lewis Rats. Some of the hormone and behavioral differences that Lewis Rats exhibit when compared to Sprague Dawley or Fisher Rats, are: (1) The Lewis Rat is easily and voluntarily addicted to any drug of abuse[8] (i.e., they will voluntarily administer almost any drug of abuse, inhibitory or excitatory in nature); (2) Lewis Rats have an exaggerated behavioral response to various psychostimulants, (3) Lewis Rats have a blunted ACTH response to various stressors, and (4) Lewis Rats do not maintain Glucocorticoid stressor hormone responses (as measured in plasma levels) to various types of stressors when compared to either Sprague Dawley or Fisher Rats (Kosten & Ambrosio, 2002; Sircar & Kim, 1999; Dhabhar, McEwen & Spencer, 1997; and Stohr, Szuran, Welzl, Pliska, Feldon & Pryce, 2000). Lewis Rats have a compromised inflammatory response and are subject to several autoimmune diseases (Whitnall & Smallridge, 1997; Dhbhar, McEwen & Spencer, 1996; Pechowicz, Tonelli &

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8. Cross-sensitization studies have shown that reward and stress systems are cross-linked; the same neurotransmitters, neurohormones and brain nuclei are involved in effector pathways (Amygdala-VTA-NucA-Hypothalamus), with differences in the perception of Stress/Reward *state* seeming to involve a type of vector-analysis-shift in weighting of coactivation within the systems; such weighting occurs on a moment-to-moment basis, and generates the positive perception of reward and the negative perception of stress.

Sternberg, 2003; and Wei, Listwak & Sternberg, 2003). There have been conflicting reports from studies, some of which show that Lewis Rats have a slower glucocorticoid response to stress challenges than the other two groups. However, measurement of glucocorticoid depends on how the response is stimulated, what the target response is, and how the measurement is made. For instance, male Lewis Rats actually have a higher magnitude of change from baseline of plasma glucocorticoid when subjected to a strong stressor (i.e. cocaine) than do Sprague Dawley's or Fisher Rats, but once stimulated, Lewis Rats cannot maintain CRF release, or plasma levels of glucocorticoid, to the degree that Fisher Rats or Sprague Dawley Rats do (Simar, Saphier & Goeders, 1996). Various studies have shown a faulty HPA negative feedback function at various loci within the feedback loop (Calogero, Sternberg, Bagdy, Smith, Bernardini, Aksentijevich, Wilder, Gold and Chrousos, 1992).

Although there are many more hormonal strain differences in Lewis Rats enumerated in the literature, what might be seen as a corollary here is the behavioral response of Group EXP#4 (No-Auditory); it's consistently exaggerated response to the psychostimulant stress-primer, and conversely, its seemingly dampened behavioral response to the mild stress (i.e., an injection of Saline and placement into a novel open field box.) Most unfortunately, in this study no measurement of plasma hormones could be made and, so, that opportunity to gather information that might have offered physiological support for the corolorary offered here has not happened. There is, however, in the behavioral data generated by this study, (and which is consistent across all measures) strong similarities in the open field box behavior of EXP#4 subjects (both when exposed to psychostimulant (strong stressors) and to saline (mild stressor) the behavior of Lewis Rats under similar test conditions and which have been reported in published research.

Reflecting on the results of the weight measurements for the EXP#4 Group, for the first 4-days of life this group actually weighed more than the Auditory Isolation Treatment animals, but as the No-Auditory Isolation Treatment progressed, they continuously gained less weight than the Auditory Isolation Treatment animals through PND , and lost more weight *during each daily Isolation Treatment.* Also, this No-Auditory Group maintained that lower weight relationship up until the first day of testing in adulthood. These results combined with the results from the Postbaseline measurements, could support a developmental change that has its origins within the manipulated Isolation Treatment itself. It is only the Experimenter's speculation, however, that whatever the underlying physiological mechanism is that is being changed, it is being changed *inside that test chamber (*no doubt in an epigentic process), and to a degree that cannot be compensated for by (1) the pup-Dam-sibling interaction, or (3) any increase (resulting from the pups' absence) in the attention of the dam to the pups upon the pups' return to the nest, no matter how strong a developmental regulatory effect that maternal-pup-sibling relationship normally has---**which is considerable** -- during the perinatal period (as has been shown by the work of the Meaney-Plotsky Group and, (lest we forget) which originally had its beginnings in the behavioral studies conducted by Harry Harlow.

Further, based on the results of the analysis of the data produced herein, it can be surmised that the inclusion of the auditory variable produced a qualitatively and quantitatively different developmental change from those experiencing the Isolation Treatment without the auditory mask. Several ideas as to what the underlying mechanisms are that could account for these differences, drawn from the published literature, are included in the final Discussion section of this dissertation.

One could develop some interesting and eminently testable hypothesis about the behavioral results of this study using a Levels Theory approach and the regulation of

multi modal stimulation during specific time periods in development. Regarding any speculation about strain similarities and the induction or reversal of behavior profile changes in perinatal epigenesis within one generation, until this study is repeated (and with hormone assay), any similarity between any living Lewis Rat to any living EXP#4 subject portrayed herein is, as they say, merely coincidental

9.0 Subinquiry No. 1: Investigation Regarding Necessity of Drug Primer.

This is an Investigation of whether or not it was necessary to administer a psychostimulant drug to adult animals which underwent the perinatal Isolation Treatment in order to elicit the behavioral changes predicted in the Main Inquiry, Experiment No.1.

As stated earlier, changing a guideline embedded in your perspective will change the questions you are asking, and therefore change methods, procedures and depending on which levels of organization you predominantly focus —where in the system you look for the informative answers. Denenburg combined behaviorism and comparative approaches, Hofer combines behavioral observation and endocrinology, the Meaney-Plotsky group investigates pup-dam interactions similar to Hofer and then applies behavioral-endocrine testing and, more recently, this group is looking for proximate genetic mechanisms underlying the hormone changes involved in the pup-dam co-activational behaviors. Levine and colleagues historically have utilized molecular neuroendocrinology as the primary tool in addressing ontological issues without testing at a behavioral level. Many smaller labs make important contributions, sometimes *because* they are less constrained, approach a solution from a different perspective, or initiate a change in methodology[9].

One of many groups working in the EH, ED paradigm is that of Kehoe, McCormick, Mallinson and Bronzino, et al (1995; 1996; 1998; 1999; 2000; 2002; 2003).

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9. On rare occasions, Methodologists become Tinkerers and not Mechanists. Hopefully what is in play during those sometimes fortuitous moments is of the nature "less serendipity and more synchronisity"–a cognitive process generated from the (yet) unknown Black Box, formerly known as "The Cognitive Daemon". These are insights for which there exists no current vocabulary, no map; only a ***Direction***. (?Philosophy and NOT (as yet) Science). I remind myself often that Black-Box terminology is not only where phenomena (with undiscovered underlying mechanisms) reside, but also where unexamined time-dependant co-activational processes, and their underlying relationships and probabilities are temporiarly housed.

The early deprivation procedure they used is the same procedure which some of this dissertation work is modeled after. The timing is unique to their procedure in that it removes the pup from the dam and litter mates and places it in another environment for only one hour a day, for eight consecutive days, under tightly controlled experimental conditions. Kehoe and colleagues have published various results of the impact of the Early Deprivation procedure when using behavioral assessments and measures of changes in neurohormones levels in response to various types of stressor challenges, and at several developmental stages. This ED procedure is severe in that it removes the pup from both the dam and its litter mates for eight consecutive days and during "prime-time" nursing hours (i.e. studies have shown that for 12:12 day/night cycled dams, very young pups have frequent nursing bouts during early morning hours and even later into the morning while the dam sleeps)(Leon, Croskerry & Smith, 1977; and Pryce, Bettschen & Feldon, 2000). However the procedure is only 60 minutes a day. The Kehoe groups' early work conducted behavioral tests (1) shortly after the conclusion of the procedure, (2) upon reaching the juvenile stage; and (3) upon reaching young adulthood (PND 7 0-90). As researchers Robinson, Spear, Smotherman, Kuhn, working separately and also in collaboration with other groups have shown, Time-to-Test can be an important variable since developmental compensatory mechanisms may ameliorate or appear at a behavioral level to ameliorate any long term effects (Levine & Banich, 1990; Levine, 1993; Spear, L.,1987, 1990; and Paulson & Robinson, 1995). If so, then uncovering the original reorganization (if any) would involve breaking through or getting beyond any subsequent compensatory reorganization of neural networks (whether anatomical or chemical). At the adult stage such discovery could only be done by using a stressor-challenge large enough or having sufficient strength to *unmask or* **uncover** the *original underlying dysfunctional change in perinatal organization* (Spear, 1994). Determining just exactly what is a strong enough stressor-challenge could require multiple attempts.

It is considered a standard practice to test for an ontological effect obtained by a perinatal experimental manipulation by conducting behavioral tests at multiple time points prior to reaching adulthood so as to not miss the effect. Using a psychostimulant drug, (and varying the acute dose levels), *does* function as a strong elicitor of the HPA stress response (Spear, 1990).

In the Kehoe et al., 1998, publication it was stated that in a measure of Locomotion behavior, significant differences were seen when comparing Isolation Treatment subjects with Control No-Isolation subjects only if a strong challenge (i.e., psychostimulant primer or a 1-hour restraint environmental pre-stressor) was administered before placing subjects in the Open Field Chamber. When analyzing the behavioral data gathered on these non-pre-stressed and drug-naive groups during habituation trials (10 minutes in the Open Field apparatus), analysis of the Between-Group differences indicated that only a statistically non-significant trend was produced between the Isolation Treatment subjects v. No-Isolation Control subjects. Further, during later tests, for those Treatment or Control subjects that received only a mild stressor, that is, a saline injection and placement in the open field box, no significant differences in the scores obtained on the behavioral measures were found when comparing the Isolation Treatment subjects to either of the Control Groups. It was conjectured that "...this level of challenge may not be strong enough to reveal statically different behaviors due to a prior isolation experience." (P 123.)

The question of the necessity of administering a drug primer when testing in the open field measurement is examined.

9.1 Statistical Treatment.

Since a large number of animals were not available, and a Categorical Imperative exists to be parsimonious particularly in the use of animal life, separate animals could not be used to establish behavioral baselines prior to the first adult open field test.

Therefore, a separate statistical analysis was carried out on the already collected data to address this inquiry.

The analysis was carried out on the two cohort groups: (1) No-Auditory Isolation Treatment Group EXP#4 v. CG3, and (2) the Auditory Isolation Treatment Group EXP#5 v. CG3. All three of these litters were from dams supplied by the same vendor, delivered on the same date, and pups were born on the same date (i.e., within 24 hours). All testing was done on these groups within one week of the round of tests beginning for each group. (It can be noted, however, that the results for EXP#5 litter are extremely similar to the Main Inquiry Auditory Isolation Group, as can be seen in Table No. 9.)

In order to determine if a significant difference in behavior resulted from the perinatal Isolation Treatment in combined-conditions which did *not* receive psychostimulant drug at the time of the Open Field Test (instead they received an injection of saline (only) prior to testing in the Open Field chamber), a separate analysis of variance was run holding the absence of drug constant (i.e., 2 x 2 ANOVA; Isolation/No-Isolation x Sex). See Table 8 which sets forth the distribution of subjects for this analysis.

9.2 Statistical Treatment:

All of the same procedures listed in Paragraph 7.0a (1) through (5) above were also applied to this analysis. A 2 x 2 ANOVA (Isolation/No-Isolation x Sex) was done on all four behavior measurements.

(1) No-Auditory Isolation Treatment Group EXP#4 v. No-Isolation Control Group CG3:

LINECROSS:

There were two Between Subject Main Effects: Isolation $F(1,8) = 48.059$, $p < .0001$; and Sex $F(1,8) = 9.038$, $p < .017$. There were no significant interactions.

In the Isolation Condition, female Mean scores were 97.00 (SEM, 15.220); and male Mean scores were 82.500 (SEM, 10.762). In the No-Isolation Condition, female Mean scores were 213.50 (SEM, 15.220); and male Mean scores were 148.75 (SEM, 10.76). The combined scores for males and females in the Isolation Condition were significantly lower that the Linecross scores produced by the No-Isolation Condition subjects. The effect of the experimental No-Auditory Isolation Treatment was to produce fewer linecrosses than that produced by the Control No-Isolation subjects.

REARING:

There was one Between Subject Main Effect: Isolation $F(1,8) = 17.286$, $p < .003$, and no significant interactions. In the Isolation Condition, female Mean scores were 31.50 (SEM, 17.84); and male Mean scores were 31.250 (SEM, 12.62). In the No-Isolation Condition, female Mean scores were 119.50 (SEM, 17.84); and male Mean scores were 71.75 (SEM, 12.62). The combined scores for males and females in the Isolation Condition were significantly lower than the Rearing scores produced by the No-Isolation Condition subjects, and the effect of the experimental No-Auditory Isolation Treatment was to produce lower behavior counts than the Control No-Isolation subjects.

LOCOMOTION:

There were two Between Subject Main Effects: Isolation $F(1,8) = 32.96$, $p < .0001$; and Sex $F(1,8) = 5.51$, $p < .047$. There were no significant interactions. In the Isolation Condition, female Mean scores were 128.50 (SEM, 9.19); and male Mean scores were 113.75 (SEM, 44.04). In the No-Isolation Condition, female Mean scores were 333.00 (SEM, 38.18); and male Mean scores were 220.50 (SEM, 52.64). The combined scores for males and females in the Isolation Condition were significantly lower that the Locomotion scores produced by the No-Isolation Condition subjects and the effect of the experimental No-Auditory Isolation Treatment was to produce lower Locomotion scores than those produced by the Control No-Isolation subjects.

STEREOTYPY:

There was one Between Subject Main Effect: Isolation $F(1,8) = 11.74$, $p < .009$, and no significant interactions. In the Isolation Condition, female Mean scores were 12.50 (SEM, 2.23); and male Mean scores were 12.75 (SEM, 1.597). In the No-Isolation Condition, female Mean scores were 19.50 (SEM, 2.23); and male Mean scores were 19.00 (SEM, 1.58). The combined score for males and females in the Isolation Condition was significantly lower than the combined male/female Stereotypy score produced by the No-Isolation Condition subjects. **It should be noted however, that the Mean scores achieved by either group do not reflect actual "stereotypy" as any score under 5.0 reflects the lack of stereotypy (just movement/behavior). There were six bins with a possible score of 10.0 per bin.** The highest Mean score above was 19.50 +/- 2.23, or a possible 21.73, which is still 14.27 points under a score that would actually reflect stereotypy behavior.

(2.) Auditory Isolation Treatment Group EXP#5 v. No-Isolation Control Group CG3:

LINECROSS:

There was one Between Subject Main Effect: Sex $F(1,7) = 12.283$, $p < .01$. There were no significant interactions. In the Isolation Condition, female Mean scores were 199.50 (SEM, 26.60); and male Mean scores were 98.33 (SEM, 21.72). In the No-Isolation Condition, female Mean scores were 213.50 (SEM, 26.60); and male Mean scores were 148.75 (SEM, 18.81). The combined scores for males and females in the Isolation Condition were not significantly different from the those produced in the No-Isolation Condition.

REARING:

There was one Between Subject Main Effect: Sex $F(1,7) = 9.501$, $p < .018$. There were no significant interactions. In the Isolation Condition, female Mean scores

were 127.50 (SEM, 21.80); and male Mean scores were 55.67 (SEM, 17.80). In the No-Isolation Condition, female Mean scores were 119.50 (SEM, 21.80); and male Mean scores were 71.75 (SEM, 15.42). The combined scores for males and females in the Isolation Condition were not significantly different from those produced in the No-Isolation Condition.

LOCOMOTION:

There was one Between Subject Main Effect: Sex $F(1,7) = 12.349$, $p < .01$. There were no significant interactions. In the Isolation Condition, female Mean scores were 327.00 (SEM, 245.66); and male Mean scores were 154.00 (SEM, 37.27). In the No-Isolation Condition, female Mean scores were 333.00 (SEM, 45.65); and male Mean scores were 220.50 (SEM, 32.28). The combined scores for males and females in the Isolation Condition were not significantly different from the those produced in the No-Isolation Condition.

STEREOTYPY:

There were no significantly different scores and none of the Means were high enough to reflect a true stereotypy score or stereotypic behavior.

9.4 DISCUSSION of Subinquiry No. 1:

The inquiry was whether it was necessary to administer a psychostimulant priming stressor (to adult subjects just prior to the Open Field Test) in order to elicit the effects produced by the perinatal Isolation Treatment. Could any enduring behavioral effects of the perinatal Isolation Treatment be revealed by only administering a mild stress primer, that is, saline injection before placement into the Open Field Box. This question is really addressing the strength of the perinatal Isolation Treatment effect and its ability to have a long-term impact on adult stress response behavior. The rationale is that the stronger the perinatal Treatment Effect, the milder the test "challenge" will have

to be in order to illicit the enduring perinatal Isolation Effects on adult stress-response behavior.

This analysis shows that a psychostimulant primer **was necessary** to detect significant differences between the Isolation Treatment and No-Isolation Control Groups when perinatal Isolation Treatment was carried out using the Auditory Isolation Treatment method (i.e. in both Main Inquiry and EXP#5 groups). However, analysis of the subjects that received saline only in the **No-Auditory Isolation Treatment** (EXP#4 v. CG3) *showed significant Between Group effects*, across all behavioral measures, for subjects that were exposed perinatally to the No-Auditory Isolation *Treatment without* the necessity of using a psychostimulant primer.

Also, there were two prominent differences in the scores of behavioral performance between these two methodologically different isolation procedures:

(1) The No-Auditory Groups exhibited a *greater* response to the psychostimulant drug challenge/priming stressor than the Auditory Groups did;

(2) In *both* the No-Auditory Group and the Auditory Group, the Isolation/Saline Condition subjects produced *lower* test scores *across all measures* than the No-Isolation/Saline Condition subjects. *However, it was only in the No-Auditory Isolation Treatment Group that the difference in scores between the Isolation/No-Isolation Conditions receiving saline reached statistical significance.*

In the Kehoe study, when tested using a 2.0 mg/kg dose of Amphetamine, the neonatal Isolation Treatment subjects had significantly higher scores on total locomotor activity than the No-Isolation subjects, with no sex differences reported. Their second experiment, using a 1.0 mg/kg dose of Amphetamine, when comparing the Isolation/Amph v. No-Isolation/Amph Conditions, females had significant isolation effects with Isolation subjects achieving higher Locomotion scores than No-Isolation females; but males did not have locomotion distances that differed significantly between

Isolation Treatment groups. In their third experiment, the priming stressor used was a 60-minute restraint procedure (in place of a drug-pre-stressor). When comparing the Locomotion scores of Isolation Treatment subjects with the No-Isolation Treatment "handled" subjects (these would meet the definition of Animal Facility Reared animals), Females' scores showed significant Isolation effects, but males did not. But what was of interest here is that both males and females that experienced neonatal isolation produced Locomotion scores that were *lower* than the No-Isolation subjects. And, as stated previously in the Paragraph 9.0 introduction to this section, the results of the Kehoe group did not show statically significant differences in scores of Isolation Treatment subjects compared against the No-Isolation Control Subjects when using only a mild saline stressor.

Both the Kehoe group and this dissertation work utilized the Early Deprivation Isolation Treatment of 1-hour isolation for 8-consecutive days. The Kehoe group utilized somewhat different behavior measures, and they used an automated testing apparatus. For instance, their Locomotion measure was defined as "time spent in movement" and was measured as distance in centimeters, whereas movement was defined as "number of discrete movement events". These measures are combined in a total activity measure, "Locomotion." Because of these differences in measuring methods between that study and these dissertation results, it is difficult (if not impossible) to make a 1:1 correlation between the individual activity findings, and this may account for some of the differences in the direction of the results. However, overall, several findings in this study could be considered to be a "constructive" replication of the 1-hour for 8-consecutive days Early Maternal Deprivation paradigm used by the Kehoe group, and there are convergent results. Certainly the results of the behavioral analysis herein lend support to the idea that a 1-hour deprivation from both the dam and siblings, repeated over 8-consecutive

days is sufficient to produce, what Pryce-Feldon terms a "...sufficiently severe form of deprivation to alter the trajectory of neurobehavioural development." (p 8)

Further, the results of this analysis shows that the effects of perinatal Isolation Treatment can be shown to (1) have a long-term impact on stress-induced behavior, even into adulthood, and (2) depending on the specific methodology used in the Isolation Treatment, the perinatal Isolation Treatment effects can be elicited in the adult by using only a mild saline pre-stressor before testing.

10.0 EXPERIMENT No. 3, Postbaseline Open Field Test

The utility of having a baseline measure is well-established, the point being to have a basis from which you can measure absolute change in behavior. However, some designs cannot support a baseline measure because to expose the animal to the test chamber prior to the actual first trial could modify the variables measured. When using a drug-primer for instance, only the first injection is acute; after that point the neural systems are irrevocably changed (Ahmed, Stinus, LeMoal, & Cador, 1995) and what you are then studying are chronic drug effects and not initial increases in behavior due to acute stress factors (i.e., change in response levels of HPA axis hormones.) If no drug injection is used, then some researchers will expose the animal to the test chamber the day prior to testing to eliminate any novelty effects (increases or decreases in behavior resulting from being in an environment not experienced before), which might be a competing source of changes in behavior. However, in developmental paradigms it is often important to record the naive animal's behavior in the initial test environment (that is, naive to adult test administration and not naive to perinatal experimental manipulation. In hormone research pre-exposure to novel test apparatus has been reported to change base levels of stressor hormones. Even though novelty is attenuated by the pre-exposure procedure, the resulting raised levels of hormones such as CRF and Gluc have been reported not to subside for the next 2-3 weeks. If the subjects are tested within two weeks from the pre-exposure, the hormone residue then becomes an uncontrolled for stress- primer (Bardo, Rowlett & Harris, 1993; Walsh, et al. 1976; Tzschentke, 1998; Sorg, 1992; Koob, Heinrichs, Pitch, Menzaghi, Boldwin, Miczek & Britton, 1993; and Sircar, 1999).

An alternative approach to a pre-exposure to the test apparatus is to count the first 10-15 minutes of the initial open field test as a time period which includes increases (or decreases) in behavior resulting from novelty effects. After this period of

attenuation to the new environment elapses, any novelty effects drop out of the analysis and what remains is the pure behavioral effect which is then theoretically attributable to just the prior experimental manipulation (Kelley, 1998).

This study involved the use of an acute drug primer for the stress response and no pre-exposure to the test apparatus. Subsequently, a Postbaseline measure was conducted prior to the elapse of two weeks from the original Open Field Test. The Postbaseline Test was done to assess if there were differences in behavior between groups that could be attributed to (1) receiving the psychostimulant (stress primer) drug exposure at the time of the original open field test, which would be –if detected at Postbaseline–a type of "residual drug effect"; or (2) to assess any behavioral difference which could be attributed to increases in stressor hormones caused by the original test and which have not yet returned to baseline (appropriate for saline conditions only). Several comparisons could be done on scores , both between conditions and within-subjects within the Postbaseline test measure itself, and/or (depending on the condition) comparisons with the original Test behavioral measurements.

10.1 Methods

10.2 Subjects.

Isolation Treatment Groups (Litters No. 1, 3, 5 and EXP#4) (except subject #55), all received post-baseline testing. Also the Control Group (Litters No. 2 and 3), received post-baseline tests; however Control Litter No. 1 did not receive Post-baseline tests as the equipment was not available when the first group was ready for testing.

10.3 Post-Baseline Test Procedures.

The same order of testing was followed as when conducting the first Open Field Test, matching as closely as possible the time of test to the original testing hour and testing order for cagemates, so as to repeat as closely as possible the original trial conditions (note: all of the original test orders were done by random assignment - see

Experiment No. 1-Procedures for further details,). No injections of drug or saline were administered.

On the day of testing, the animal was taken from the cage and weighed in the colony room; a mark was placed on its tail and it was put back into the home cage. After 15 minutes, the animal was hand carried to the testing room next door and placed in the lower right corner of the open field box. The trials lasted 30 minutes and they were videotaped for scoring later. Between trials, the box was cleaned first with an enzymatic solution that removed any possible trace of pheromones or urine traces, and then cleaned with a diluted Equinox labware cleaning solution, then wiped thoroughly dry with paper towels. Animals receiving saline followed the same procedure.

10.4 Statistical Treatment.

All of the same preliminary screening was carried out on the data from these Postbaseline Trials as was done on the data from the first Trials and Postbaseline Trials for the Main Inquiry Auditory Isolation Treatment Group (Litters No. 3 and 5) v. No-Isolation Control Group (Litters No. 2 and 3). Since no Postbaseline testing was done on Control Litter No.CG1, the Auditory Isolation Treatment Group Litter No. EXP#1 (its cohort litter) was also not included in the analysis so as to balance the Main Inquiry Groups being analyzed. Identical analysis were also carried out on the No-Auditory Isolation Treatment Group, EXP#4 v. Control Group CG3. The following were run:

1) Three-way ANOVA'S were run on each of the Postbaseline measures for Main Inquiry Groups; and also on EXP#4 v. CG3. Those results can be found summarized in Table 10.

2) Also, Repeated Measures ANOVA's were carried out on the scores from the Postbaseline measures for the Main Inquiry Groups; and also on EXP#4 v. CG3, treating them as separate studies from the original Tests, and the results can be found summarized in Table 11, the Means for each bin are shown in Table B for the Main

Inquiry groups and Table D for the No-Auditory EXP#4 v. CG3; and also they are set out below.

3) Repeated Measures were done comparing scores on the original Tests with scores on the Postbaseline measures; results can be found summarized in Table 11, and also are set out below.

10.5 Results of Analysis.

In an attempt to make an organized presentation of the this experimental data two Tables have been constructed which contain the Mean scores for each combined-condition. Postbaseline test means for the Main Inquiry Auditory Isolation Treatment Group v. No-Isolation Control Group are contained in Table B; and the bin Means for the No-Auditory Isolation Treatment Group (EXP#4) v. No-Isolation Control Group (CG3) are contained in Table D.

(1.) Table B–Main Inquiry Auditory Isolation Treatment Group Litters No. EXP#3,5 v. No-Isolation Control Group Litters No. CG#2,3: *Postbaseline*:

A. *LINECROSS*:

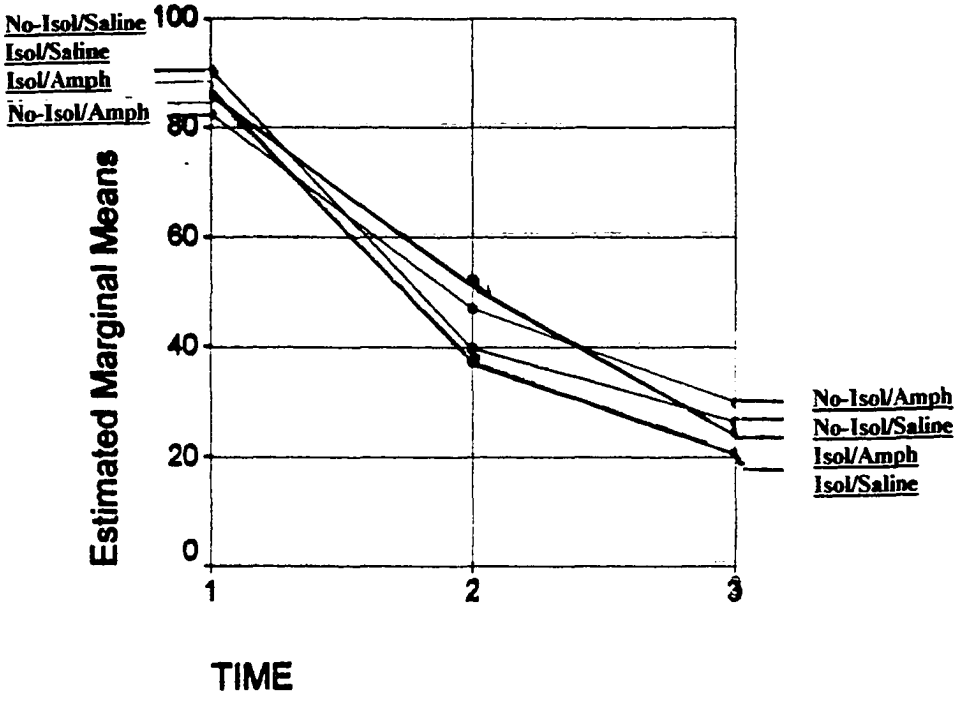
First, reference is made to Table A, for the first open field trial measurements. The following comparisons are those between the combined-condition groups within each measure, but are discussed (generally) compared with the original Trial-scores for each individual behavioral measurement.

Second, as can be seen in Table 11, and Figure 18, there are significant Within interactions for Within: Time, $F(1.939, 77.560) = 219.249$, $p < .0001$; Time x Drug, $F(1.939, 77.560) = 3.308$, $p < .043$; Time x Sex, $F(1.939, 77.560) = 3.456$, $p < .038$; Time x Drug x Sex, $F(1.939, 77.560) = 4.767$, $p < .012$; and Between effects for Drug x Sex, $F(1, 40) = 4.298$, $p < .045$. Since no drug is actually administered prior to Postbaseline Trials, the Isolation/Amph and No-Isolation/Amp "Drug" combined-condition scores actually reflect a difference in the scores of subjects who did receive psychostimulant but only at the original Trial. The Mean and SEM's are reported in Table B for each bin.

BEHAVIOR MEASURED	TIME 1 end 10 min	Hierarchy of Group	10min	Hierarchy of Group	TIME 2 end 20 min	T-3 end 30 min	T-4	T-5	TIME 6	Hierachy of Group
LINECROSS-- (PostBaseline)	(7.897) 90.450	No-Isol/Saline		Isol/Amph	50.937 (7.753)	29.917 (5.488)	→→→→→→	→→→→→→	→→→→→→	No-Isol/Amph
	(7.897) 87.267	Isol/Saline		No-Isol/Amph	47.021 (6.837)	26.417 (6.153)	→→→→→→	→→→→→→	→→→→→→	No-Isol/Saline
	(7.987) 85.500	Isol/Amph		No-Isol/Saline	39.700 (7.660)	23.062 (6.223)	→→→→→→	→→→→→→	→→→→→→	Isol/Amph
	(7.044) 82.458	No-Isol/Amph		Isol/Saline	37.050 (7.666)	20.283 (6.153)	→→→→→→	→→→→→→	→→→→→→	Isol/Saline
	end 5 min				end 10 min	end 15 min	end 20 min	end 25 min	end 30 min	
REARING--	(2.704) 26.500	No-Isol/Saline		No-Isol/Amph	23.979 (2.216)	17.125 (2.594)	15.000 (2.828)	11.813 (1.900)	7.708 (1.951)	No-Isol/Amph
(Postbaseline)	(2.411) 22.104	No-Isol/Amph		Isol/Amph	23.562 (2.513)	15.938 (2.288)	13.938 (2.494)	9.617 (2.131)	5.650 (2.188)	No-Isol/Saline
	(2.704) 21.767	Isol/Saline		Isol/Saline	20.067 (2.485)	13.300 (2.565)	11.567 (2.796)	7.875(2.155)	5.313 (2.212)	Isol/Amph
	(2,734) 21.125	Isol/Amph		No-Isol/Saline	19.567 (2.485)	10.833 (2.565)	8.500 (2.796)	5.267 (2.131)	5.283 (2.168)	Isol/Saline
	end 10 min				end 20 min	end 30 min				
LOCOMO	(11.485) 136.517	No-Isol/Saline		Isol/Amph	83.062 (12.261)	49.438 (8.572)	→→→→→→	→→→→→→	→→→→→→	No-Isol/Amph
(Postbaseline)	(11.615) 130.187	Isol/Amph		No-Isol/Amph	76.896 (10.813)	41.683 (9.611)	→→→→→→	→→→→→→	→→→→→→	No-Isol/Saline
	(11.458) 129.100	Isol/Saline		No-Isol/Saline	64.567 (12.124)	36.250 (9.719)	→→→→→→	→→→→→→	→→→→→→	Isol/Amph
	(10.243) 128.512	No-Isol/Amph		Isol/Saline	56.383 (12.124)	30.833 (9.611)	→→→→→→	→→→→→→	→→→→→→	Isol/Saline
	end 5 min				end 10 min	end 15 min	end 20 min	end 25 min	end 30 min	
STEREOTYP-	(101) 4.104	No-Isol/Amph		No-Isol/Amph	4.167 (.286)	3.687 (.361)	3.854 (.327)	3.200 (.394)	2.625 (.336)	No-Isol/Amph
(Postbaseline)	(113) 4.083	Isol/Saline		Isol/Amph	3.687 (.325)	3.450 (.357)	3.250 (.371)	3.063 (.352)	2.375 (.381)	No-Isol/Saline
	(114) 4.062	Isol/Amph		Isol/Saline	3.533 (.321)	3.250 (.318)	2.917 (.367)	2.375 (.399)	2.233 (.377)	Isol/Amph
	(113) 4.017	No-Isol/Saline		No-Isol/Saline	3.464 (.321)	2.900 (.357)	2.567 (.367)	2.367(.394)	1.733 (.377)	Isol/Saline
EXP#1,3,5 vs.CG#1,2,3–POSTBASELINE					**TABLE(FLOW CHART) "B"**-MAIN INQUIRY GROUPS					

Postbaseline LINECROSS

isolation/no isolation



Within:

Time, F(1.939, 77.560) = 219.249, p<.0001;
Time x Drug, F(1.939, 77.560) = 3.308, p<.043;
Time x Sex, F(1.939, 77.560) = 3.456, p<.038;
Time x Drug x Sex, F(1.939, 77.560) = 4.767, p<.012;

Between:

Drug x Sex, F(1, 40) = 4.298, p<.045.

FIGURE 18

FIGURE 18(a)

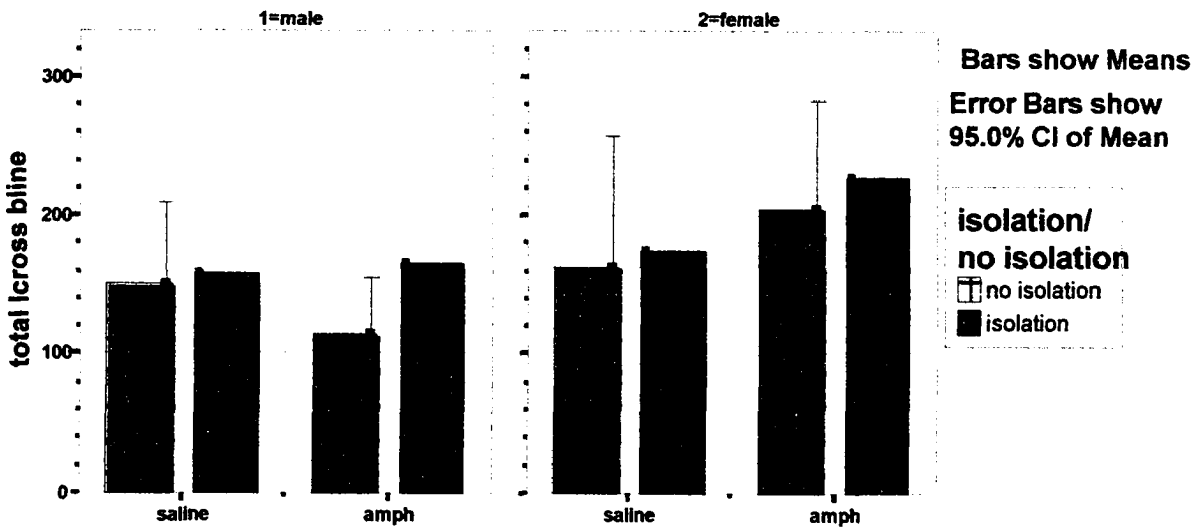
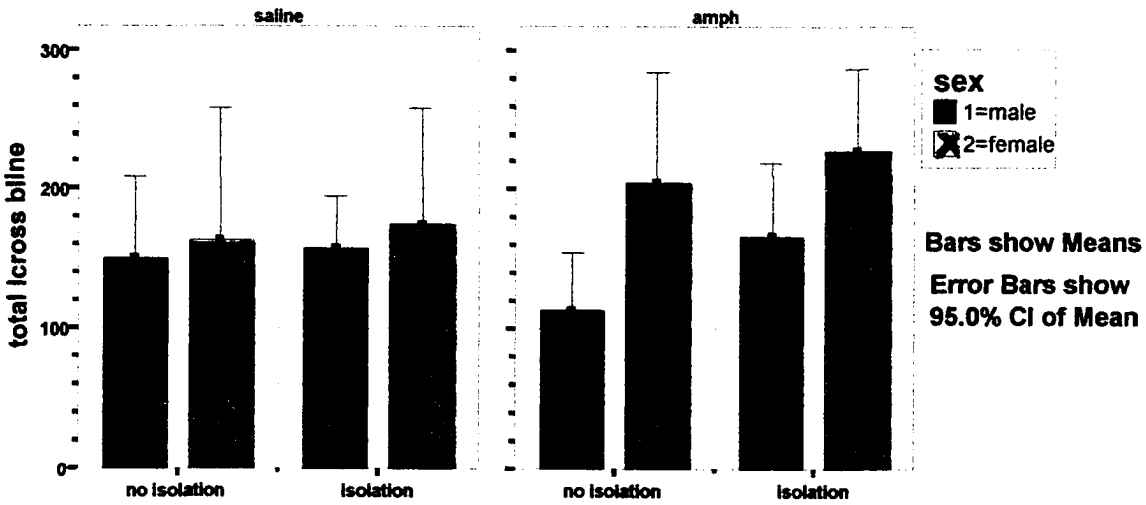


FIGURE 18(b



As can be seen in Figure 18(a)(b), males scored lower than females across all combined-conditions, scoring the least linecrosses in the No-Isolation/Amph condition. Females scored highest in the Isolation/Amph conditions, next highest in the No-Isolation/Amph condition. Since no drug is administered at Postbaseline, the greater activity levels could be attributed to "residual effects" of the drug at original Trial (i.e., unsubsided drug-induced increases in stressor hormones), which appear to be highest in the Isolation Treatment condition for both males and females when compared to the No-Isolation condition. However, from this it would appear that females have a much greater Postbaseline *Residual Drug Effect* than males in both these conditions.

B. *REARING.*

As can be seen in Table 11, and Figure 19, there is a significant Within effect for Time, $F(4.417, 176.66) = 56.219$, $p < 0001$; Time x Sex, $F(4.417, 176.66) = 3.643$, $p < .0005$; and Between effects for Sex, $F(1,40) = 4.278$, $p < .045$ only. As can be seen in Figures 19, 19 (a), 19(b) and 19(c), females had higher rearing scores than males across all combined-conditions regardless of drug/saline pre-stressor.

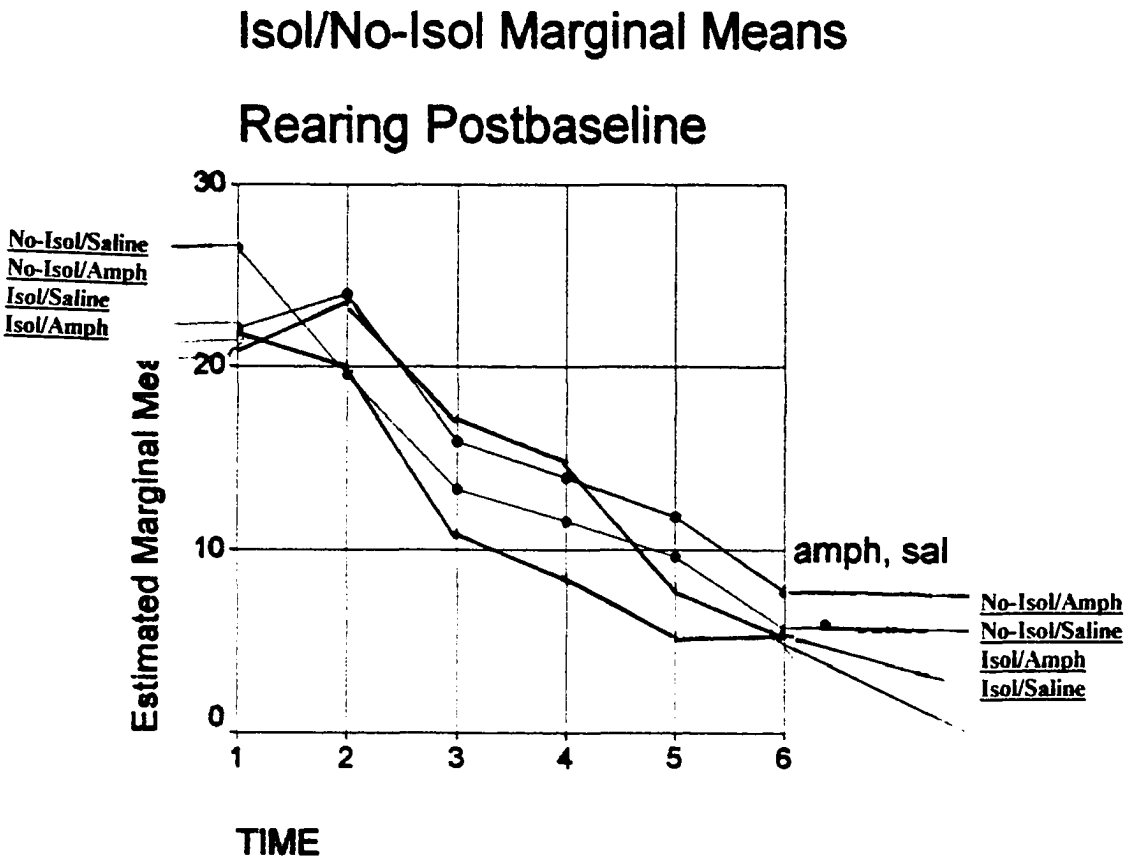
C. *LOCOMOTION.*

As can be seen in Table 11, and Figure 20, there are significant Within effects for Time, $F(4.417, 176.66) = 56.219$, $p < 0001$; Time x Sex, $F(4.417, 176.66) = 3.643$, $p < .0005$; and Between Group effects for Sex, $F(1,40) = 4.278$, $p < .045$. As can be seen in Figure 20(a), females had higher locomotion scores than males across all combined-conditions, but there were no other significantly different Between Group effects.

D. *STEREOTYPY:*

As can be seen in Table 11, and Figures 21, 21(a), there is a significant Within effect for Time, $F(5,200) = 31.107$, $p < 0001$; and no Between effects. As can be seen in Figure 21, the highest scores were obtained in the No-Isol/Amph condition; second

EXP.#1,3,5 vs. CG.#1,2,X



Within:

Time, F(4.417, 176.66) = 56.219, p<.0001;
Time x Sex, F(4.417, 176.66) = 3.643, p<.0005;

Between:

Sex, F(1, 40) = 4.278, p<.045;

FIGURE 19

FIGURE 19(a) Postbaseline Rearing Main Inquiry EXP3,5v. CG2,3

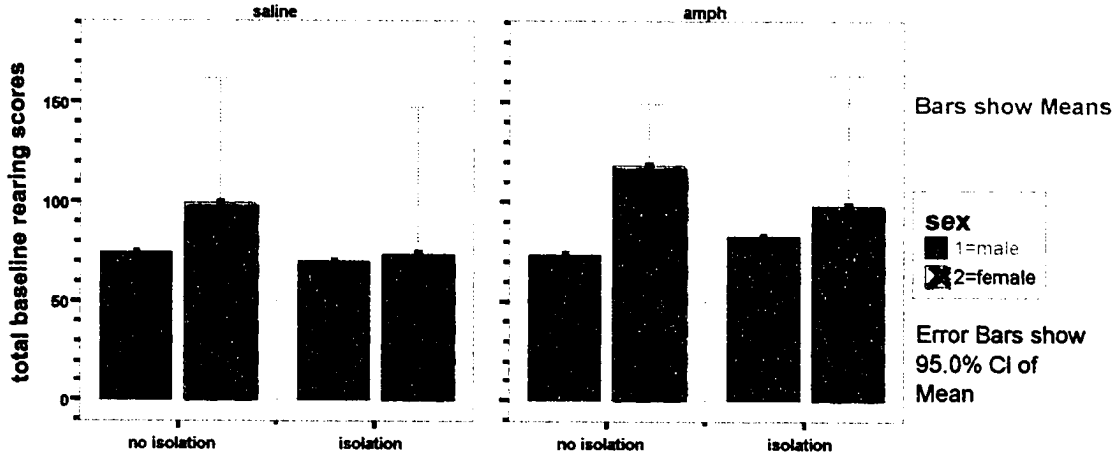


FIGURE 19(b) POSTBASELINE REARING Main Inquiry EXP3,5 v. CG2,3

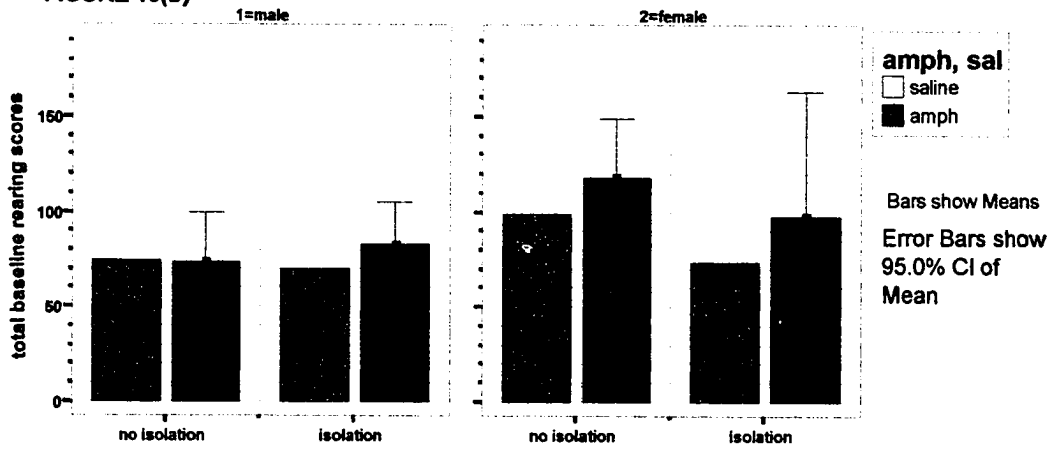
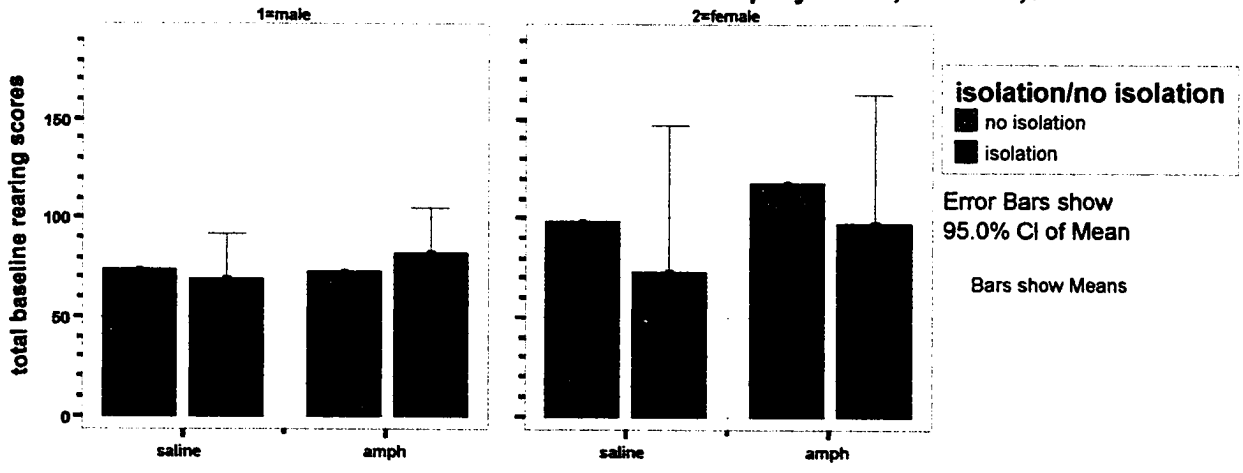
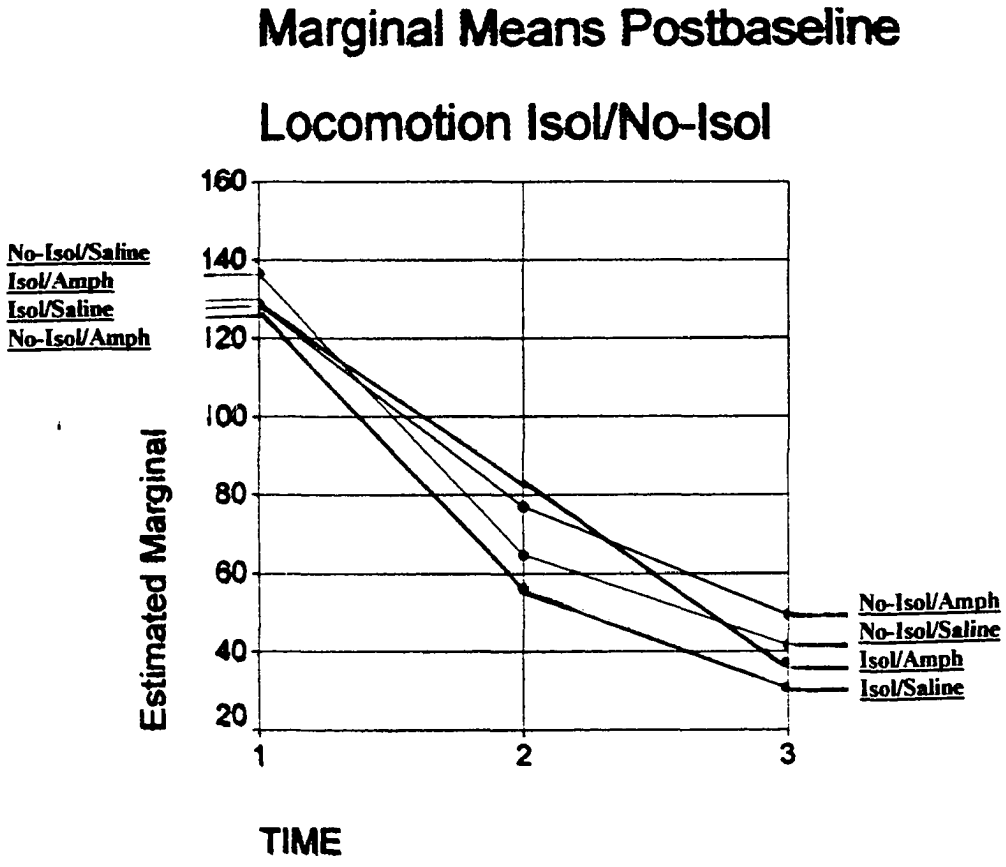


FIGURE 19(C) Postbaseline Rearing

Main Inquiry EXP3,5 v. CG2,3



EXP.#1,3,5 vs. CG.#1,2,X



Within:

Time, F(1.948, 77.938) = 200.977, p<.0001;
Time x Drug, F(1.948, 77.938) = 3.064, p<.054;
Time x Sex, F(1.948, 77.938) = 5.013, p<.009;

Between:

no sig.

FIGURE 20

FIGURE 20(a)

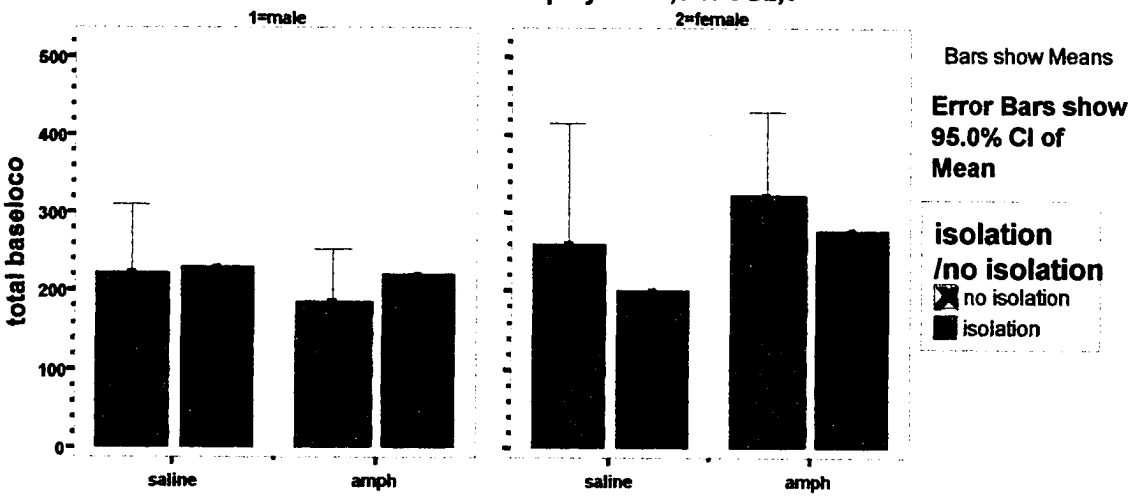
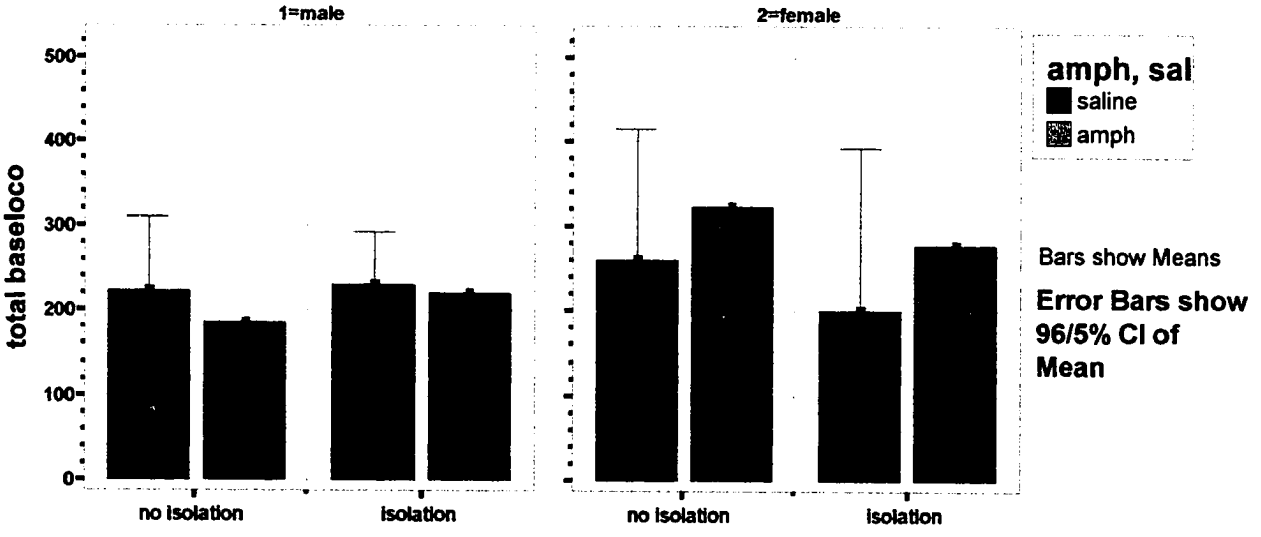
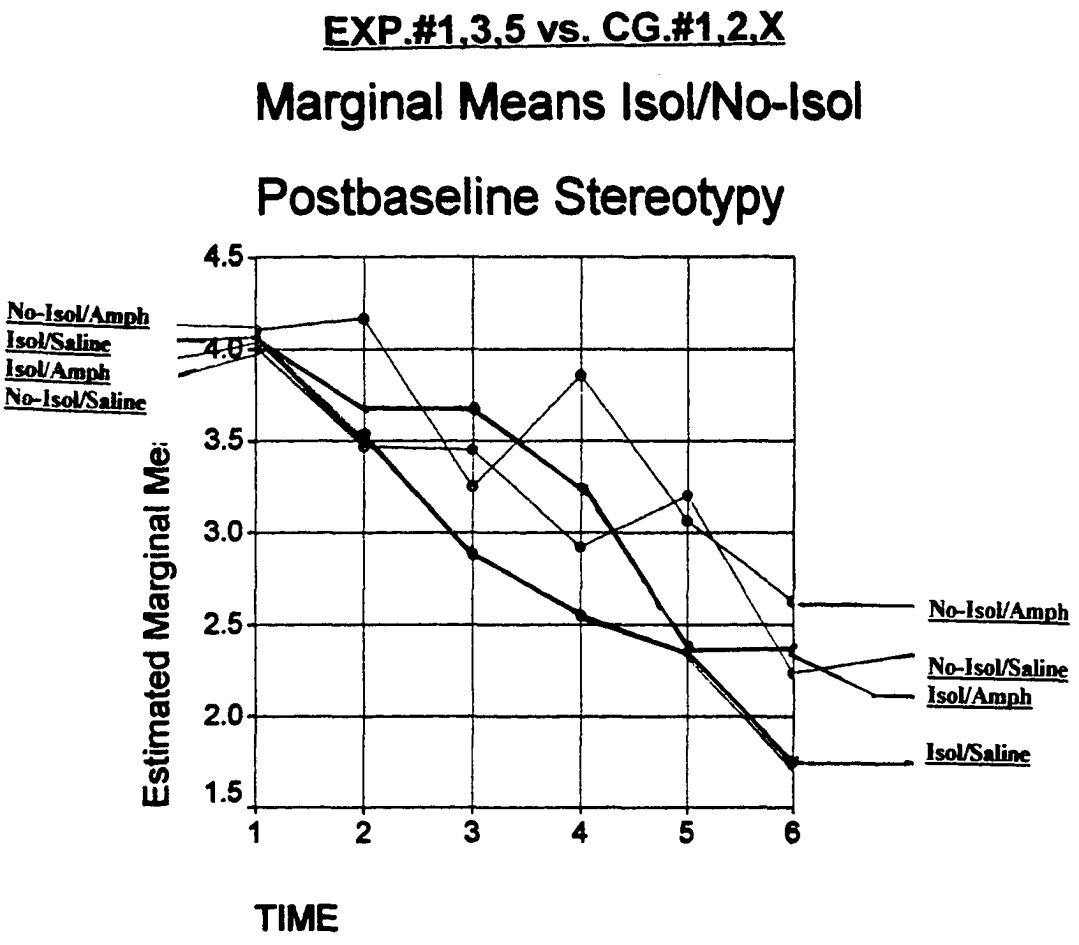


FIGURE 20(b)





Within:

Time, F(5, 200) = 31.107, p<.0001;

Between:

no sig.

FIGURE 21

FIGURE 21(a)

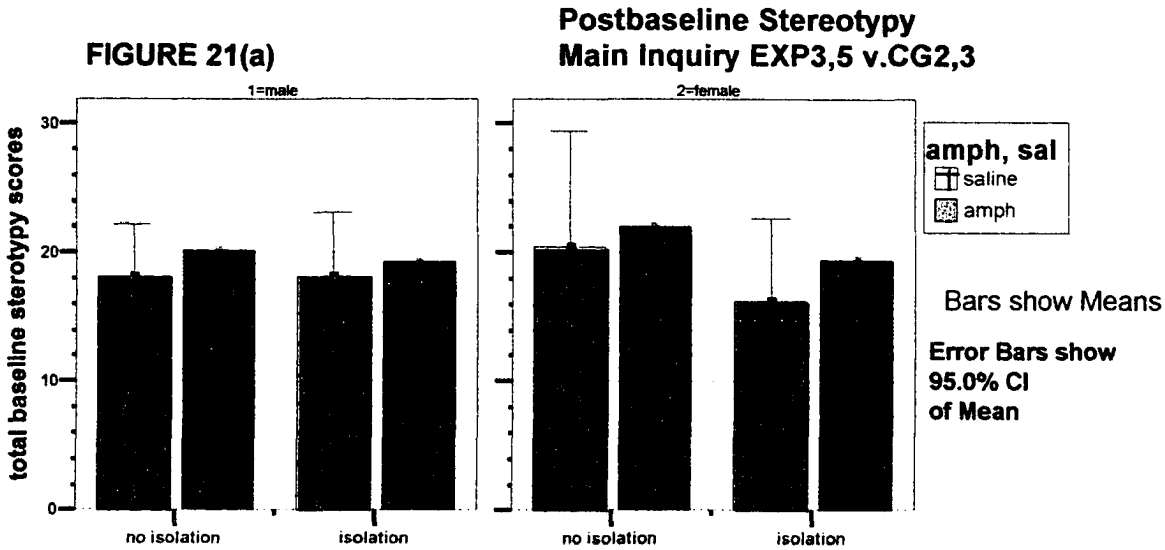
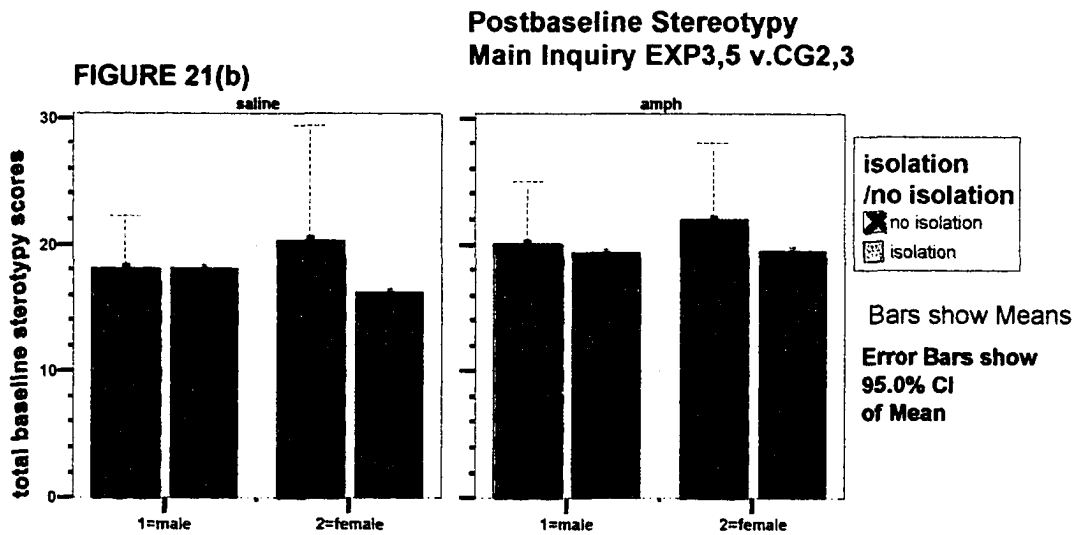


FIGURE 21(b)



highest was the Isol/Amph condition, followed by No-Isol/Saline, and last, Isol/Saline. It might be noted that the hierarchical ordering of groups at the end of the trial was maintained in that order for groups and across all measures.

(2.) Table D -No-Auditory Isolation Treatment Group EXP#4 vs. No-Isolation Control Group CG3– Postbaseline.

Attached to Table D are four graphed-figures 22,.23, 24 and 25, with three Figures each attached. Generally the individual combined- conditions of the No-Auditory Isolation Treatment Group and Control Group at this Postbaseline test exhibited a 15-20 minute behavioral shift which was not present at the original Test trials, however by T-3 the ordering of performance for all combined condition groups was fixed through the end of the Postbaseline Trials.

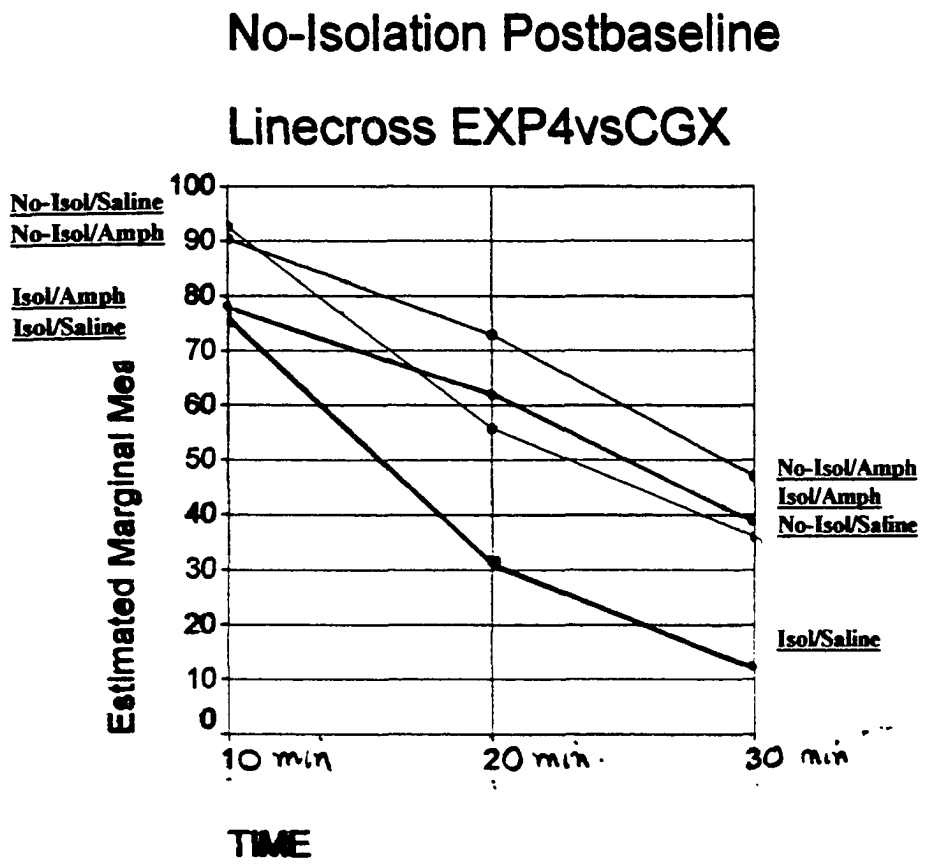
A. *LINECROSS:*

As can be seen in Figure 22, and Figure 22(a)(b), there were significant interactions for Within effects: Time, $F(2,34) = 102.175$, $p < 0001$; Time x Drug, $F(2,34) = 6.383$, $p < .004$; Time x Sex, $F(2,34) = 4.551$, $p < .018$; and in the Between effects for Isolation, $F(1,17) = 4.230$, $p < .055$; Sex, $F(1,17) = 4.535$, $p < .048$; and Isolation x Sex, $F(1,17) = 5.785$, $p < .028$. Males had opposite residual drug effects from females. Males that had received amphetamine at the original Trial scored lower than males in who received saline in those conditions at the original Trial. Whereas, females who received amphetamine at the original Trials, in either the Isolation or No-Isolation condition had higher scores in this Postbaseline measure than those females who received saline.

B. RE*ARING:*

As can be seen in Figure 23, there were significant Within effects for Time, $F(1,85) = 21.648$, $p < .0001$; Time x Drug, $F(1, 85) = 3.434$, $p < 007$; and Between effects for Drug, $F(1, 17) = 7.219$, $p < .028$; Isolation.x Sex, $F(1, 17) = 7.219$, $p < .016$. Females and males had higher scores in the residual drug effect/amphetamine conditions

BEHAVIOR MEASURED	TIME 1 end 10 min	Hierarchy of Group		Hierarchy of Group	TIME 2 end 20 min	T-3 end 30 min	T-4	T-5	TIME 6	Hierachy of Group
LINECROSS-- (PostBaseline)	(9.223) 92.625	No-Isol/Saline		No-Isol/Amph	72.900 (7.999)	➡	➡	➡	➡	No-Isol/Amph
	(8.910) 90.350	No-Isol/Amph		Isol/Amph	62.333 (7.806)	➡	➡	➡	➡	Isol/Amph
	(8.695) 78.167	Isol/Amph		No-Isol/Saline	55.750 (8.280)	➡	➡	➡	➡	No-Isol/Saline
	(9.223) 76.500	Isol/Saline		Isol/Saline	31.750 (8.280)	➡	➡	➡	➡	Isol/Saline
	end 5 min				end 10 min	end 15 min	end 20 min	end 25 min	end 30 min	
REARING--	(4.284) 23.625	No-Isol/Saline		No-Isol/Amph	29.050 (2.593)	➡	➡	➡	➡	No-Isol/Amph
(Postbaseline)	(4.039) 22.500	Isol/Amph		Isol/Amph	25.333 (2.530)	➡	➡	➡	➡	Isol/Amph
	(4.289) 18.125	Isol/Saline		No-Isol/Saline	20.875 (2.684)	➡	➡	➡	➡	No-Isol/Saline
	(4.139) 15.650	No-Isol/Amph		Isol/Saline	16.500 (2.684)	➡	➡	➡	➡	Isol/Saline
	end 10 min				end 20 min	end 30 min				
LOCOMOTION	(14.240) 137.125	No-Isol/Saline		No-Isol/Amph	114.650 (13.266)	➡	➡	➡	➡	No-Isol/Amph
(Postbaseline)	(13.757) 135.050	No-Isol/Amph		Isol/Amph	102.333 (12.947)	➡	➡	➡	➡	Isol/Amph
	(13.757) 126.000	Isol/Amph		No-Isol/Saline	87.875 (13.732)	➡	➡	➡	➡	No-Isol/Saline
	(14.240) 111.125	Isol/Saline		Isol/Saline	50.125 (13.732)	➡	➡	➡	➡	Isol/Saline
	end 5 min				end 10 min	end 15 min	end 20 min	end 25 min	end 30min	
STEREOTYPY-	(.209) 4.250	No-Isol/Amph		No-Isol/Amph	5.200 (.374)	4.200 (.388)	➡	➡	➡	No-Isol/Amph
(Postbaseline)	(.217) 4.125	No-Isol/Saline		Isol/Amph	3.833 (.365)	4.000 (.402)	➡	➡	➡	Isol/Amph
	(.204) 4.000	Isol/Amph		No-Isol/Saline	3.750 (.388)	3.833 (.379)	➡	➡	➡	No-Isol/Saline
	(.217) 3.750	Isol/Saline		Isol/Saline	2.875 (.388)	2.625 (.402)	➡	➡	➡	Isol/Saline
➡	arrow indicates there are no further changes in hierarchy				**TABLE (FLOW CHART) "D."-POSTBASELINE for EXP#4 v. CG3**					



Within:

Time, $F(2, 34) = 102.175$, $p<.0001$;
Time x Drug, $F(2, 34) = 6.383$, $p<.004$;
Time x Sex, $F(2, 34) = 4.551$, $p<.018$;

Between:

Isol., $F(1, 17) = 4.230$, $p<.055$;
Sex, $F(1, 17) = 4.535$, $p<.048$;
Isol. X Sex, $F(1, 17) = 5.785$), $p<.028$).

FIGURE 22

FIGURE 22(a)

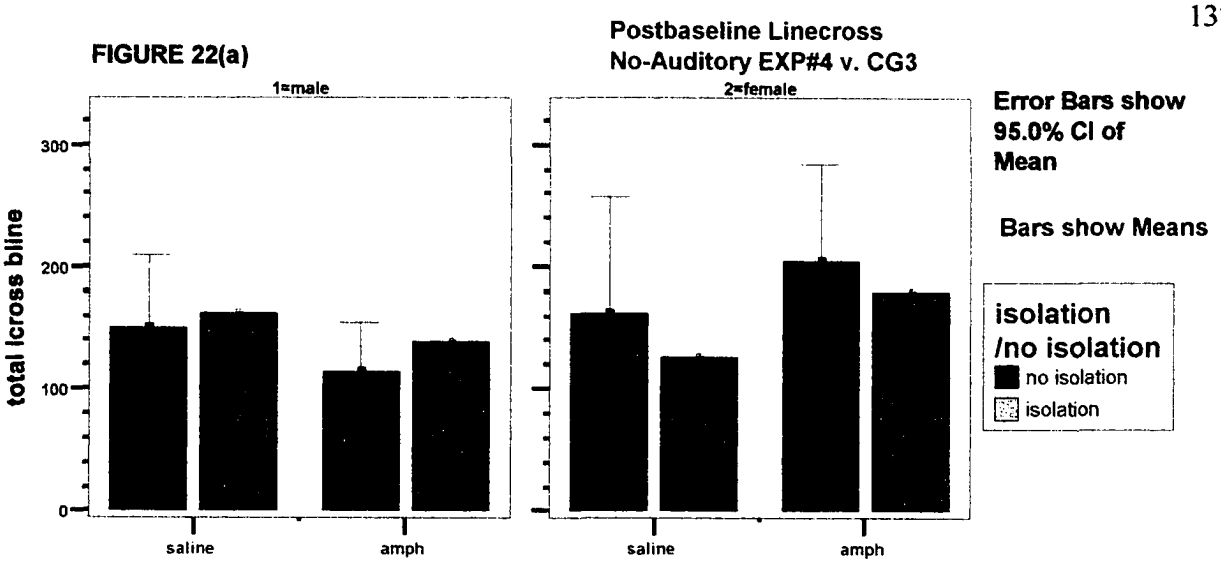


FIGURE 22(b)

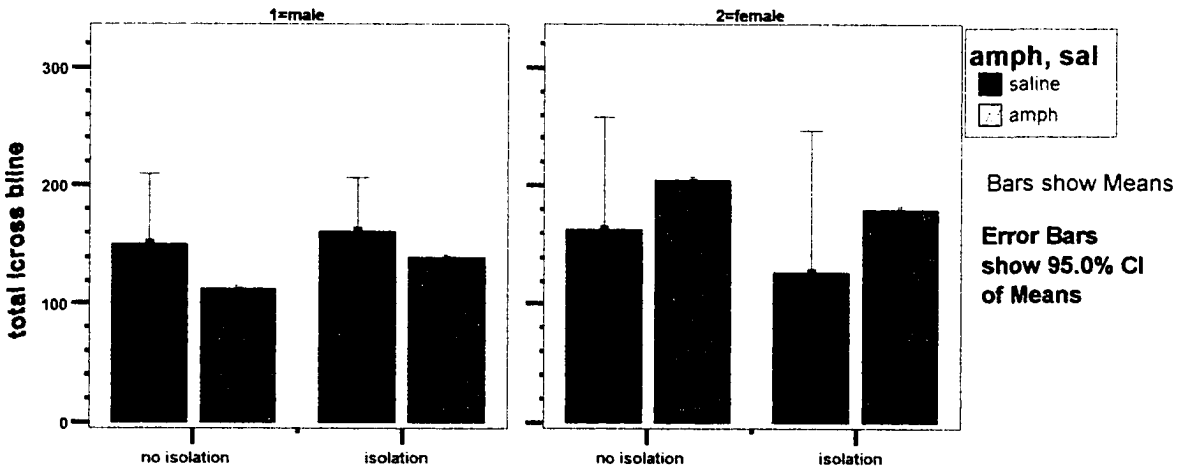
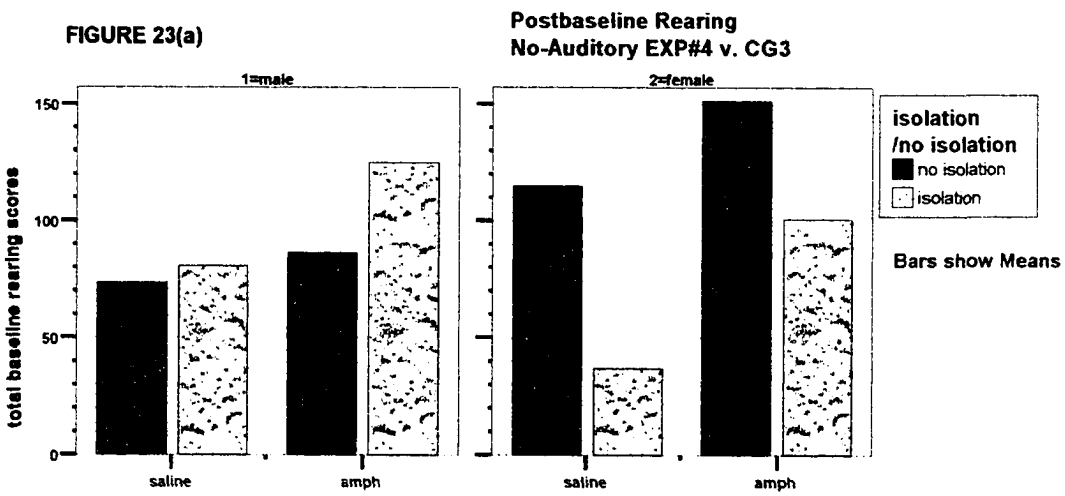
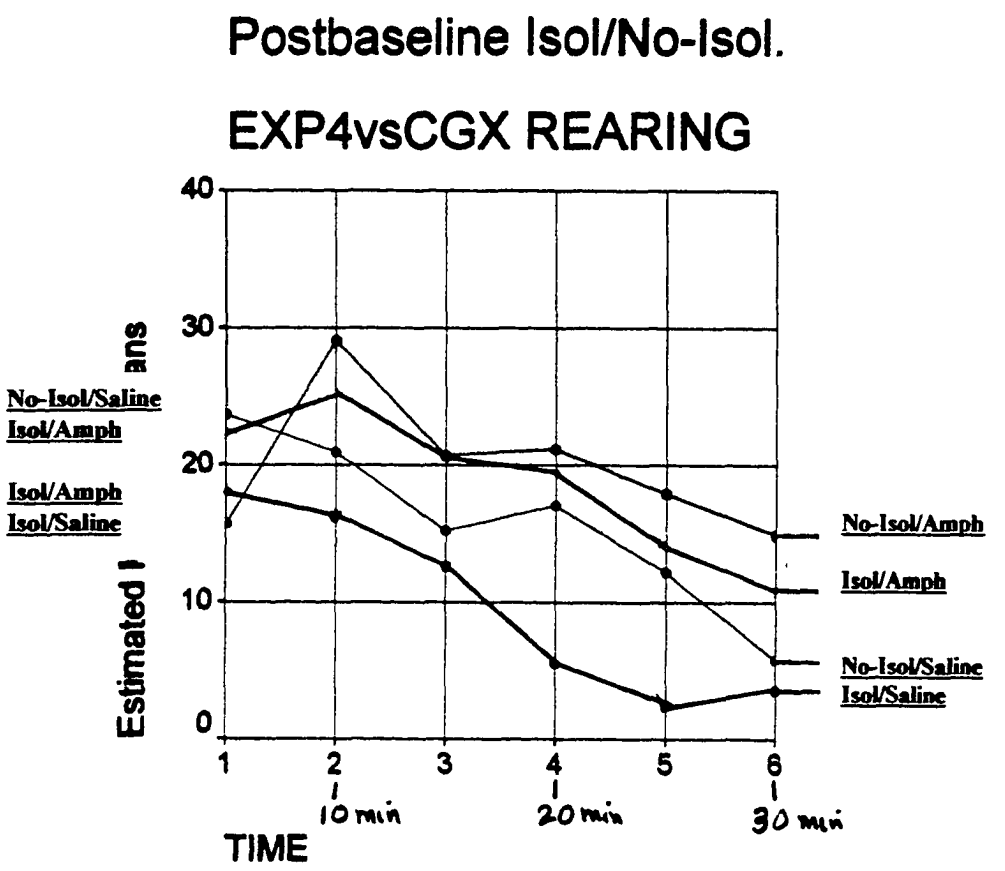


FIGURE 23(a)





Within:

Time, F(1, 85) = 21.648, p<.0001;

Time x Drug, F(1, 85) = 3.434, p<.007;

Between:

Drug, F(1, 17) = 7.219, p<.028;

Isol.x Sex, F(1, 17) = 7.219, p<.016.

FIGURE 23

FIGURE 23(b)

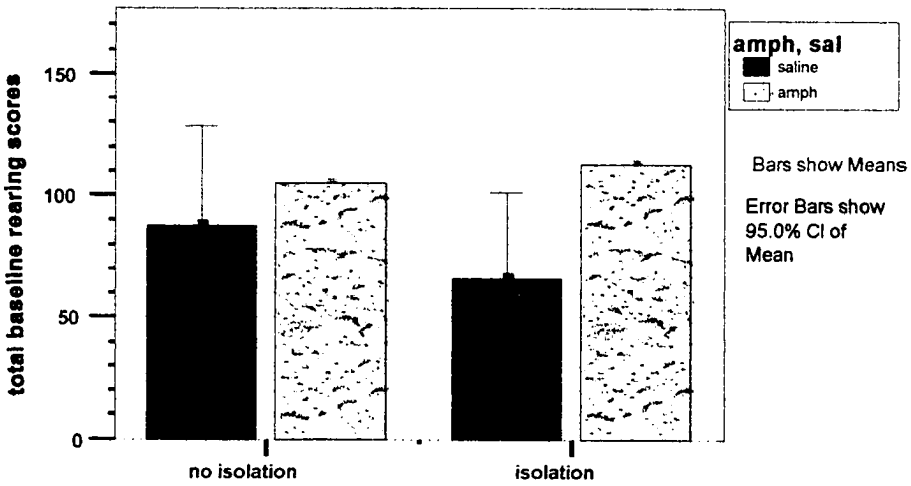


FIGURE 24(a)

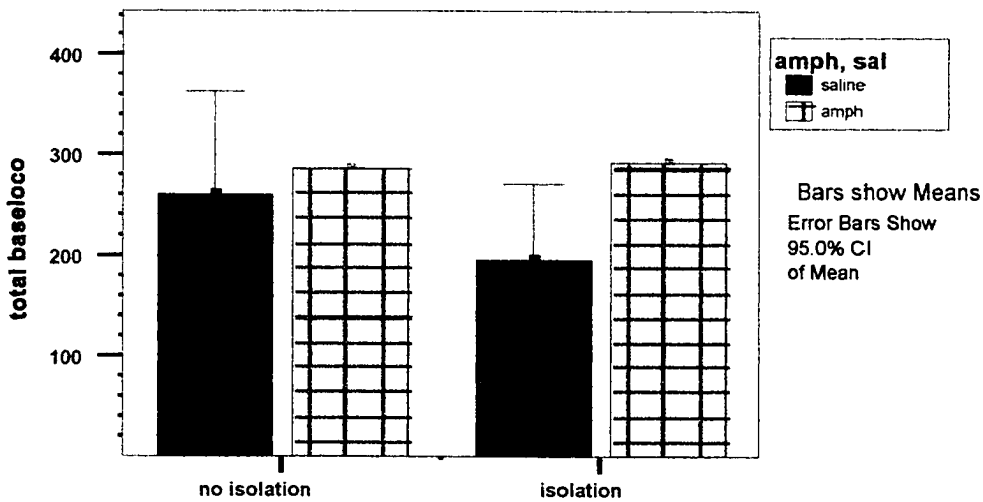
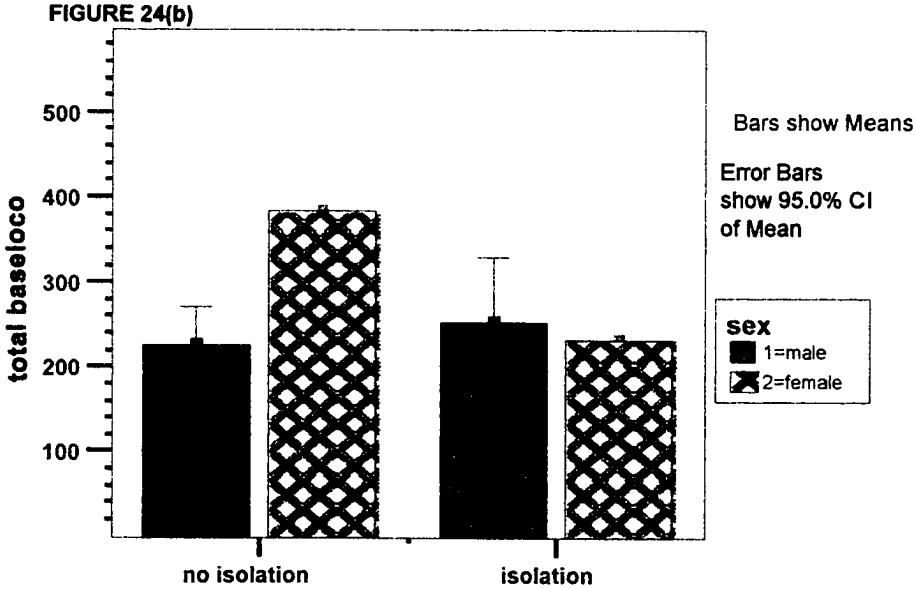


FIGURE 24(b)



than in the saline conditions; however females had higher scores in the No-Isolation conditions, and males had higher scores in the Isolation conditions.

C. *LOCOMOTION:*

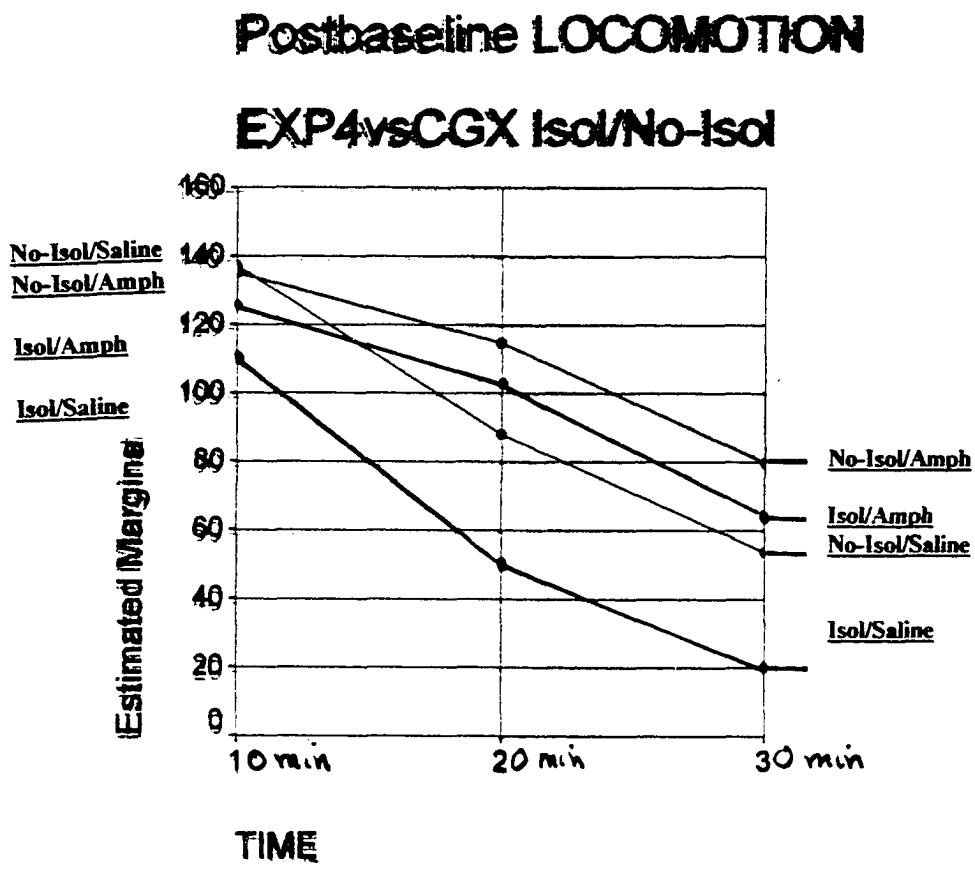
As can be seen in Figure 24(a)(b), there were significant Within effects for Time, $F(2, 34) = 108.121$, $p < .0001$; Time x Drug, $F(2, 34) = 6.566$, $p < .004$; Time x Sex, $F(2, 34) = 3.201$, $p < .053$; and Between Group effects for Drug, $F(1, 17) = 4.599$, $p < .047$; and Isolation x Sex, $F(1, 17) = 6.754$, $p < .019$. Males and females scored highest in the residual drug/amphetamine conditions. Males scored higher in the Isolation conditions over females, and females scored higher in the No-Isolation conditions than males.

D. STEREOTYPY:

As can be seen in Figure 25, there were significant Within effects for Time, $F(5, 85) = 9.763$, $p < .0001$; and Between: Drug, $F(1, 17) = 6.956$, $p < .017$, Isolation, $F(1, 17) = 12.345$, $p < .003$; Isolation x Sex, $F(1, 17) = 5.786$, $p < .028$. Females scored higher than males in the No-Isolation condition for both saline and amphetamine; males scored higher than females in the Isolation condition for both saline and amphetamine. In all conditions, those in the amphetamine conditions scored highest, which means that there was a significant "residual drug effect" for both sexes (Figures 25(a)(b)(c)).

(3.) Comparisons Between Scores on the Original Trial Measures v. Scores on the Postbaseline Measures for (1) the Main Inquiry Auditory Isolation Treatment Group (Litters No. 3, 5) v. No-Isolation Control (Litters No. 2, 3); and for (2) No-Auditory Isolation Treatment Group EXP#4 v. No-Isolation Control CG3.

(1) Main Inquiry Groups: Figure 26(a), is a bar graph which plots the total Mean scores attained by females in the Isolation/Saline and the No-Isolation/Saline conditions in both the Original Trial and the Postbaseline Trial; Figure 26(b), plots the same information for the Isolation/Amphetamine and the No-Isolation/Amphetamine conditions. Figure 26(c) and 26(d) sets out the same information for males in the respective conditions.



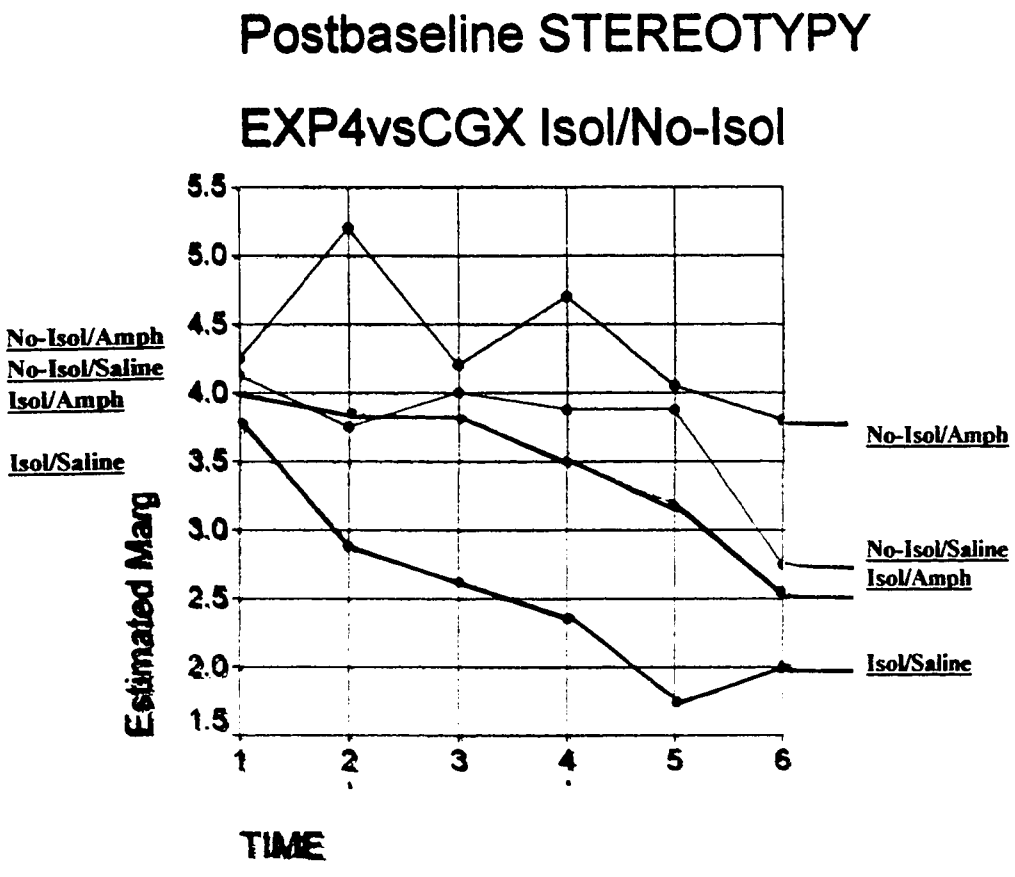
Within:

Time, F(1, 34) = 108.121), p<.0001;
Time x Drug, F(1, 34) = 6.566, p<.004;
Time x Sex, F(1, 34) = 3.201, p<.047;

Between:

Drug, F(1, 17) = 4.599, p<.047;
Isol. X Sex, F(1, 17) = 6.754, p<.019.

FIGURE 24



Within:

Time, $F(5, 85) = 9.763$, $p<.0001$;

Between:

Isol., $F(1, 17) = 12.345$, $p<.003$;

Drug, $F(1, 17) = 6.956$, $p<.017$;

Isol.x Drug, $F(1, 17) = 5.786$, $p<.028$.

FIGURE 25

FIGURE 25(a)

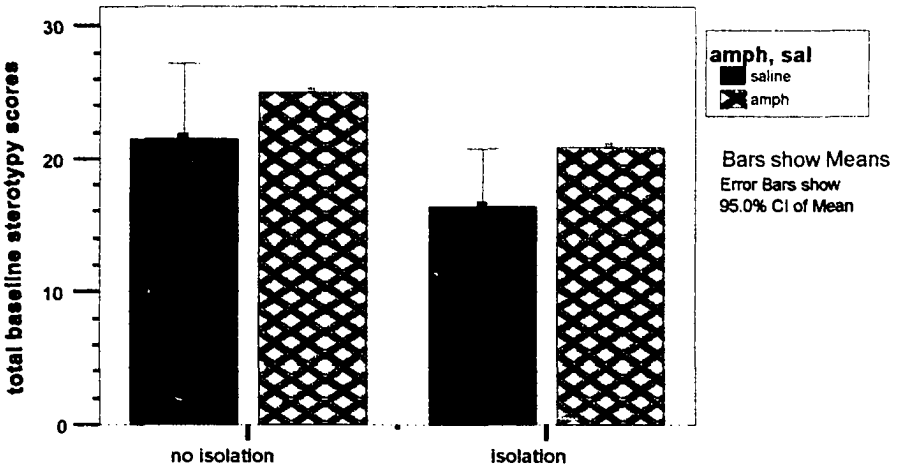


FIGURE 25(b)

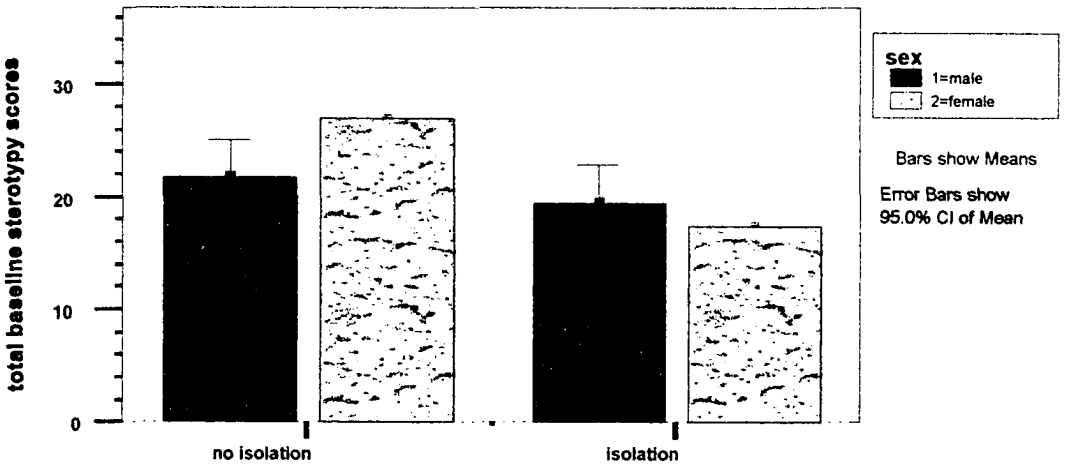


FIGURE 25(c)

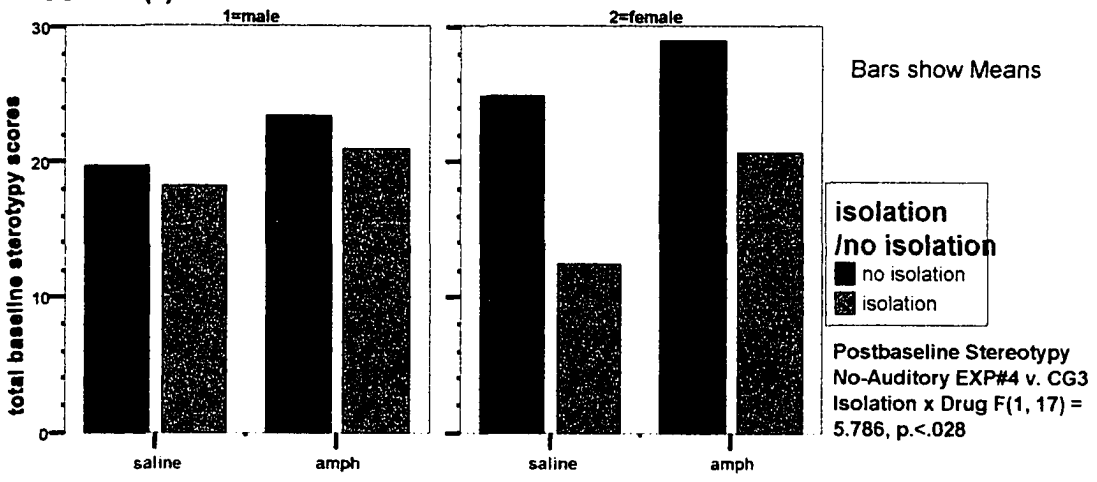


FIGURE 26(a)--Females/Saline Only

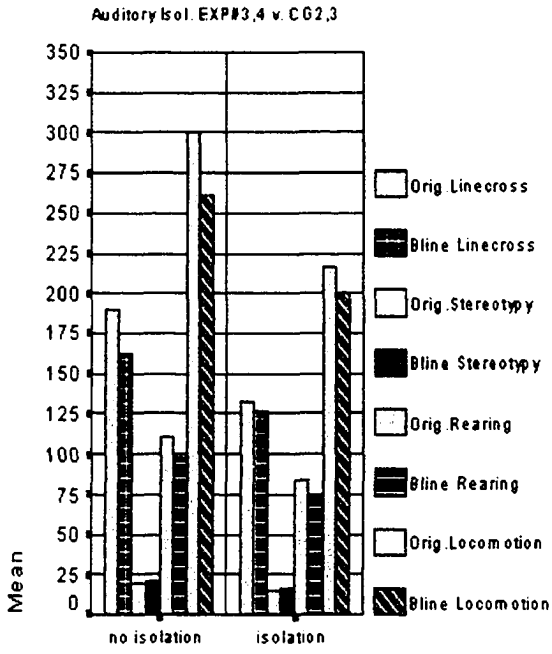


FIGURE 26(b)--Females/Amph Only

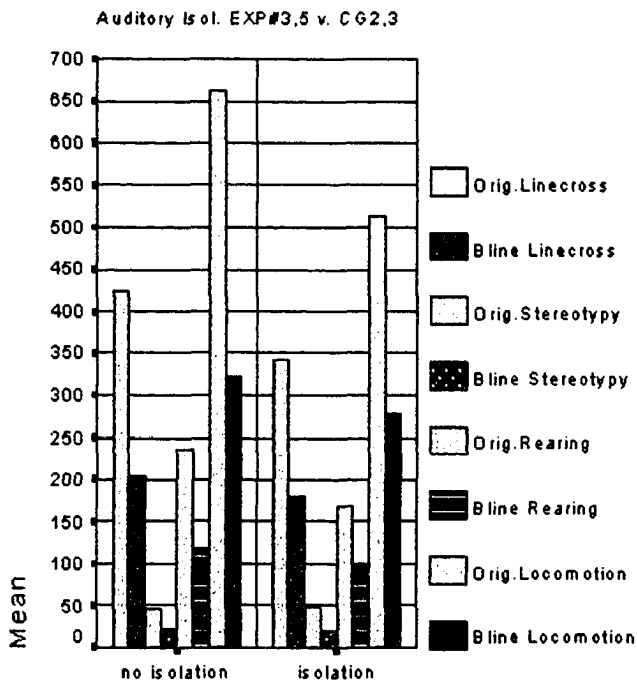


FIGURE 26(c)--Males/Saline Only

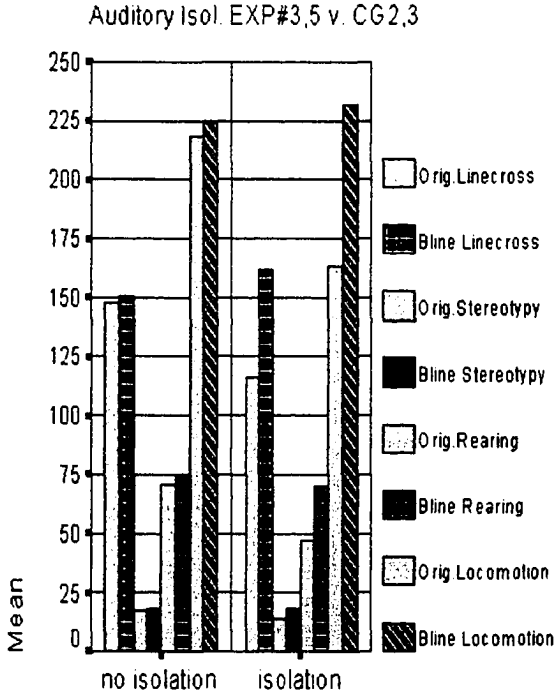
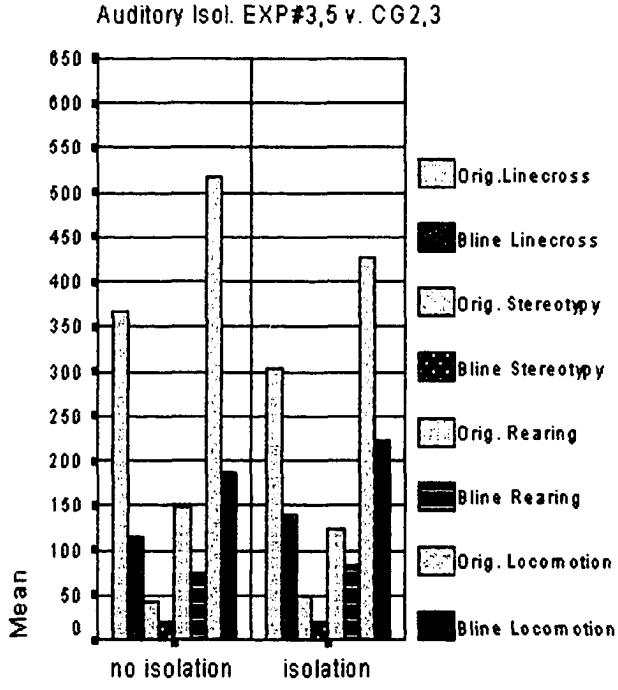


FIGURE 26(d)--Males/Amph Only



(2) Auditory EXP#4 v. CG3: Figure. 27(a), is a bar graph which plots the total Mean scores attained by females in the Isolation/Saline and the No-Isolation/Saline conditions in both the Original Trial and the Postbaseline Trial; Figure 27(b), plots the same information for the Isolation/Amphetamine and the No-Isolation/Amphetamine conditions. Figure 27(c) and 27(d) sets out the same information for males in the respective conditions.

Section 10.6 which follows directly has a brief discussion of this information.

10.6 Discussion of Experiment No. 3.

Main Inquiry, Auditory Isolation Treatment

(1) As can be seen in Figures 26(a) and 26(c), in the original trial Saline/Isolation Condition, females had higher Linecross, Rearing and Locomotion scores than males; however, in an opposite effect, males had much higher postbaseline scores than females. Again, Stereotypy scores were approximately equal. The same relationship held in the Saline/No-Isolation Condition, with females scoring higher at trials than males, but lower than males at postbaseline.

(2) As can be seen in Figures 26(b) and 27(d), in the Amphetamine/Isolation Condition, females had higher original test and postbaseline Linecross, Rearing and Locomotion scores than males; Stereotypy scores were similar between males and females with greater test than postbaseline scores. The same relationships held for the Amphetamine/No-Isolation Condition with both males and females in this condition having higher scores on all measures except Stereotypy.

No-Auditory Isolation Treatment

(1) As can be seen in Figures 27(a) and 27(d), in the Saline/Isolation Condition, females had higher scores than males on Linecross, Rearing and Locomotion at trial; in an opposite effect males had higher postbaseline scores on these same measures. Stereotypy scores were equal for males and females at trial and males had higher postbaseline scores.

(2) As can be seen in Figures 27(b) and 27(c), in the Amphetamine/Isolation Condition, at trial females scores higher than males on Linecross, Stereotypy and Locomotion, and males scored

FIGURE 27(a)--Female/Saline Only

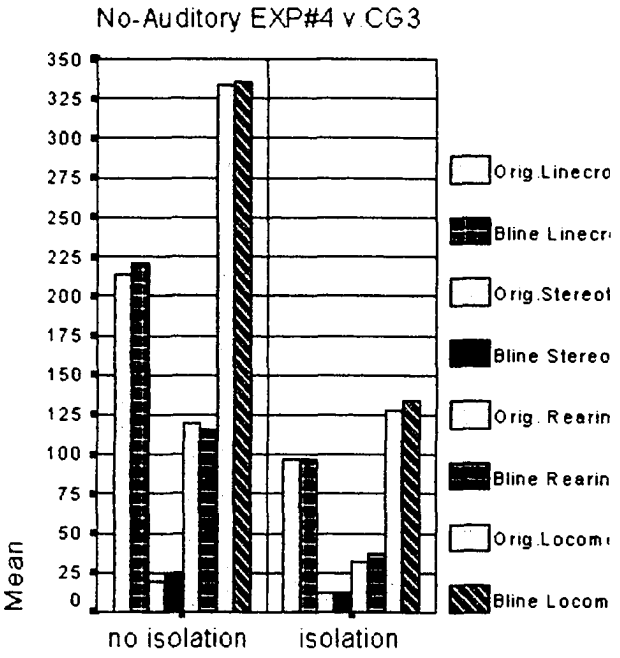


FIGURE 27(b)--Female/Amph Only

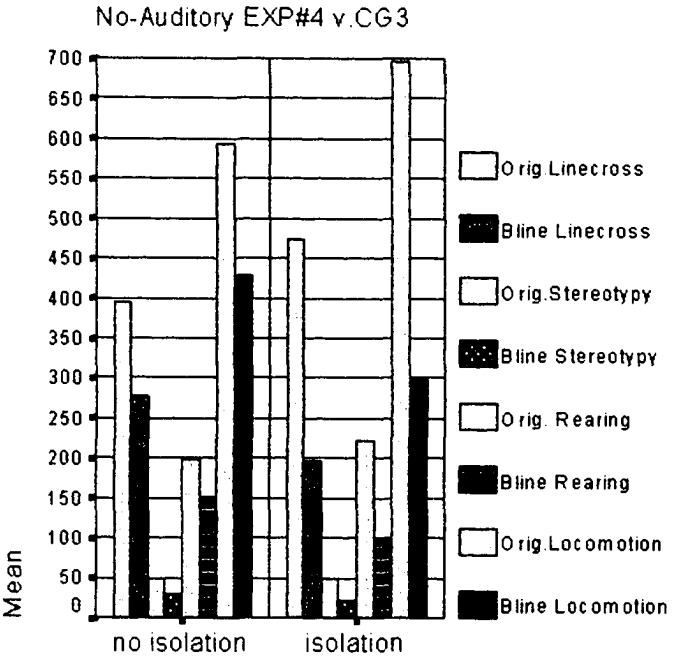


FIGURE 27(c)--Male/Saline Only

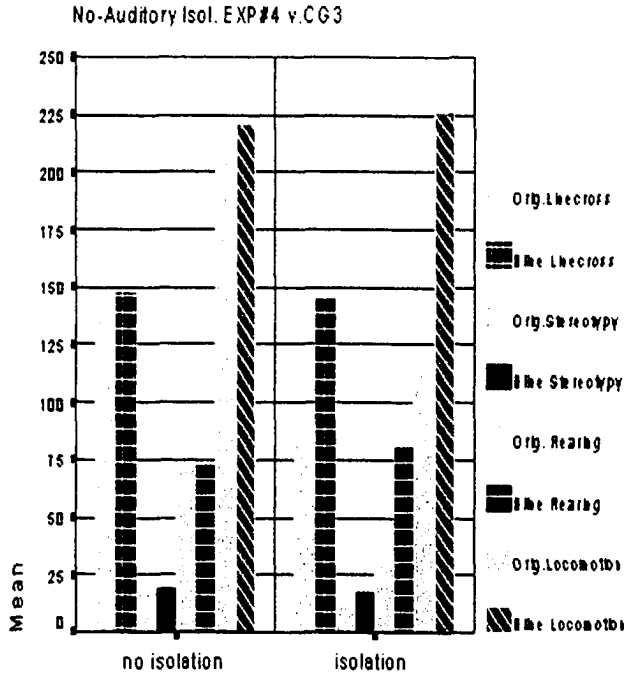
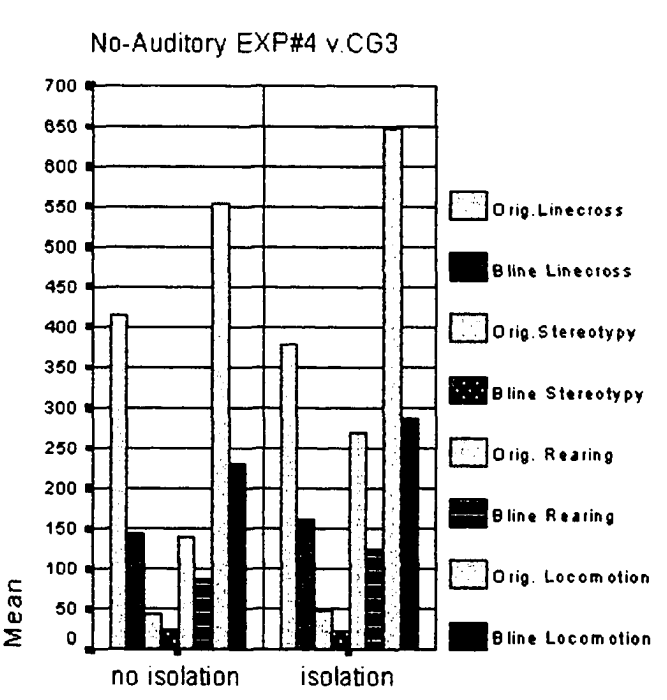


FIGURE 27(d)--Male/Amph Only



higher than females on Rearing. At postbaseline males had higher scores than females on Rearing, and females had higher scores on each of the other measures.

Comparison of Auditory Isolation Treatment v. No-Auditory Isolation Treatment

(1) In the Saline/No-Isolation Control Conditions, males had approximately equal scores at trial and at baseline for both of the different Isolation Treatments. Females had somewhat higher scores in the EXP#4 No-Auditory Condition than in the Main Inquiry Auditory Group at trial and at baseline.

(2) In the Saline/Isolation Conditions, males had lower scores at trial and postbasline in the EXP#4 Treatment relative to the Main Inquiry Treatment; Females also had lower scores at trial and baseline in the EXP#4 Treatment

(3) In the Amphetamine/No-Isolation Condition, males had higher scores at trial and postbaseline in the EXP#4 condition relative to the Main Inquiry Treatment groups; and females were opposite to males and had higher scores in the Main Inquiry Treatment groups at trial and postbaseline.

(4) In the Amphetamine/Isolation Condition, males had much higher scores at trial and postbaseline in the EXP#4 condition relative to the Main Inquiry Treatment groups, and females had much higher scores at trial and postbaseline in the EXP#4 condition relative to the Main Inquiry Treatment group

Looking at just the male Saline conditions, there is very little difference between trial and postbaseline scores in the No-Isolation condition for either of the types of Isolation Treatments. This would seem to evidence very little "novelty" effect. However, in both types of Isolation Treatments males produced considerably elevated scores in the Isolation postbaseline trials relative to the original trials. And the greater differences in the same direction were in the EXP#4 scores. This would argue for a "novelty" effect occurring only for Isolated male subjects, and with the apparent effect of

causing a reduction of activity at the original trial, and the most reduction happening for the No-Auditory group

Looking at just the female Saline conditions, there is little difference between trial and postbaseline scores in the No-Isolation condition for either types of Isolation Treatments, however, the direction of change (if any) is different between types of Isolation Treatments. In the Main Inquiry group the postbaseline scores are lower than the trial scores, but in the EXP#4 group the postbaseline scores are higher than the trial scores. These differences are very minimal, which does not say much for a novelty effect here. In the EXP#4 Isolation/Saline condition, there are no trial v. postbaseline differences, and in the Main Inquiry group, again, the trial v. postbaseline differences are minimal and showing a reduction at postbaseline. Again, this does not argue for much of a novelty effect for any group, for either type of Isolation Treatment in the Saline Condition..

Comparing the Amphetamine conditions, in all groups and all conditions, the postbaseline scores were much lower than the trial scores as would be expected, for both males and females. The greatest reduction in scores appears to be in the EXP#4 postbaseline scores in the Isolation condition. In the Main Inquiry groups, for males and females, the greatest reduction in scores appears to be in the No-Isolation condition. It is difficult to address novelty effects here since these conditions all received amphetamine at the original trial and it would be difficult to access residual drug effects from novelty effects.

11.0 Subinquiry No. 2 - Inquiry regarding Weight Gain of Pups and Adults With a View Towards Possible Treatment Effects.

The ED Isolation Procedure was carried out during what would have been "prime time" nursing (lights went on in the colony at 5:00 AM EST, and the Isolations were run from approximately 6:00 AM through 10:30 AM). For several reasons, it would be predicted that the pups experiencing the Isolation Treatment would weigh less than the Control pups who remained with their dams and therefore were nursed during this period of time. Studies done by various researchers have reported significant between group differences in weights on a daily basis, while others have reported no differences between the experimental and control groups either when using daily group weights, or when weighed on PND 9 --which is the last day of the treatment (Pryce et al., 2001; Lehrmann et al., 2003). Furthermore, some groups that reported weight differences on PND 9 also reported that the differences were ameliorated at weaning age (exact day of weaning varies across studies; this study weans at PND 25). As can be seen in Table 1, other groups have reported that there were ***no*** significant between-group differences in weight (except for the expected sex differences, that is, males attain higher weights than females when the animals reached juvenile age, or adulthood (i.e., approximately PND 30, and PND 70-90, respectively)(Lehrmann et al, 2000).

Not only is it important to be able to make a comparison between the experimental and control groups on weight gain or loss, but some indication of whether or not weight is being lost by the pups during the Isolation Treatment Procedure could aid in directing an inquiry as to what the underlying mechanisms, endogeneous, exogeneous or coactivational, taking place during the 60 minute separation period from the dam actually involves, and whether those variables contributes in an additive or non-additive way to the deprivation of being away from the dam and from potential nursing bouts. One of the current beliefs is that the "deprivation factor" contained in this

Isolation Procedure is that of the pup being "deprived" of the lactation and grooming manipulations of the dam (which are believed to be those maternal actions regulating the suppression of GLUC levels during SHRP)(Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky & Meaney, 1997). Little attention has been paid to the possibility that any weight loss *during* the procedure could have an additive or synergistic stress effect on the physiology of the pups (i.e., "energy deprivation") in connection with the psychological stress of separation from the dam (and whatever specific behavioral factors that "deprivation" includes) or to any possible synergy between the two (accepting the theoretical assumption that they are, in fact, separable events).

11.1 Procedure - Pups, General.–Before Treatment Weights.

Beginning on PND 2, and through PND 9, pups were removed from the dam and placed together into one round, 5" in diameter plastic container with no bedding in it; half the litter was removed at a time. They were placed under a warming lamp and temperature was measured inside the pup huddle to see that it did not get warmer than 31 °C. Each pup was removed one at a time, weighed, and numbered with a fine-line permanent Sharpie marker, color coding each 6-pup group, and rotating the colors used each day between groups. To further control for order effects, the order in which the 6-pup groups were taken from the litter were rotated each day. The newly marked pup was then returned to its siblings' huddle until all pups had been weighed and marked (less than 3-4 minutes). At this point, control pups were returned to the mother, and the remaining half of the litter was given the same procedure and then returned to the mother.

Experimental pups experienced the same weighing and marking procedure, but instead of being returned to the dam and litter mates, they were then placed into individual round containers and carried all at once into the isolation chamber in the

adjoining room; the individual cups with the pup inside were placed into the test chamber about ten inches apart, and the cover replaced and audio started. After one hour passed, they were gathered into a one-group-cup while still in the isolation chamber and then carried back to the colony room and returned to the dam. The second half of the testing-litter was then given the same procedure as the first.

11.2 Statistical Treatment.

All of the same procedures listed in Paragraph 7.0a (1) through (5) above were also applied to this analysis as far as screening of the data. (1) A (2 x 2) ANOVA was done comparing total Mean weights between experimental and control groups. (2) Then, Repeated Measures ANOVA (Time x Isolation/No-Isolation) was done on the weight for all groups (PND 2 through PND 9). (3) Simple ANOVA's were done to verify significant differences in weights at each time point . (4) Further, paired t-tests were done comparing weights on PND 2 with weights on PND 9 for the Isolation Treatment pups, and for the No-Isolation pups. (5) A simple correlation, and scatter plot with a fitted regression line, was done to estimate the rate of change in weight gain within each group, and then the results were compared between groups.

11.3 Results of Analysis–Before Isolation Treatment Pup Weights.
(1.) Main Inquiry Auditory Isolation Treatment Group (Litters No. EXP 1,3,5 vs. No-Isolation Control Group (Litters No. CG1, 2, 3).

As can be seen in Figure 28 (which sets out Main Inquiry Group Litters No. EXP#1, EXP#3, EXP#5 vs. Control Group Litters No. CG1, CG2, CG3), all pups gained weight on PND 2 through PND 9. There was a significant difference in total weight gain between the Isolation Treatment Cond. pups and the No-Isolation Condition (control) pups, (2 x 2 ANOVA), $F(1, 69) = 35.192$, $p < .0001$. Repeated Measures ANOVA had a significant Within-Subject effect for Time $F(7, 5.819) = 1232.387$, $p < .0001$), and a significant Between-Subjects effect for the Isolation v. No-Isolation Condition, $F(1, 69) = 35.192$, $p < .0001$) (see Figure 28(a)). Simple ANOVAs were run at each time point

FIGURE 28--PUP Wts. Before Isol.

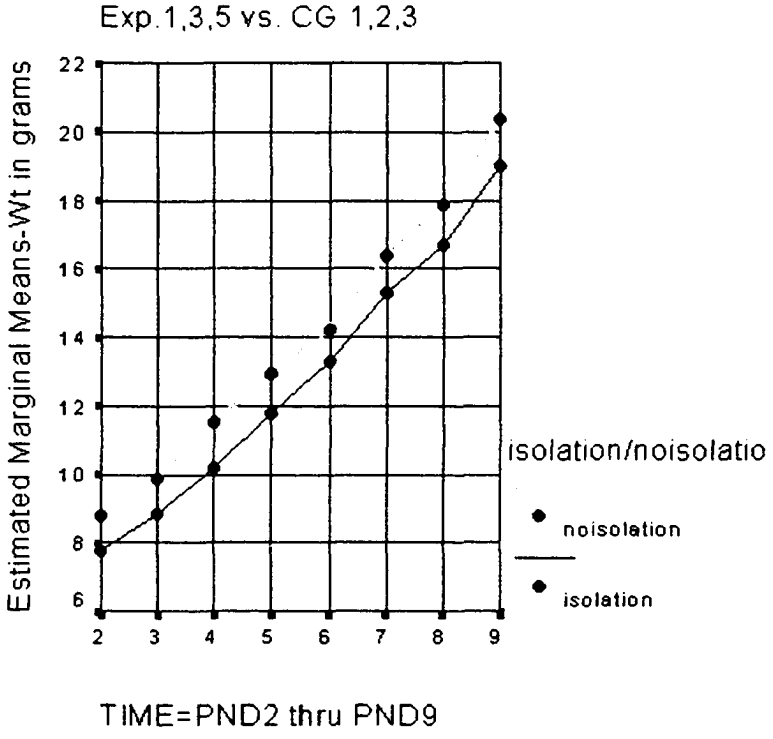
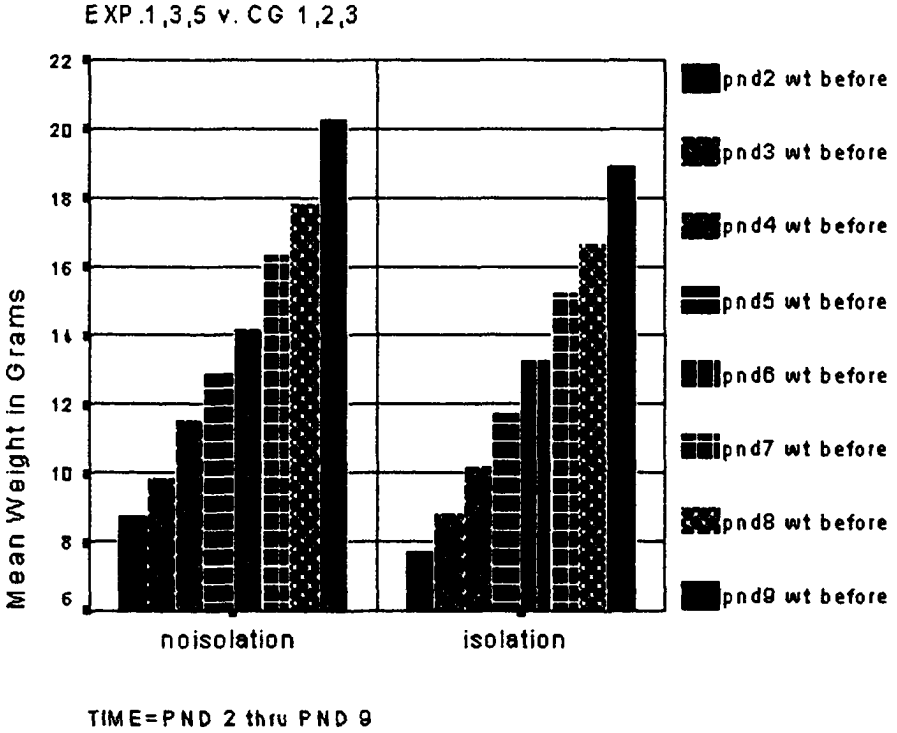


FIGURE 28(a)-PUP WTS. Before Isolation



(i.e.: post natal day), which indicated that the No-Isolation Condition pups remained significantly greater in weight than the Isolation pups on each consecutive day, PND 2 through PND 9 . The No-Isolation Control Group (combined litters) had 36 pups, and the Isolation Treatment Group (combined litters) had 35 pups. The Mean weight for the No-Isolation Control Group already higher on PND 2 than the Isolation Treatment Group (i.e., No-Isolation Mean weight was 8.769 grams, and the Isolation Treatment Group's Mean weight was 7.767 grams). To adjust for this unequal number of pups per group (36 vs. 35), a paired t-test was carried out on the Mean weights for the Experimental Group and Control Group:

(1) Experimental Group: T-test compared the within-group average Mean weight on PND 2 (7.766 grams), with their average Mean weight on PND 9 (18.987 grams). The result indicated a significant within-group total weight gain of 11.221 grams ($t(34) = 41.046$, $p < 0001$ (2-tailed)).

(2) Control Group: The same procedure was run for the No-Isolation Control Groups, comparing their within-subject average Mean weight on PND 2 (8.769 grams), with their average Mean weight on PND 9 (20.349 grams); they experienced a total Mean weight gain (between PND 2 and PND 9) of 11.570 grams ($t(35) = 45.034$, $p < 0001$ (2-tailed)).

(3) A simple ANOVA was done comparing the EXP v. Control groups for the Mean weight on PND 2 v. PND 9 between subject; this was significant for PND 2, $F(1,70) = 43.972$, $p < .0001$; and PND 9; $F(1,70) = 13.446$, $p < .0001$.

Not only did the Isolation Treatment pups gain *less* overall weight when compared with the No-Isolation Control pups, *but their rate of weight gain* **was less**.

(2.) During *No-Auditory* Isolation Treatment Group (EXP#4) vs. No-Auditory Control Group CG3.

BEFORE Isolation Treatment Weights:

The same procedures were followed as in Paragraph "11.4" and Paragraph "11.5". This Experimental treatment is analyzed separately because it has a change in the experimental design from the Main Inquiry Experimental Group (Litters No.1, 3, and 5) and the Main Inquiry Control Group (Litters No. 1, 2, and 3). This Experimental No-Auditory Isolation Treatment Group - EXP#4 has the deletion of the auditory mask; details are in the Methods paragraph..

A. As can be seen in Figure 29, the No-Auditory Isolation Group (EXP#4) weight *before* Isolation Treatment on days PND 2 through PND 9, when compared to the No-Isolation Control Group CG3, was significantly *less* on each day of that period, (Repeated Measures ANOVA), Within Group effect for Time, $F(7, 4.995) = 646.811$, $p < .0001$, Time x Isolation, $F(7, 4.995) = 14.091$, $p < .0001$; and a Between Group effect for Isolation, $F(1, 23) = (1600.800)$, $p < .0001$. Simple ANOVAs were run at each time point (i.e.: post natal day), which indicated that the No-Isolation Condition group remained significantly heavier in weight than the Isolation Group on each consecutive day, PND 2 through PND 9 . The No-Isolation Condition group had 13 pups, and the Isolation Treatment group had 12 pups. This placed the Mean weight for the No-Isolation Control groups already heavier on PND 2 than the Isolation Treatment group (i.e. No-Isolation Control's Mean weight was 14.498 grams, and the Isolation Treatment Group Mean weight was 11.796 grams). To adjust for this unequal number of pups per group (i.e., 13 vs. 12), paired t-tests were carried out on the Mean weights for the Experimental Groups and Control Groups:

(1) **No-Auditory** Isolation Treatment Group EXP#4: The t-test compared the within-group average Mean weight on PND 2 (8.12 grams +/-SEM .1291 grams), with its average Mean weight on PND 9 (17.086 grams +/- SEM .3221 grams). The result indicated a significant within-group total weight gain of 8.966 grams over the eight day Isolation Treatment ($t(11) = -23.165$, $p < .0001$ (2-tailed)

FIGURE 29--EXP#4 vCGX

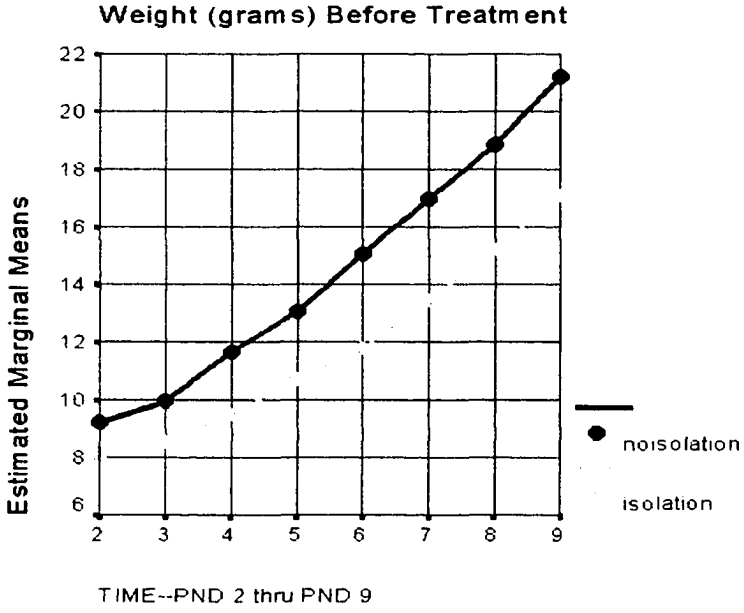
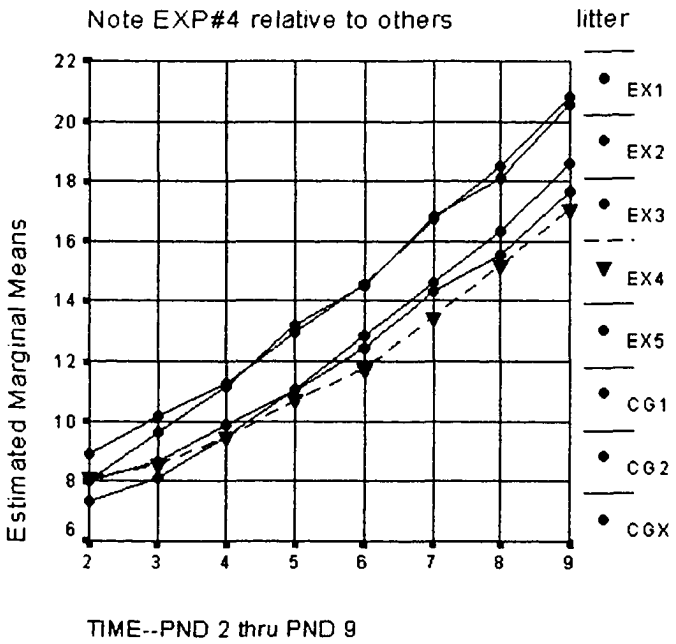


FIGURE 30---PUP Wts (Grams)--All Litters



(2) No-Isolation Control Group CG3: The same procedure was run for the No-Isolation Control Group, comparing their within-group average Mean weight on PND 2 (9.208 grams, +/- SEM .1022 grams), with its average Mean weight on PND 9 (21.202 grams, +/-SEM .2040 grams); the pups experienced a total Mean weight gain (between PND 2 and PND 9) of 11.995 grams, ($t(12.) = -52.249$, $p < .0001$ (2-tailed).

(3) A simple ANOVA was done comparing the EXP#4 v. Control groups for the Mean weight on PND 2 v. PND 9 between groups; this was significant for PND 2 ($F(1, 23) = 44.321$, $p < .0001$); and PND 9 ($F(1, 23) = 120.476$, $p < .0001$). Not only did the No-Auditory Isolation Treatment group (EXP#4) gain significantly *less overall weight* when compared with the No-Isolation Control group (a Mean total of 3.029 grams) $t(24) = -27.965$, $p < .0001$ (1-tailed), but their rate of weight gain was less (See Figure 29(a)).

It can be noted that just as this No-Auditory Isolation Treatment Experimental Group, EXP#4, was shown to be different from all remaining groups on *adult* behavior measures (planned apriori contrasts, Newman Kuels post hoc, and several types of ANOVA's), the EXP#4 pup weights were shown to be significantly different (they weighed ***less*** at each time point from PND 3 forward, even adjusting for any group pup number difference*)* from every other group including: (1) those experimental groups that included an auditory Isolation Treatment (Main Inquiry, EXP#1, EXP#3, and EXP#5); and, (2) from the control groups (Main Inquiry Auditory No-Isolation Treatment) CG1, CG2, and CG3.

As Figure 30 shows clearly, the EXP#4 group start out on PND 2 weighing the same as the other experimental groups (and more than EXP#5), but after two No-Auditory Isolation Treatments, they fall to the lowest weight of any group and remain the lowest weight until PND 9 . Also note that these are before treatment weights (i.e. weights taken prior to being place in the isolation treatment chamber).

Following Are Analysis for after Isolation Treatment Pup Weights:

11.4 Procedure - Pups, Selected Groups for an Experimental Subgroup of After Treatment Weights.

A measure of *after* isolation treatment weight was made on *three* of the groups: Experimental No-Auditory Isolation Treatment Group EXP#4, and Experimental Auditory Isolation Treatment Group EXP#5, and, No-Isolation Treatment Control Group CG3.

(1) For the Experimental Treatment Groups, at the end of the Isolation Treatment session, the scale was brought into the test room, cover lifted from the chamber and the scale set inside. Each pup was lifted out of their individual cups, placed on the scale for a few seconds for a weight read and then placed into the group-cup that contained the rest of the pups. Six pups were tested during each session, so this weighing before joining the litter mates in the group-cup took a total of three minutes, for the entire group

(2) For the Control Group-CG3, immediately after Isolation Treatment pups were returned to the Mother, six of the CG3 pups were taken from their litter and weighed; with the remaining six being weighed when the other six Experimental pups were returned to their Mother. Weights for all of the CG3 pups were taken on only one day, PND 5 (approximately half the way through the 8-day run) in order to keep any variation at a minimum.

11.5 Statistical Treatment

All of the same procedures listed in Paragraph 7.0a (1)through (5) above were also applied to this analysis as far as screening of the data. An ANOVA was done comparing total Mean weights between experimental and control groups. Repeated Measures ANOVA (Time x Isolation/No-Isolation) was done on the weight for all groups (PND 2 through PND 9). Simple ANOVA's were done to verify significant differences in weights at each time point. Further, paired T-tests were done comparing weights on PND 2 with weights on PND 9 for each Isolation Treatment group, and for the No-

Isolation Control Group A simple correlation, and scatter plot with a fitted regression line, was done to estimate the rate of change in weight gain within each group, and then was compared between groups.

11.6 Results of Analysis–AFTER Treatment Pup Weights

Following Are Analysis for a Selected Subgroup of Experimental and Control Groups That Had 'After' Isolation Treatment Weights Measured:

(1.) Auditory Isolation Treatment Group EXP#5 vs.CG3.

As was explained earlier, Auditory Isolation Treatment Litter EXP#5 is the "cohort" of No-Auditory Isolation Treatment Litter EXP#4 and of No-Auditory Control Litter CG3. All three dams were delivered from same vendor on the same day; pups were born on the same day, and testing as pups and behavioral tests in adulthood were conducted either simultaneously or within a week from each other. This cohort relationship also provides a rationale for comparing the EXP#4-No-Auditory Isolation Treatment conditions with the EXP#5-Auditory Isolation Treatment conditions directly. As can be seen in Table 8a, the subject numbers and other subject variables are also similar. Since only a subpopulation of the Main Inquiry Group would be tested, this is the appropriate Auditory Isolation Treatment litter to use for comparison with EXP#4 (No-Auditory) and with the No-Isolation Control litter CG3.

A. As can be seen in Figure 31, a simple ANOVA, (weight x Isolation/No-Isolation Condition), showed a significant difference in the after treatment weights for Experimental group EXP#5 when compared to Control group CG3, $F(1, 23) = 107.363$, $p < .0001$. The No-Isolation Group had heavier post-treatment weights than the Isolation Group (Mean for CG3 = 13.068 grams; Mean for EXP#5 = 11.049 grams).

B. As can be seen in Figure 31a, an ANOVA showed a significant difference in the after treatment weights for Auditory Group EXP#5 when compared to EXP#4 (No

FIGURE 31

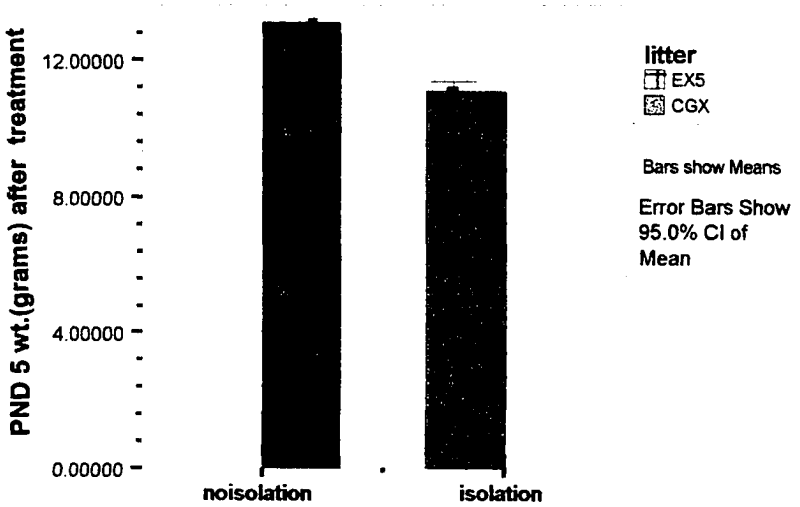
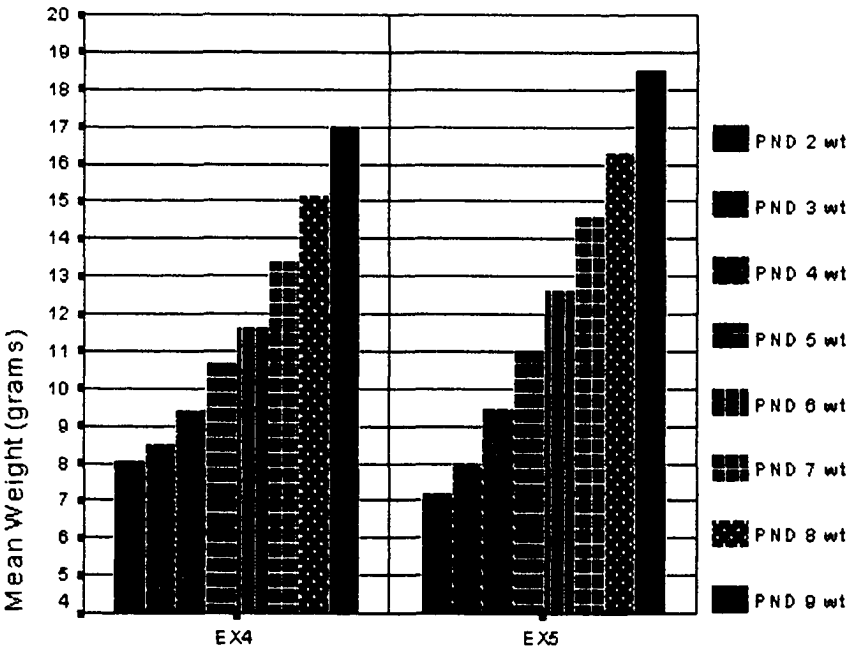


FIGURE 31(a)--Wts.After Isol. Treatments

No-Auditory EXP#4 v. Auditory EXP#5



Auditory Isolation Treatment group), $F(1,21) = 38.807$, $p < .0001$. The EXP#5 Isolation Treatment group had higher post-treatment weights than the EXP#4 Isolation Treatment group (Mean for EXP#5 = 12.233; Mean for EXP#4 = 11.746).

C. As can be seen in Figure 31b, a Paired-Samples t-test showed a significant difference in the before and after Isolation Treatment weights for EXP#5, taken on PND 5, ($t(11) = 12.320$, $p < .0001$ [2-tailed]). The pups weighed significantly heavier before the treatment than after the treatment.

(2.) No-Isolation Control Group CG3 Planned Comparisons.

A. For CG3 v. EXP4, see (3.)(C.) Below in this section.

B. For CG3 v. EXP#5, see (1.)(B.) Above, (Figure 31a).

C. As can be seen in Figure 32, a Paired-Samples t-test showed a significant difference in the before and after No-Isolation Control group weights for CG3 (Within Group Comparison), taken on PND 5, ($t(12) = -2.978$, $p < .012$ [2-tailed]). The pups weighed significantly less before the simulated treatment time period than after it. The *before* Mean weight was 13.056 grams, and the *after* Mean weight was 13.067 grams, which reflects a weight gain during the time these pups spent nursing while the experimental group was Isolated (see Figure 32a, and No. 32b, before and after for CG3 pups on PND 5 only).

(3.) EXP#4-No-Auditory Isolation Treatment Group

***AFTER Isolation Treatment Weights* For this No-Auditory EXP4 Group, and Within-group Comparisons:**

A. As can be seen in Figure 33, a simple ANOVA, (weight x Isolation/No-Isolation Condition), shows a significant difference in the after treatment weights for Control group CG3 when compared to No-Auditory Isolation Treatment Group EXP#4, $F(1,23) = 64.198$, $p < 0001$. The No-Isolation Control group had heavier post-treatment

FIGURE 31(b)--Auditory EXP#5 on PND 5 only

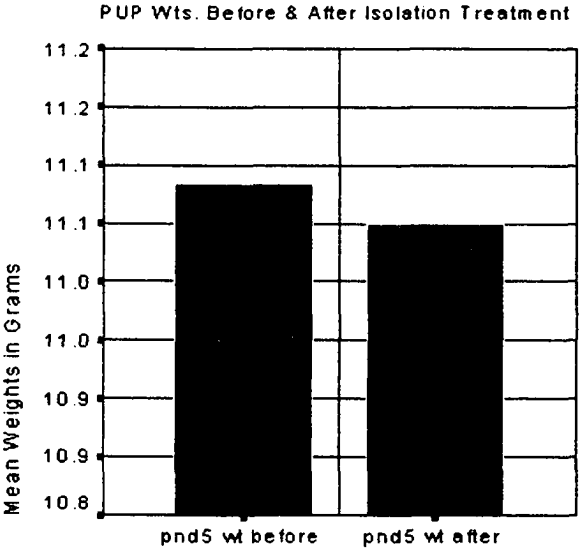


FIGURE 32--No-Isol. Treatment Controls

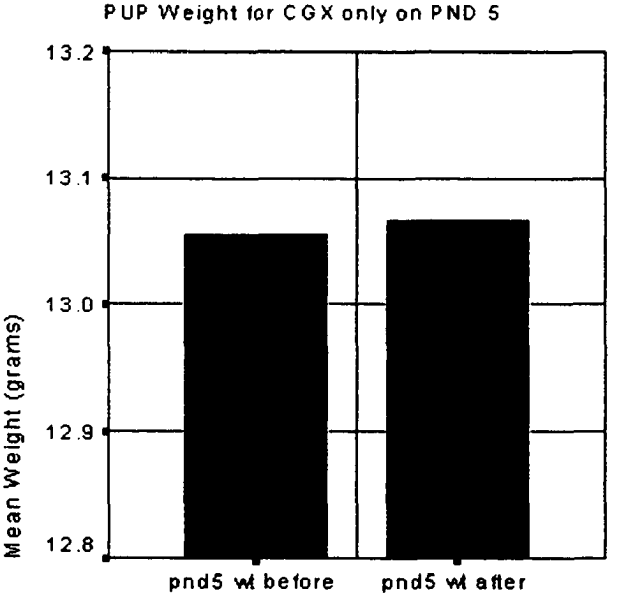


FIGURE 32(a)-No-Isol. Controls CGX

"Before" PUP Weights on PND 5

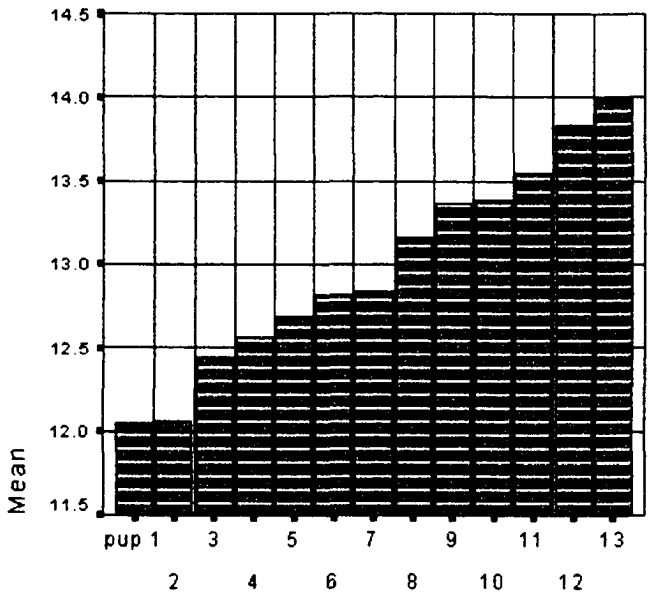
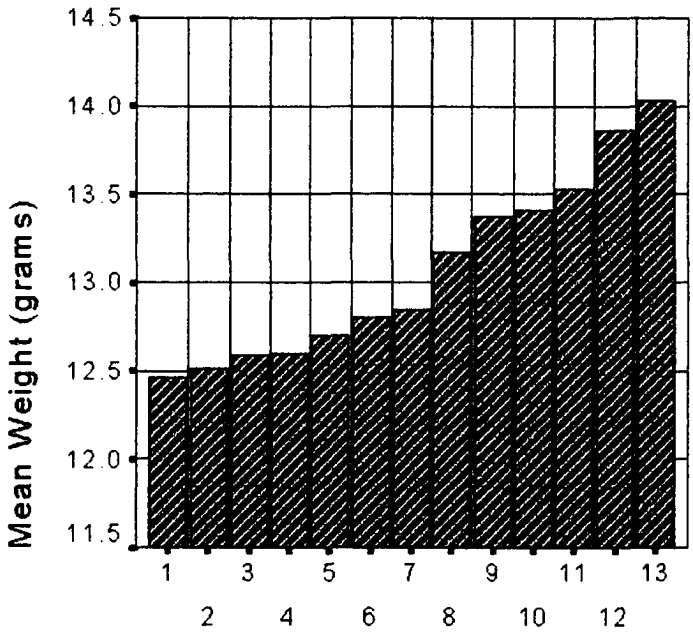


FIGURE 32(b)--No-Isol. Controls CGX

"After" PUP Weights on PND 5



weights than this Isolation No-Auditory Isolation Treatment Group (Mean for CG3 = 13.214 grams; Mean for EXP#4 = 10.687 grams) on PND 5.

B. As can be seen in Figure 33a, a Repeated Measures ANOVA comparing EXP#4 (No-Auditory) with EXP#5 (Auditory) on after isolation treatment weights, had a significant Within-Group effect for Time, $F(1, 5.329) = 543.722$, $p < .0001$), and a significant Between-Subjects effect for Litter (EXP#4-no-auditory v. EXP#5 auditory) Condition, $F(1, 21) = 38.807$, $p < .0001$). The EXP#4 No Auditory group weighed less than the EXP#5-Auditory group after their respective isolation treatments after PND 3 forward thru PND 9.

C. A simple ANOVA, (weight x Isolation/No-Isolation Condition), showed a significant difference in the after treatment weights for Control Group CG3 when compared to Experimental Isolation Group EXP#5, $F(1, 23) = 92.974$, $p < 0001$. The No-Isolation Control Group had higher post-treatment weights than the Isolation Group (Mean for CG3 = 13.068 grams; Mean for EXP#5 = 10.687 grams).

D. Finally, as can be seen in Table 12, a Paired Sample t-test was made comparing pup weights within the EXP#4 group only:

(1) comparing day PND 2 **before** weight with PND 9 **before** treatment weights;

(2) comparing day PND 2 **after** weights with PND 9 **after** treatment weights;

(3) comparing day PND 2 **before** with PND 2 **after** treatment weights;

(4) comparing day PND 9 **before** with PND 9 **after** treatment weights.

All were statically significant differences, $p < .0001$, indicating the following within-group relationships:

(1) Comparing the weights taken prior to being placed into the Isolation Chamber, EXP#4-No-Auditory Isolation Group *gained weight* from day PND 2 through PND 9; a Mean weight gain of 8.966 grams;

FIGURE 33

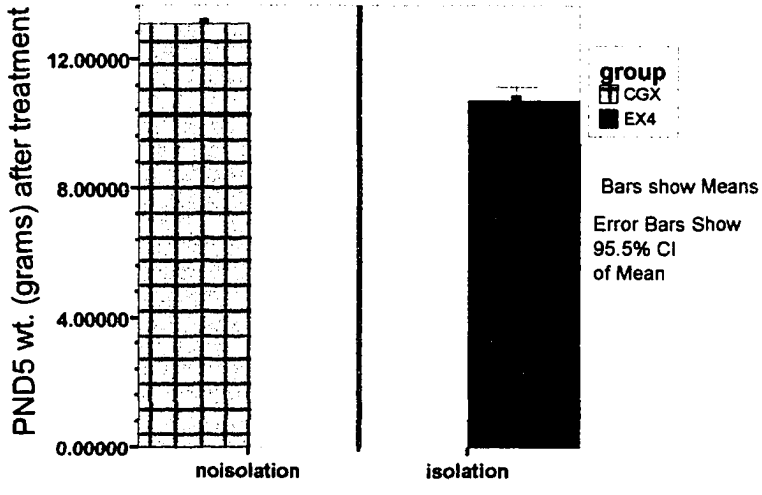


FIGURE 33(a)--PUP Weights "After" Treat.

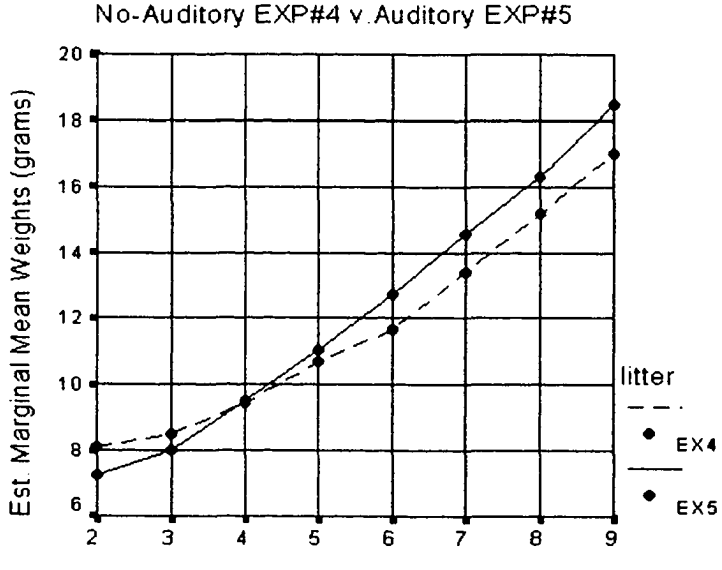


TABLE 12 –Pup Weights

Descriptive Statistics-EXP#4 only No-Auditory Isolation Treatment Group
Paired Sample t-tests, Pup Weights Before and After Treatments

	litter	Mean	Std. Deviation	SEM	N
		Wt. In Grams			
pnd2 wt before	EX4	8.1200000	.44739651	.129	12
pnd2 wt after	EX4	8.0808333	.44159748	.127	12
pnd3 wt before	EX4	8.5500000	.50542511	.146	12
pnd3 wt after	EX4	8.5166667	.49144191	.142	12
pnd4 wt before	EX4	9.4833333	.59518268	.172	12
pnd4 wt after	EX4	9.4333333	.57577352	.166	12
pnd5 wt before	EX4	10.7077500	.70457713	.203	12
pnd5 wt after	EX4	10.6870000	.69925441	.202	12
pnd6 wt before	EX4	11.7714167	.76014932	.219	12
pnd6 wt after	EX4	11.6523333	.82938360	.239	12
pnd7 wt before	EX4	13.4530000	.71004993	.205	12
pnd7 wt after	EX4	13.4093333	.72299658	.209	12
pnd8 wt before	EX4	15.2165000	1.39621010	.403	12
pnd8 wt after	EX4	15.1663333	1.38557801	.400	12
pnd9 wt before	EX4	17.0855833	1.11581543	.322	12
pnd9 wt after	EX4	17.0234167	1.12037011	.323	12

TABLE 12(a) - -PAIRED SAMPLES T-TEST, PUP WEIGHTS FOR EXP#4, NO-AUDITORY

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	pnd2 wt before - pnd9 wt before	-8.9655833	1.34069605	.38702561	-9.8174210	-8.1137457	-23.165	11	.000
Pair 2	pnd2 wt after - pnd9 wt after	-8.9425833	1.33040803	.38405572	-9.7878843	-8.0972824	-23.285	11	.000
Pair 3	pnd2 wt before - pnd2 wt after	.0391667	.03918681	.01131226	.0142686	.0640648	3.462	11	.005
Pair 4	pnd9 wt before - pnd9 wt after	.0621667	.01478841	.00426905	.0527706	.0715628	14.562	11	.000

(2) Comparing the weights taken *after* the Isolation Treatment, EXP#4 *gained weight* from day PND 2 through PND 9; a Mean weight gain of 8.943 grams;

(3) Comparing weights taken before and then after the treatment on PND 2, there is a Mean weight *loss* of .040 grams;

(4) Comparing weights taken before and then after the treatment on PND 9, there is a Mean weight *loss* of .062 grams.

Although EXP#4-No-Auditory Isolation Group gained weight from PND 2 through PND 9, at the before-treatment weighing, (see Figure 30) specifically on PND 2, and PND 3 relative to two of the Main Inquiry Auditory Isolation Groups, the No-Auditory EXP#4 pups weighed *more* than they did; and at PND 4, EXP#5 and EXP#4 matched weight *before* the treatments. A change then occurs from PND 5 through PND 9, and the EXP#4 pups weigh *less* than any other group, experimental or controls when weighed before the isolation treatment procedures.

ALSO, when weighed *after* the isolation procedure, and compared to the EXP#5-Auditory Isolation Treatment group, the same pattern as above was followed between these two groups: EXP#4 weighed *more* than EXP#5 pups *after* isolation treatment on PND 2 and PND 3; on PND 4 both groups weighed similar *after* the treatment; however, from PND 5, PND 6, PND 7, PND 8 and PND 9, the EXP#4 group –when weighed *after* the Isolation Treatment, EXP#4 (no-auditory mask) weighed *less* than EXP#5-(with auditory Isolation Treatment group)(See Figure 33a). Also, EXP#4 weighed after the isolation treatment significantly *less* than the CG3-Control (No-Isolation) group on PND 5.

11.7 ADULT WEIGHTS

11.7a Procedure for Adults - Weights Measured Prior to Original Open Field Test;

All adult subjects were weighed in the colony room approximately 20-25 minutes prior to behavioral testing in the Open Field Box.

11.8 Statistical Treatment.

All of the same procedures listed in Paragraph 7.0a (1) through (5) above were also applied to this analysis of all data in this Section as far as screening of the data. An ANOVA was done comparing total Mean weights between Adult experimental and control groups. Further, paired post hoc contrasts or paired t-tests were done comparing weights for the Isolation Treatment groups, and for the No-Isolation Groups.

11.9 Results of Analysis for BEFORE-(First) Trial Measurements (1.)For Main Inquiry Auditory Isolation Treatment Group (Litters No. EXP 1,3,5) vs. No–Isolation Control Group (Litters No. CG1,2,3.).

A.. The results of the 2 x 2 (Isolation/No-Isolation x Sex x Weight at test) ANOVA showed the expected significant Main Effect for Sex ($F(1, 36) = 607.689$, $p < .0001$), and a Sex by Isolation Interaction ($F(1, 36) = 4.533$, $p < .040$. (See Figure 34.) Post hoc pairwise comparisons showed males weighed more than females at the time of the first open field test, however the weight of the **males** depended on whether or not they received Isolation Treatment, i.e. males receiving Isolation Treatment as pups weighed significantly **less** than males in the control-No-Isolation condition (Isolation males had a Mean weight of 460.726 grams +/- SEM 6.185 grams; No-Isolation males had a Mean weight of 486.517 grams +/-SEM 6.047 grams). Female Mean weight at the time of the first open field test apparently did not depend on Isolation Treatment Condition (Isolation = 290.889 grams +/- SEM 6.330 grams; No-Isolation = 299.352 grams +/1 SEM 6.103 grams).

FIGURE 34--Auditory Isolation Treatment EXP#1,3,5 v. No-Isol.Control CG1,2,3

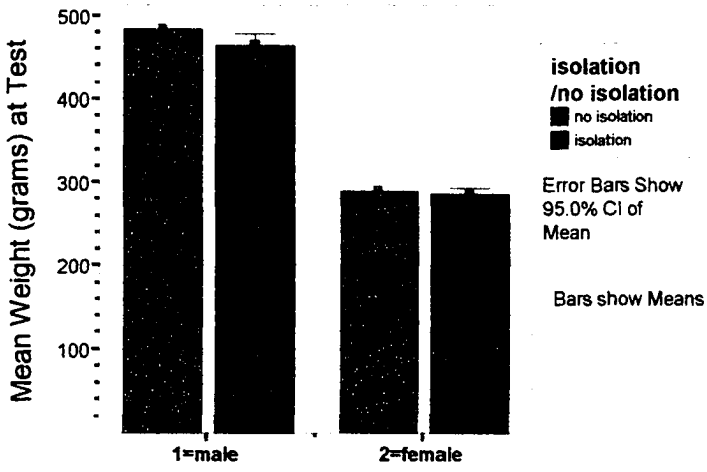
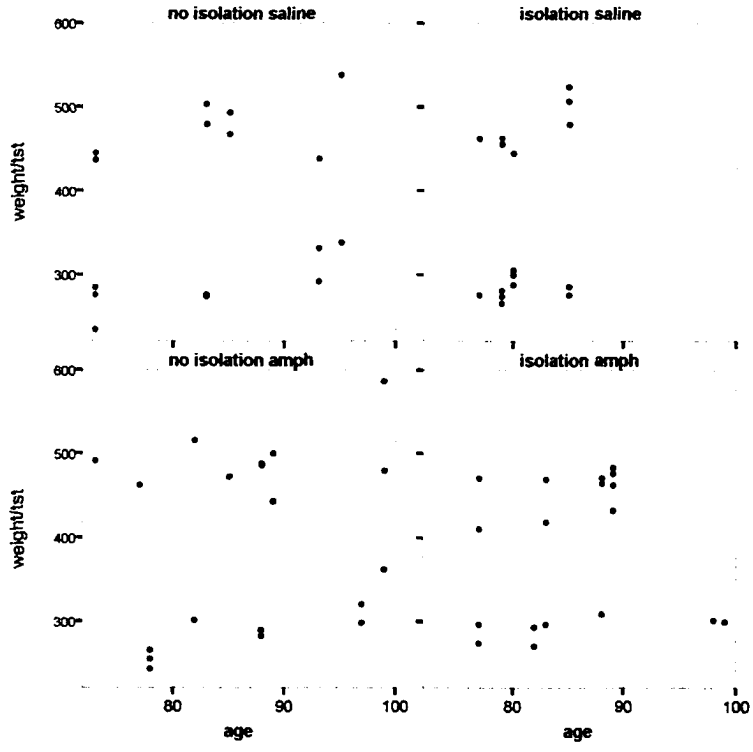


FIGURE 34(a)--ANCOVA-Adult weight on test date by age at test date



B. A separate ANCOVA (Weight-at-test x Isolation/No-Isol WITH Age as covariant) was run to test whether age at time of test had any significant impact on the results of the above ANCOVA (Figure 34a). ANCOVA results for Age-at-time-of-test was not significant as a factor ($F(1, 68) = 2.332$, $p < .131$).

(2.) EXP#5 vs. CG3 (Auditory Isolation Treatment Group).

A. As can be seen in Figure 35, a (2 x 2) ANOVA, (weight x Isolation/No-Isolation Condition x Sex), showed the expected significant Main Effect for Sex ($F(1, 21) = 440.605$, $p < .0001$). No other Main Effects or Interactions were significant.

(3.) EXP#4 (*No-Auditory Isolation Treatment*) vs. CG3).

A. The results of the 2 x 2 (Isolation/No-Isolation x Sex x Weight at test) ANOVA showed the expected significant Main Effect for Sex ($F(1, 21) = 572.392$, $p < .0001$), and a Main Effect for Isolation ($F(1, 21) = 13.404$, $p < .001$. (See Figure 36.) There were no Interaction effects. Males, of course, weighted more than females, however both males and females in the No-Isolation Control condition weighed significantly more at the pre-trial weighing than the male and female subjects in the Isolation Treatment Condition did at the pre-Trial weighing (No-Isolation males = 480.778 grams +/-SEM 6.365 grams; females = 280.250 grams +/- SEM 9.547 grams)(Isolation males = 442.571 grams +/-SEM 7.217 grams; females = 259.800 grams +/-SEM 8.539 grams). It should be noted that this finding in adulthood repeats the pattern set with the (1) analysis of the pup weights before and after Isolation Treatment, and the (2) comparison between Auditory vs. No-Auditory Isolation Treatment group (see Paragraph "11.6(3.)[A.]{2.}" above for discussion). Again repeating the pup-weight findings, this No-Auditory Isolation Treatment Experimental Group EX#4 weighs *less* than any other group *in adulthood.* As is noted later, this pattern holds for the Postbaseline Weights also.

FIGURE 35--Auditory Isol. EXP5 v. CG3-
Adult Weights at Original Test

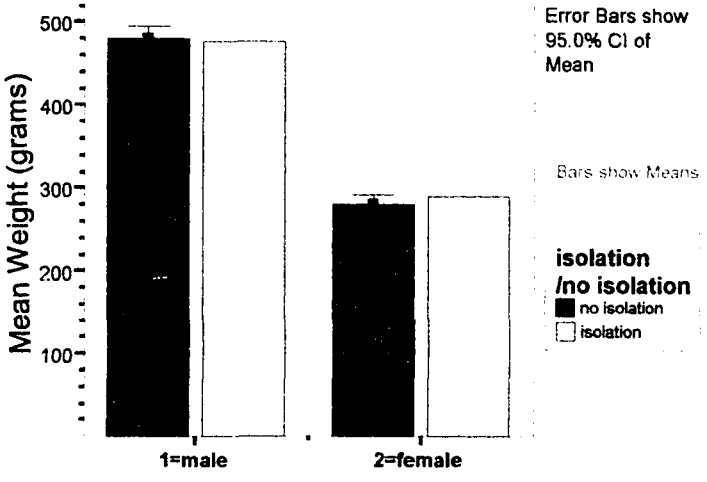


FIGURE 36-Adult --No-Auditory EXP#4
Original Test Weights

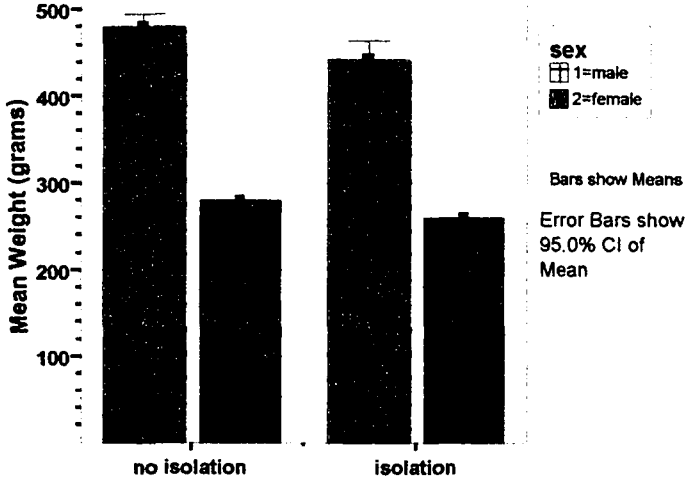
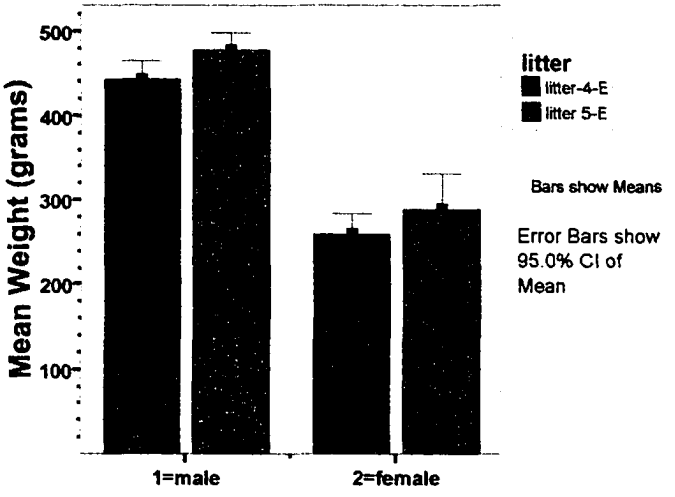


FIGURE 37-Adult Weight at Original Test
No-Auditory EXP#4 v. Auditory EXP#5



Postbaseline ADULT weights
Main Inquiry Auditory Isolation EXP3,5 v. CG2,3

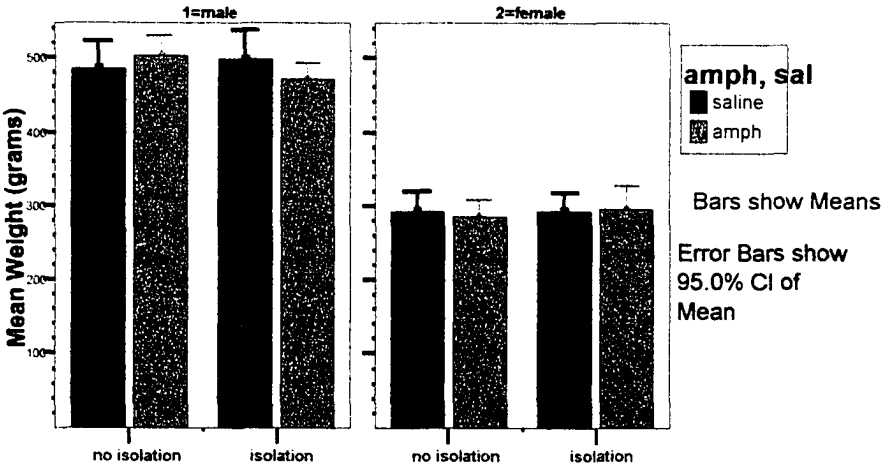


Figure 38

B. As can be seen in Figure 37, a 2 x 2 (Weight x Sex x Litter) ANOVA comparing EXP#4 (No-Auditory) with EXP#5 (Auditory) on pre-trial weights, had a significant Between-Subjects Main Effect for Litter (EXP#4-no-auditory v. EXP#5 auditory) Condition $F(1, 20) = 9.802$, $p < .005$), and a Main Effect for Sex $F(1, 20) = 323.883$, $p < .0001$. Again, repeating the pup-weight findings, this No-Auditory Isolation Treatment Experimental Group EX#4 weighs *less* than the Auditory Isolation Treatment group EXP#5 in adulthood. (EXP#5-Auditory Isolation males' Mean = 477.111 grams +/-SEM 7.728 grams; females' Mean = 289.667 grams +/- SEM 13.385 grams) (EXP#4-No-Auditory males' Mean = 442.571 grams +/-SEM 6.763 grams; females' Mean = 259.800 grams +/-SEM 10.368 grams) As noted later, this pattern holds for the Postbaseline Weights also.

11.9 Procedure for Adults - Prior to *Postbaseline* Testing.

Results of Analysis of Adult Postbaseline Weight Measurements(a):

(1.)For EXP 1,3,5 vs. CG1,2,3. *Adult* Groups (Groups with Auditory Isolation Treatment During Pup Isolation).

A. As can be seen in Figure 38, a (2 x 2) ANOVA, (weight x Isolation/No-Isolation Condition x Sex), showed the expected significant Main Effect for Sex, $F(1, 4) = 785.715$, $p < .0001$. An Isolation x Sex x Drug Interaction was significant, $F(1, 47) = 3.760$, $p < .059$. The following relationship exists: Depending on whether saline or amphetamine was received at the first open field trial, and further depending on inclusion in the Treatment Condition, Isolation Treatment males who received amphetamine at Trial, had *lower* weight than any males in any of the other conditions, and higher than any of the females in any of the Conditions. Contrast this with the result for females that were in the Isolation Condition and who received amphetamine at the first Trial. When weighed at Postbaseline these Isolation/amphetamine females had *heavier* weights than females in the Isolation/Saline Condition, and higher weights than females in the

FIGURE 38(a)--Aud. Isol. EXP1,3,5v CG1,2,3

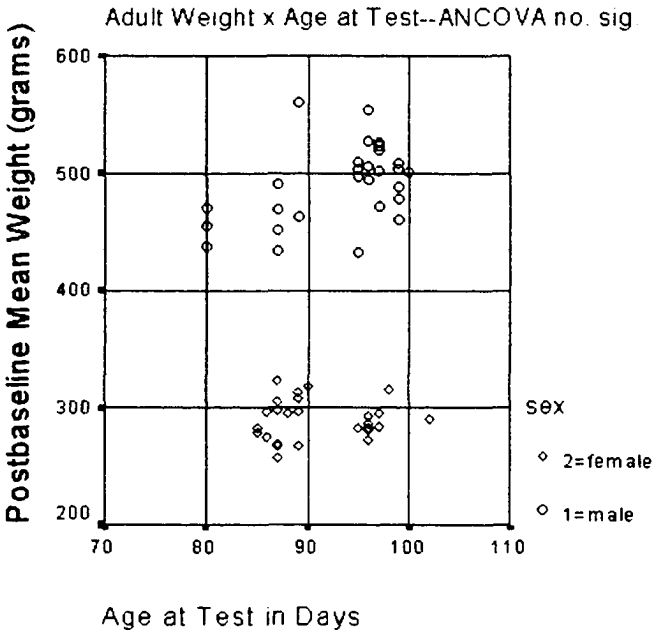
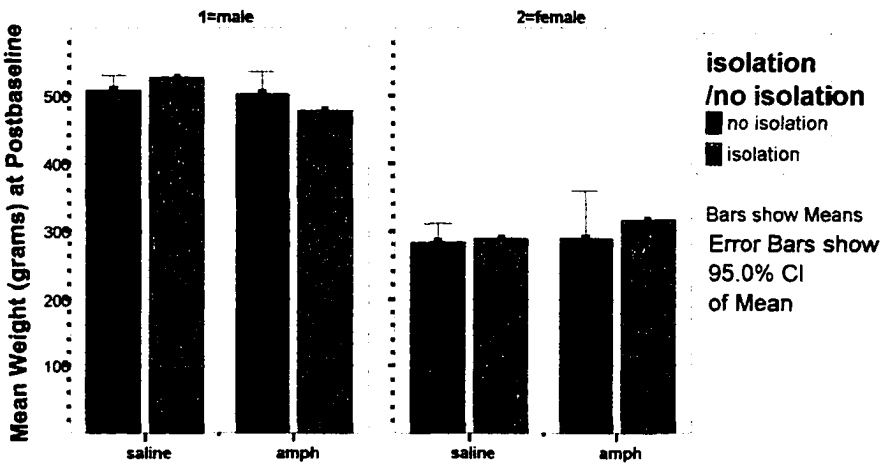


FIGURE 39-ANOVA-Drug x Sex F(1, 17) = 4.543, p <.048
Adult Postbaseline Weights for Auditory Isolation EXP#5 v. CGX



No-Isolation/amphetamine Condition. The only females that weighed more at Postbaseline were in the No-Isolation/saline Condition.

B. A separate ANCOVA (Weight-at-test x Isolation/No-Isolation with Age as covariance) was run to test whether age at time of test had any significant impact on the results of the above ANOVA (Figure 38a). ANCOVA results for Age-at-time-of-test was **not significant** as a factor ($F(1, 52) = .467$, $p < .063$).

(2.) EXP#5 vs. CG3 (Auditory Isolation Treatment Group).

A. As can be seen in Figure 39, a (2 x 2 x 2) ANOVA, (Drug x Isolation/No-Isolation Condition x Sex), showed the expected significant Main Effect for Sex ($F(1, 17) = 427.207$, $p < .0001$). A "Drug" x Sex Interaction was significant, $F(1, 17) = 4.543$, $p < .048$, which is interesting because there are no drugs administered just before or during the Postbaseline trials, and no exogenous drug is left in the plasma of subjects. What is thought by many researchers to be occurring under these circumstances is a retained drug effect, that is, changes in the animal's physiology (or a psychological conditioned effect of drug previously administered in the same test environment). Several researchers have shown that for various periods of up to two weeks after an acute dose of psychostimulant, the Hypothalamic-Pituitary Axis, and several stressor hormones, remain elevated even when no exogenous drug remained. As can be seen in Figure 39a, the Postbaseline weight of adult males depended on whether or not drug or saline was received at trial, i.e. males who received saline weighed more than those who received drug (Male Saline Mean weight = 517.292 grams +/-SEM 8.343, compared to Drug Mean weight = 490.800 grams +/-SEM 6.614). In an opposite effect from males, females' Postbaseline weight, for those females that received drug at the first open field trials, weighed *more* than those that received saline at trial (Female Drug Mean weight = 302.750 grams +/-SEM 13.378, and the female Saline Mean weight = 286.000 grams +/- SEM 10.923).

(3.) EXP#4 (*No-Auditory Isolation Treatment*) vs. CG3.

FIGURE 39(a)-Aud. Isol. EXP5 v. CGX-POSTBASELINE weights
ANOVA Drug x Sex Interaction, p. <.048

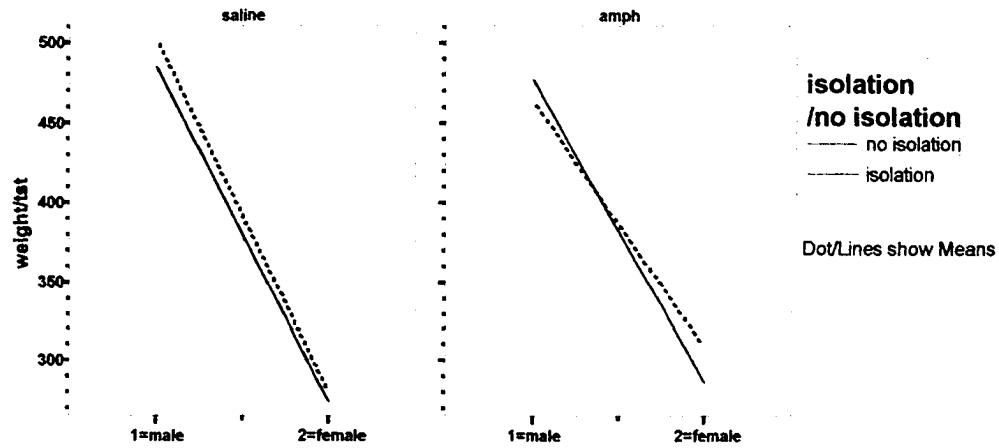
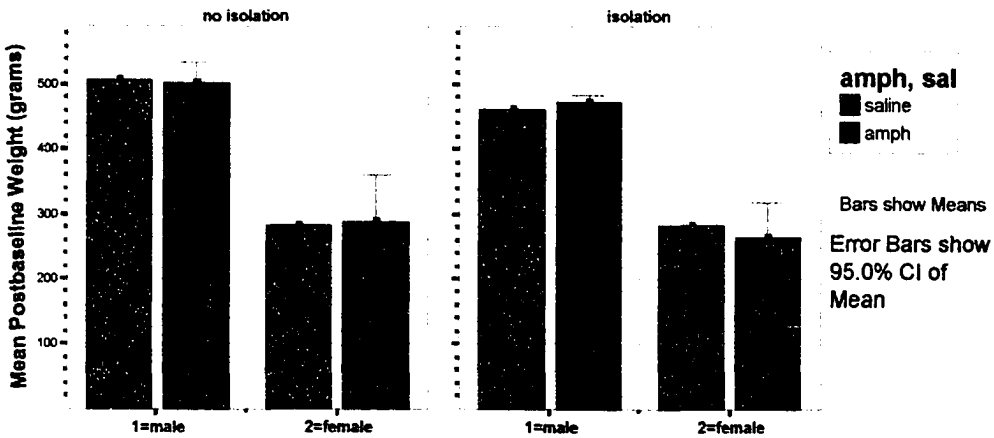


FIGURE 40--Postbaseline Weights--No-Aud. Iso. EXP#4 v. CGX
ANOVA, Sex=p.<.001; Isolation=p.<.005



A. The results of the 2 x 2 x 2 (Isolation/No-Isolation x Sex x Drug) ANOVA showed the expected significant Main Effect for Sex ($F(1, 17) = 664.774$, $p < .0001$), and a Main Effect for Isolation ($F(1, 17) = 10.531$, $p < .005$ (See Figure 40.) There were no Interaction effects. Males, of course, at Postbaseline weighed more than females, and males and females in the No-Isolation Condition weighed significantly more than males and females in the Isolation Condition (Males Mean weights No-isolation = 504.925 grams +/- SEM 6.324; Isolation = 466.625 grams +/- SEM 7.200; Female Mean weights: No-isolation = 286.750 grams +/- SEM 9.428; Isolation = 273.250 grams +/- 8.606). It should be noted that this finding repeats the pattern set with the analysis of the pup weights before and after Isolation Treatment, and also the pattern between Auditory vs. No-Auditory Isolation Treatment groups (see Paragraph "11.6(3.)[A.]{2.}" above for discussion). Again this No-Auditory Isolation Treatment Experimental Group EX#4 in adulthood weighs *less* than any of the other groups.

B. As can be seen in Figure 41, an ANOVA on Postbaseline weights for EXP#4 vs. EXP#5 shows a Main Effect for Sex $F(1, 16) = 386.720$, $p < .0001$; a Main Effect for Litter, $F(1, 16) = 10.591$, $p < .005$, and a significant Interaction for Sex by Litter# x Drug, $F(1, 16) = 6.924$, $p < .018$. Males and females in EXP#5 had higher Postbaseline weights than males and females in EXP#4, however weights in each group depended on drug or saline treatment (more accurately, the "remainder" effect from receiving either drug or saline at the first open field Trial). The effects were opposite between litters: For EXP#4, males receiving drug weighed more than males receiving saline (and more than EXP#4 females regardless of their drug/saline condition); however, EXP#4 males weighed Less than EXP#5 males (regardless of EXP#5 males drug condition). EXP#4 females in the drug condition at first trials weighed Less than all other groups. EXP#5 males in the saline condition weighed *more* than any other group;

FIGURE 41-Postbaseline No-Aud. Isol.EXP#4 v. Aud. Isol EXP#5

ANOVA, Sex x Drug x Litter, p.<018

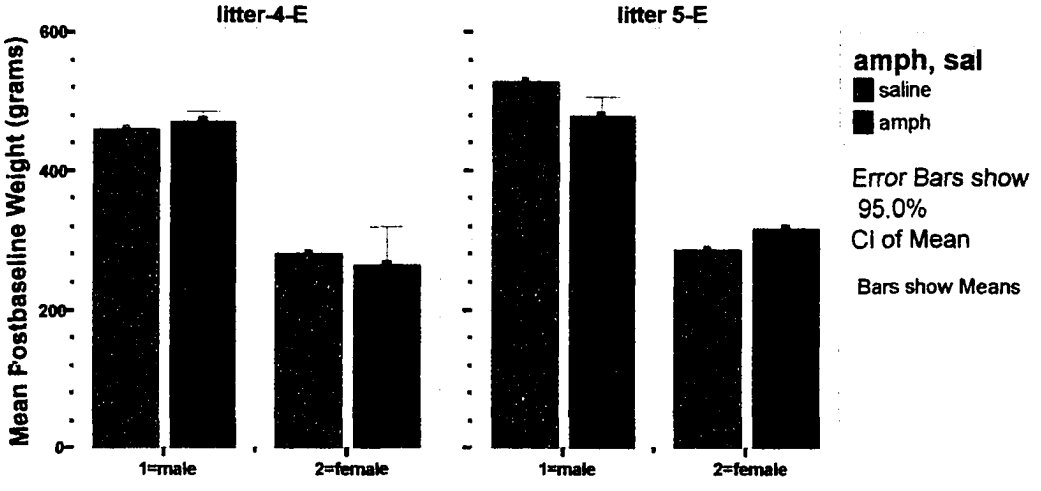
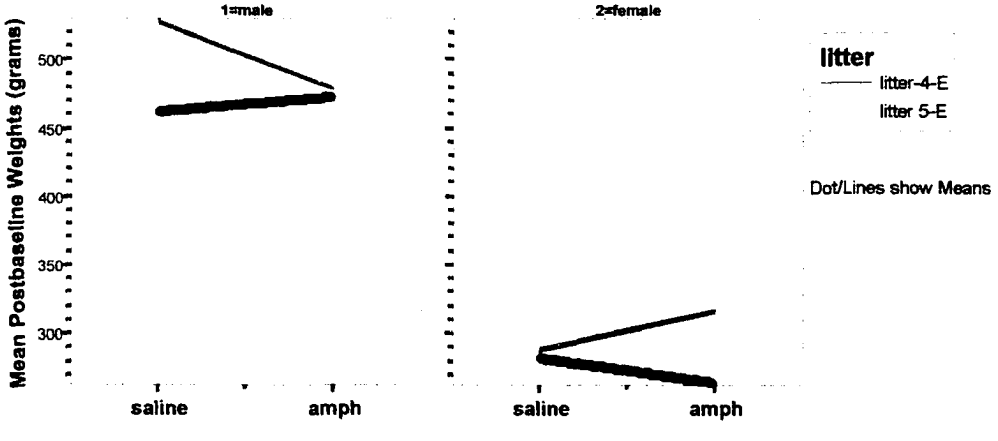


FIGURE 41(a)-No-Aud.Isol EXP#4 v. Aud. Isol. EXP5(#4=lower,dotted line)
Postbaseline ANOVA, Sex x Drug x Litter, p<.018



while EXP#5 females in the amphetamine condition weighed *more* than the EXP#5 saline females and *more* than both EXP#4 female groups.(Figure 41.) Again, this cannot be interpreted as a *direct* drug effect since no drugs are given at Postbaseline trials. Also, both of these groups(EXP#4 and EXP#5) are Isolation Treatment Groups, but one is With-Auditory-Isolation Treatment (EXP#5), and one is Without-Auditory-Isolation Treatment (EXP#4).

(4.) Repeated Measures ANOVA and other Tests Comparing Pre-Trial vs. Postbaseline Weights.

A Repeated Measures ANOVA was carried out on three comparison groups: (1)EXP#3,5 vs. CG#2,3; (2)EXP#5 vs. CG3; and (3)EXP#4 vs. CG3. Again, EXP#3,5 were compared with CG#2,3 (Auditory Isolation Treatment Group v. No-Isolation Controls) because postbaseline measures were not done on EXP#1 and CG#1, and, therefore no postbaseline weights were taken for them. The following sections have been summarized in Table 13. The tests compared adult Weights at Pre-Trial with Weights at Postbaseline. The results follow.

A. For **EXP# 3, 5 vs. CG 2, 3,** and is visualized in Figure 42(a)(b)(c)(d), there was a significant effect for Time $F(1, 40) = 47.141$, $p < .0001$; for Time x Isolation $F(1, 40) = 5.272$, $p < .027$; and for Time x Sex $F(1, 40) = 3.825$, $p < .057$.. Further, there was a Between-Subject Effect for Sex $F(1, 40) = 719.559$, $p < .0001$. At the Postbaseline weighing, all groups had gained weight compared to what they weighed on the day of the Open Field Trials. Listed from highest weight gain to lowest weight gain, the Isolation/No-Isolation-Treatment-Condition, and the Amphetamine/Saline - Treatment-Condition groups are as follows:

(1) **Isolation/Saline Treatment-Condition**: Females in this combination Condition gained an average of 2.20 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 20.66 grams.

Comparison Grp For Repeated Measures	Combined Conditions (Hierarchy same as T-2 Plot Point on Graph)	Av. Gram FEMALE Wt. Gained from Trial to Postbaseline (T-1 to T-2)	Av. Gram MALE Wt. Gained from Trial to Postbaseline (T-1 to T-2)	Mean Weight (male and female) on Pre-Trial date in Grams	Mean Weight (Male and Female on Postbaseline date in Grams	Overall Mean Weight Gain (Male and Female) Grams	Group Hierarchy ordered by weight- gain (descending)*
				Plot Point*	Plot Point*		
EXP3,5 v. CG1,2,X	Isol/Saline	02.200	20.66	384.38	395.81	11.43	No-Isol/Saline
	No-Isol/Amph	11.833	22.500	377.208	394.375	17.165	No-Isol/Amph
	No-Isol/Saline	24.000	15.733	369.400	389.817	19.87	Isol/Saline
	Isol/Amph	02.25	12.375	375.50	382.81	07.312	Isol/Amph
EXP#5 v.CGX	Isol/Saline	07.500	24.666	391.583	407.667	16.084	Isol/Saline
	Isol/Amph.	08.000	14.667	386.167	397.500	11.333	No-Isol/Saline
	No-Isol/Amph	03.500	25.200	381.700	396.050	14.350	No-Isol/Amph
	No-Isol/Saline	09.500	22.250	379.750	395.625	15.870	Isol/Amph
EXP#4 v.CGX	No-Isol/Amph	03.500	25.200	381.700	396.050	14.350	Isol/Saline
	No-Isol/Saline	09.500	22.250	379.750	395.625	15.875	No-Isol/Saline
	Iso/Saline	17.000	30.750	348.000	371.875	23.870	No-Isol/Amph
	Iso/Amph	8.000	13.333	357.333	368.000	10.667	Isol/Amph
*(highest combined condition weight-gain to lowest combined condition)							

Table 13 - Comparison of Adult Weights at Pre-Trial vs. Postbaseline Dates

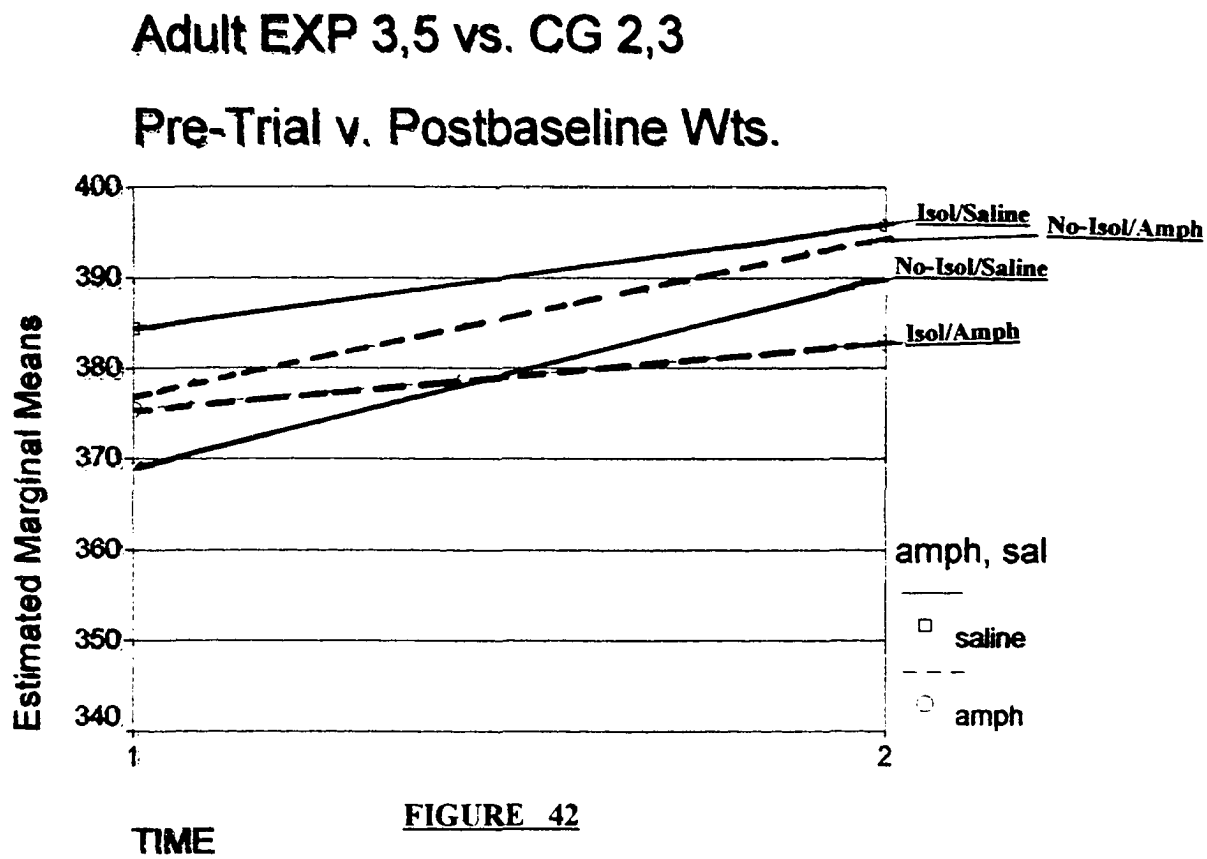
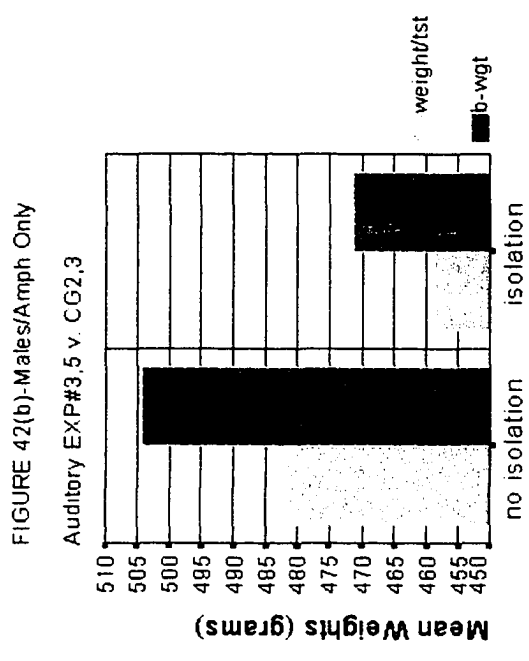
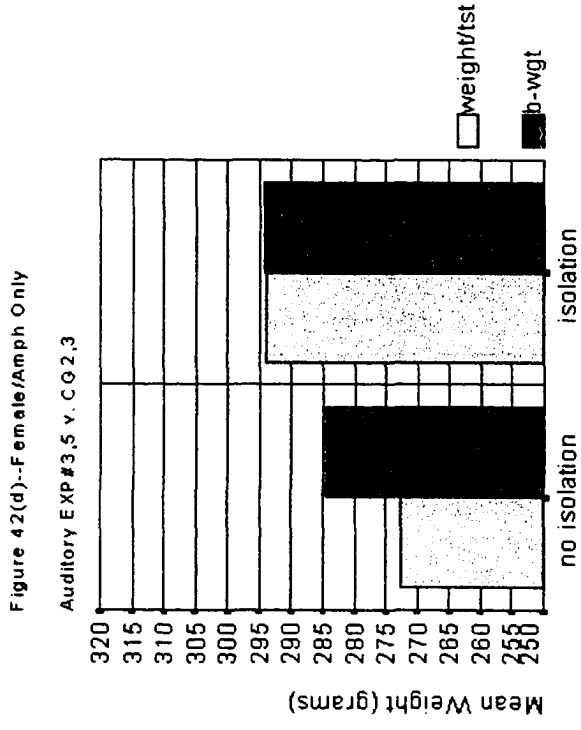
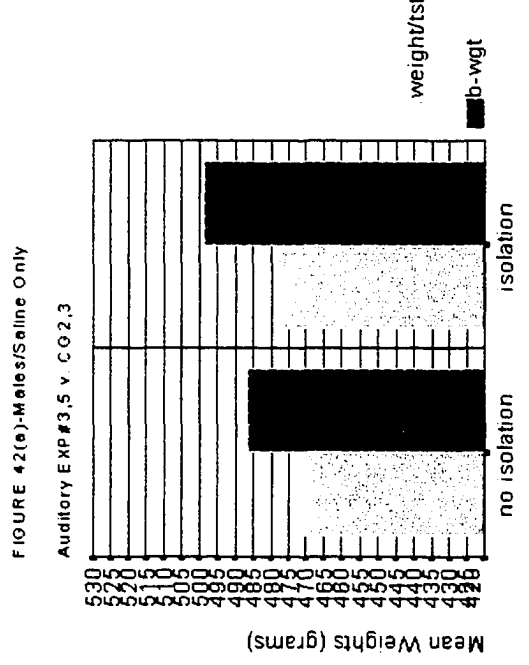
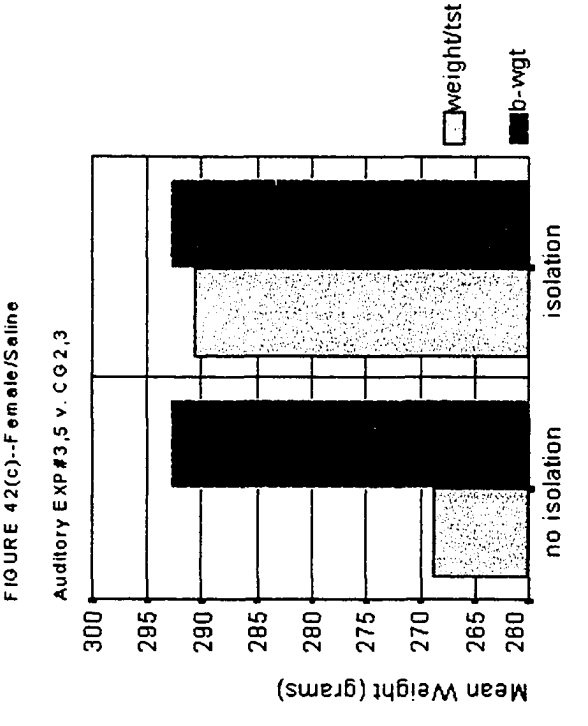


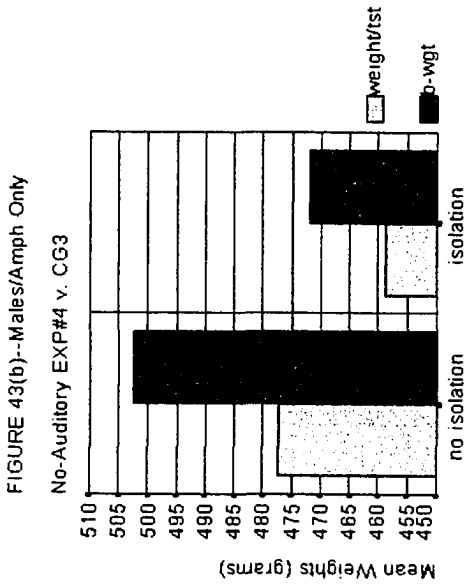
FIGURE 42

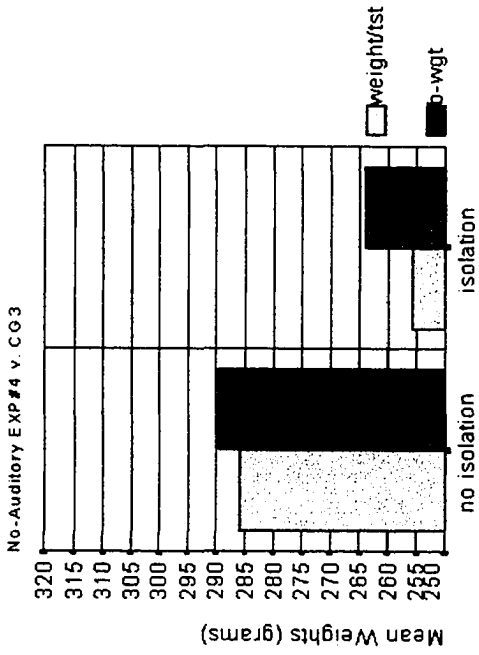


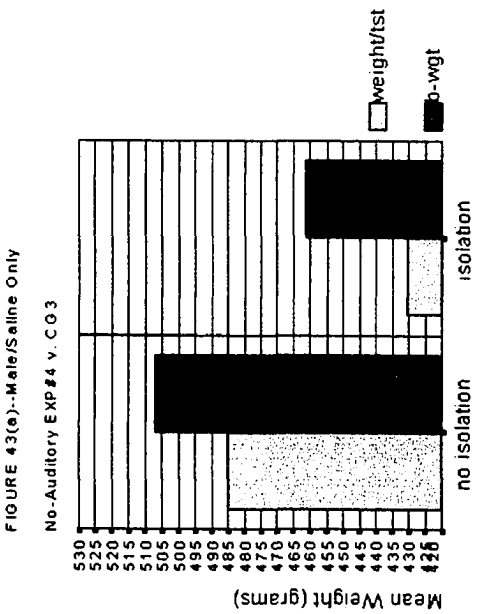


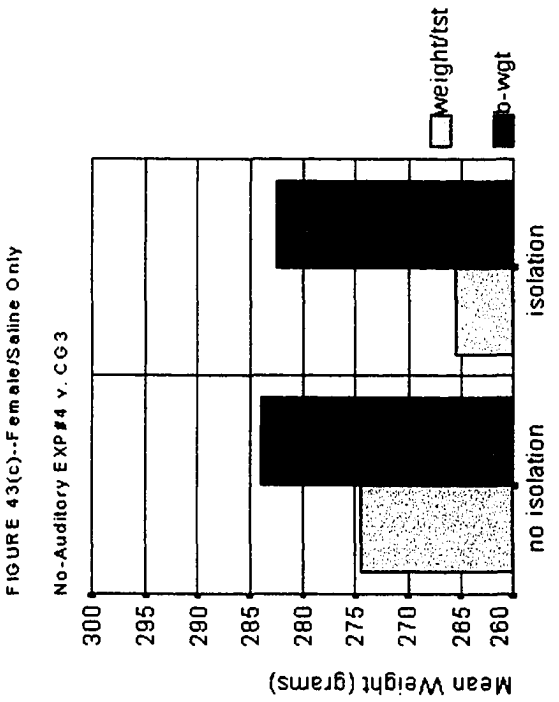












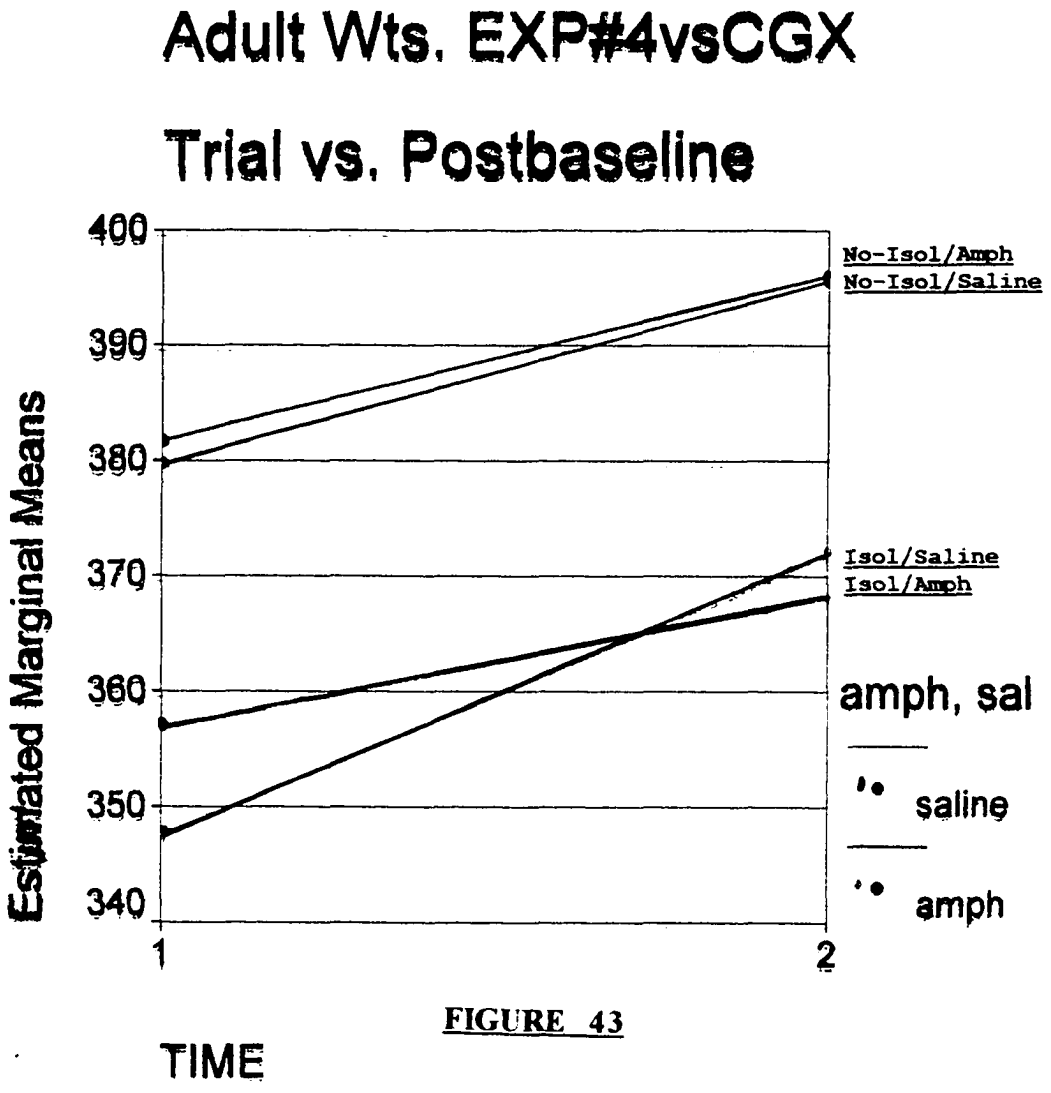


FIGURE 43

(2) **No-Isolation/Amphetamine Treatment-Condition**: Females in this combination Condition gained an average of 11.83 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 22.50 grams.

(3) **No-Isolation/Saline Treatment Condition**: Females in this combination Condition gained an average of 24.00 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 15.73 grams.

(4) **Isolation/Amphetamine Treatment Condition**: Females in this combination Condition gained an average of 2.25* grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 12.38 grams *However, it should be noted that the only "missing data" in this comparison was one postbaseline weight measurement in the Isolation/Amph Female Condition (N=4 instead of N=5) (see Figure 42(d)). Had all five animals had a postbaseline weight measured, this Mean weight gain should have been somewhat greater.

B. **For EXP#4 vs. CG3**, , as can be seen in Figure 43(a)(b)(c)(d), for this No-Auditory Isolation Treatment experimental group compared to the control group, there was a significant effect for Time, $F(1, 17) = 51.291$, $p < .0001$; for Time x Sex, $F(1, 17) = 8.760$, $p < .009$. Further, there was a Between-Subject Effect for Sex, $F(1, 17) = 683.654$, $p < .0001$, and an Isolation Effect, $F(1, 17) = 12.555$, $p < ..002$ At the Postbaseline weighing, all groups had gained weight compared to what they weighed on the day of the Open Field Trials. Listed from highest weight gain to lowest weight gain, the Isolation/No-Isolation Treatment and the Amphetamine/Saline Condition groups are as follows:

(1) **No-Isolation/Saline Treatment-Condition**: Females in this combination Condition gained an average of 3.50 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 25.20

grams.

(2) **No-Isolation/Amph Treatment-Condition**: Females in this combination Condition gained an average of 9.50 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 22.25 grams.

(3) **Isolation/Saline Treatment Condition**: Females in this combination Condition gained an average of 17.000 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 30.750 grams.

(4) **Isolation/Amphetamine Treatment Condition**: Females in this combination Condition gained an average of 8.000 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 13.33 grams.

C. **For EXP#5 vs. CG3**, , as can be seen in Figure 44(a)(b)(c)(d), for this Auditory Isolation Treatment experimental group compared to the control group CG3, there was a significant effect for Time, $F(1, 17) = 32.858$, $p < .0001$; for Time x Sex , $F(1, 17) = 8.398$, $p < .010$. Further, there was a Between-Subject Effect for Sex , $F(1, 17) = 524.288$, $p < .0001$, and a Sex by Drug Interaction, $F(1, 17) = 5.877$, $p < .027$. At the Postbaseline weighing, all groups had gained weight compared to what they weighed on the day of the Open Field Trials. Listed from highest weight gain to lowest weight gain, the Isolation/No-Isolation Treatment and the Amphetamine/Saline Condition groups are as follows:

(1) **Isolation/Saline Treatment-Condition**: Females in this combination Condition gained an average of 7.500 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of. 24.66 grams.

FIGURE 44

(2) **Isolation/Amphetamine Treatment-Condition**: Females in this combination Condition gained an average of 8.00 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 14.667 grams. .

(3) **No-Isolation/Amphetamine Treatment Condition**: Females in this combination Condition gained an average of 3.50 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 25.20 grams.

(4) **No-Isolation/Saline Treatment Condition**: Females in this combination Condition gained an average of 9.50 grams during the elapsed time from Trial to Postbaseline weighing. During this same period males gained an average of 25.0 grams.

FIGURE 44((a)--Male/Saline Only

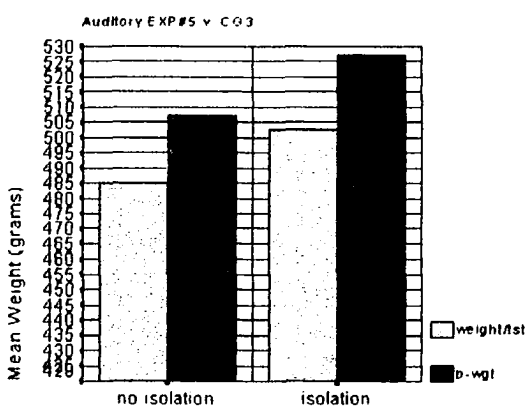


FIGURE 44(b)-Male/Amph

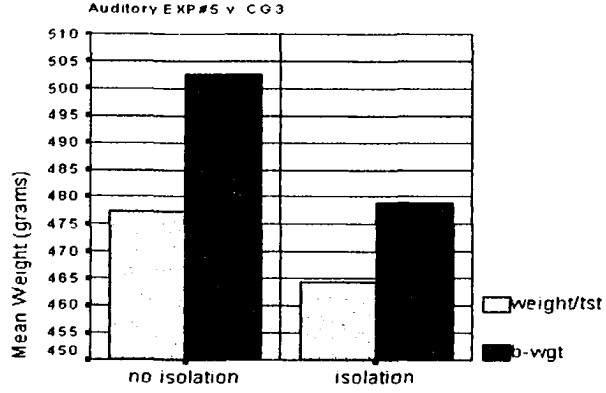


FIGURE 44(c)-Female/Saline Only

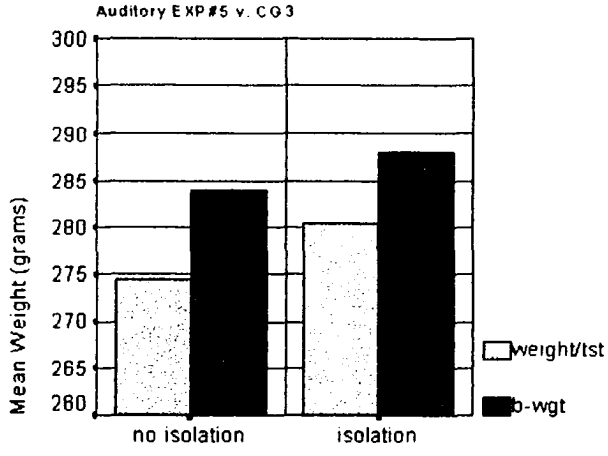
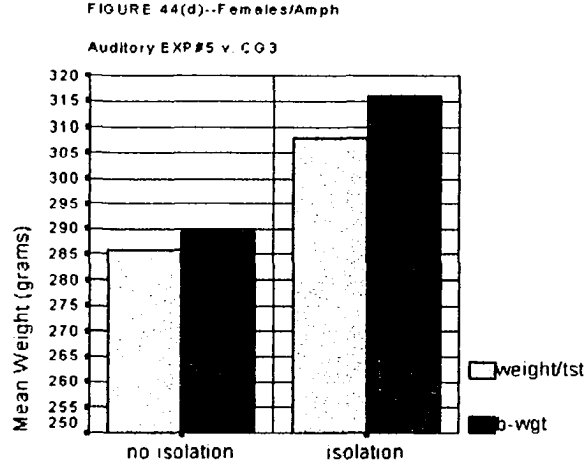


FIGURE 44(d)--Females/Amph



11.9.a Discussion of Weight Analysis:

One could ask why the last sixteen pages were spent describing (and crunching) data involving a process that might *appear* to be only a daily, minuscule, even transient (but regularly transient) amount of weight loss experienced by the pups experiencing either types of the Isolation Treatments. An answer could be proposed by making application of the previously discussed ideas of Developmental Time, and Developmental Timing.

First, Developmental Time: One hour spent away from the dam and siblings during prime time nursing hours, even with having tightly regulated controls for temperature and humidity, produces long-term change which lasts to adulthood. Certainly one pup-hour does not equate with one human neonate hour, nor would the repeated, daily loss of what in human infant terms would be a minuscule amount of weight, have an equated impact on a furless, 8-gram, neonatal rat pup (Agren, Zelenin, Hakansson, Eklof, Aperia, Nejsum, Nielsen & Sedin, 2003; Changizi, McGhee & Hall, 2002; Callahan & Rinaman, 1998; Ramirez, Wang, Kallichandra & Ross, 2002; and Hall, 1989). Quoting from Diana Dow-Edwards in her NIDA publication:

> "...the timing of the brain growth spurt or maximal expansion of the brain with respect to the day of birth is quite different for rodents and for human beings. ...For example, the cerebellum attains 50% adult weight in humans at about 12 months of age and in rat at about 15 days of age. Myelination of 50% of the corpus callosum occurs at about 18 months in humans but not until 45 days in rats...Thermoregulation, for example, takes 18 months to fully develop in humans and 18 days in rodents."
>
> And when addressing appropriate drug exposure regimens, she states:
>
> "**During perinatal life of the rat, a 3-hour exposure in the**

rat approximates a *1.1 day* exposure in humans." (P 162.)

Second, Developmental Timing: "Minuscule" is a relative term, not only to the species, age, and instant weight of the individual, but also to the ongoing developmental functional and growth processes, (i.e. body/brain/cell metabolism, production and release of glutamate, glucocorticoid, release of growth hormones, insulin balance, central brain core heat regulation, rate of thyroid/energy/oxygen/water balance and overall anabolic and catabolic processes) and, at a structural level, mylenation, synaptogenesis, pruning of receptors and number/type fixing of functional subunits, setting of (at least initial) ratios in G-protein cascades, and other homoeostatic hormonal regulators (although at this developmental time period estrogen or one of its forms is the preeminent sexually dimorphic steroid active in the brain). In keeping with the developmental theory of "More"[10] , several "fanciful" research questions could be generated , such as, if we are endogenously a water based organism, historically and, in evolutionary terms only recently evolving our way out of an exogenous water world, and, if water is so crucial to *every metabolic, chemical and energy exchange ongoing in the*

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. Linda Spear (1990) has addressed this idea, but it has a history of various advocates not only in Developmental Biopsychology alone. It is the idea that during specific periods in development the moment-to-moment compensatory physiological mechanisms which serve to modulate levels of neurohormones and neurotransmitters (including function - binding affinity, number and type of receptors and functional subunits, production of enzymatic regulators, protease, and even steroidal nuclear binding sites involved in pruning and/or cementing functional pathways) are *then* functionally qualitatively different—i.e., in the presence of "x" levels of a substance, receptors for that or other substances at the same locus or further, may not down regulate by changing either receptor numbers or binding affinity (i.e., the "general or standard" adult functional response to a [sometimes rapid] increase in production and release into the synapse), but instead "up regulate" (feed forward)the number or function of receptor sites. Considering the recent renewed focus on early developmental "switches" in the function of glutamate, GABA, glycine (and almost any substance that has the potential to regulate Immediate Early Gene gene cascades or their enzymatic trigger points), "More", as defined herein, means potentiating the ultimate up regulation of the *future processing capacity* of a substance and its *future weighted proximal impact* on overall brain function.

brain, (including Krebb and Hebb), *and therefore every homoeostatic brain process*, then how important is **dehydration** to **epigenetica**lly developing processes such as rate-fixing levels of Hasp-70-90, brain temperature regulation set points, pH metabolic levels, and the potentiation of *future* response cascades (ecosinoid inflammatory response hormones, metabotropic proteins, enzymes and its sum-time dependant disrupters–Glucocorticoid/Thyroid/Mineralcorticoid Trio.[11])? How important would *chronic dehydratio*n, for the daily equivalent of 1.1 days in human time, *be* to an organism weighing about 8 grams, or 10 grams, 20 grams, virtually naked and with an outside covering vulnerable to evaporative water loss, perceptually blind because eyelids have not as yet opened, but still able to register stimulatory light changes through translucent lids; with dampened hearing due to unopened pinna, but still possessing fully functioning auditory and vestibular nuclei sensitive to sound pressure levels and tactile stimulation; capable of cross-modal, additive stimulatory/excitatory sensory processing; temperature vulnerable; insulin and insulin-like growth factor shifting, and finally, very busy attempting to finish off a species-limited but still "plastic" altricial brain? But if this be rational revery than, at least, this researcher is not alone in it. The list of research investigators studying early stress in development is growing. And it has not escaped notice that some plasticity mechanisms in early development clearly are conserved in the adult brain and are utilized in the brain's response to major insult—such as chronic, exogenous drug administration and its then negatively disregulated stress-hypo/hyper-responsive Hypothalamic-Pituitary-Axis, and all of the potential this dysfunctional HPA stress response has for generating neuropathology and psychopathology.

12.0 GENERAL DISCUSSION. What information can be drawn from the results of the observations in conjunction with the physiological and behavioral measurements produced in this study? What support in the research literature can be gleaned or lent? At this writing, there is reason to hesitate before categorizing the collective results as definitively falling into only one of the current working research theories, approaches or paradigms. What is offered are not absolute final conclusions, but a summary of observations and comments:

First, PUP weight:

(1) Clearly, pups that received Isolation Treatment (either with OR without auditory) gained significantly less overall weight during PND 2 through PND 9 when compared to control groups (those not exposed to Isolation Treatment).

(2) Also, pups that received Isolation Treatment *without* the auditory (EXP#4) gained the *least* overall weight during PND 2 through PND 9 , when compared to: (a) its control group, and (b)when compared to pups in the Main Inquiry Group Isolation Treatment *with* the auditory Isolation Treatment .

(3) Clearly, the EXP#4 pups that did *not* experience auditory stimulation during the Isolation Treatment *lost* significantly more weight *during* the daily one-hour treatment than: (a) their comparison control group did during the comparable time period on PND 5, and (b) on PND 5 through PND 9 when compared with the Main Inquiry subjects which received Isolation Treatment *with* an auditory mask.

Second, ADULT weight: Original and Postbaseline Measurements:

(1) In the groups that experienced Isolation Treatment With Auditory, males showed an Isolation Treatment effect and they were consistent with the pup weight findings, i.e. males receiving Auditory Isolation Treatment as pups continued to weigh less than the control group pups/adults. Females however showed no significant Isolation Effects.

(2) However, in the No-Auditory Isolation Treatment Group (EXP#4), when compared to its control No-Isolation Group (CG3), BOTH males and females that received the No-Auditory Isolation Treatment weighed less than males and females in the control group This was consistent with the pup weights for EXP#4.

(3) At Postbaseline, the Main Inquiry Auditory Isolation Treatment Group males, as expected, weighed more than females (all combined-conditions and all groups). It then becomes more complicated: Adult Males that received perinatal Auditory Isolation Treatment and drug at the original trials *weighed less* than the other males in either the saline or control groups or conditions, whereas Adult Females that received perinatal Auditory Isolation Treatment and Drug at original trials weighed *more* than the other female groups (No-Isolation/Saline Condition).

(4) At Postbaseline, the EXP#4-No-Auditory Isolation Treatment group, when compared to its Control CG3-No-Isolation Group, both males and females receiving No-Auditory Isolation Treatment weighed ***less*** than males and females in the No-Isolation Control Group There were no significant Isolation x Drug interactions. Again, the adult weight relationships are consistent with the pup weights for EXP#4.

Lastly, in the Main Inquiry Auditory Isolation Treatment Group there was a long-term effect on weight in male subjects; i.e. they weighed ***less*** than control males both on the last day of Isolation Treatment (PND 9), and as Adults (just before the original trials). However, both males and females experiencing the Isolation Treatment Without Auditory (EXP#4) as Adults weighed ***less*** than males and females in their control No-Isolation Group, and also EXP#4 Treatment groups weighed *less* than any of the Main Inquiry groups, whether Isolation (with auditory) or Controls. This adult long-term effect repeats the pattern of the pup weight differences as far as it applies to Isolation Treatment v. No-Isolation Treatment conditions .

Penke, Felszeghy, Fernette, Sage, Nyakas and Burlet (2001), conducted an Early Deprivation study wherein pups were deprived from their dam on PND 5 and on PND 14 for 24-hours. Among the effects that lasted into adulthood in that study was that standard food intake was less in the deprived rats compared to controls, and a preference was seen in feeding in adulthood for carbohydrates.

Lehmann and Feldon (2000) reported on several Maternal Separation studies in their review that showed a decrease of body weight at weaning age following repeated separation periods of 2-3 hours when the pups were separated from both the dam and the siblings, and one study (McIntosh, et al. 1999) where the lower body weight carried over into adulthood. However, they also state that the comparison group was a factor: The McIntosh study compared the Maternal Separation group to Early Handled and not to Non-Handled subjects. The study herein makes multiple comparisons, and in the comparison on PND 9, all Isolation Treatment subjects weighed less than the Control groups. As stated before, the Control group in this study would be what Pryce and Feldon (2003) designate as Animal Facility Reared groups. This lower weight effect for both males and females lasted to adulthood in the EXP#4 No-Auditory Isolation Treatment groups, and for males only in the Main Inquiry-Isolation Treatment With Auditory Groups.

This dissertation study is the only study this researcher is aware of that reports an adult long-term effect for lower weight resulting from an Isolation Treatment lasting only 1- hour on repeated days, and when comparing the Isolation Treatment Group to an Animal Facility Reared Control Group It is the only study that reports differences in weight effects (measured at pup and adult ages) arising out of Auditory v. Non-Auditory Isolation Treatments. Also, no other Isolation Treatment Study has measured the weights of pups before and after the actual daily Isolation Treatments (with or without Auditory) and reported the results of multiple comparisons.

Third, **performance on the behavioral measures**:

(1.) Clearly there are sex-related differences in the performance scores attained on the various behavioral measures, both at original Trial and at Postbaseline Trials and which can be attributed to the Isolation Treatment, to Drug received at Trial, and to the interaction between Sex, Isolation Treatment and the Drug or Saline Condition. When amphetamine was administered prior to the first Trial in the Open Field Box, the Drug Main Effects cut across Isolation Treatment Main Effects but differentially for male or female. As far as sex-dependent differences, the following generalizations held:

A.) In the **Main Inquiry study (Auditory Isolation Treatment),** original Trials, in the measures of Linecross, Locomotion and Rearing, **females** produced higher scores than males in both the Isolation Treatment/No-Isolation Treatment Condition and the Drug/Saline Condition. Stereotypy scores for Saline/Isol, or Saline/No-Isol Conditions were almost equal for male and female subjects, and females scored higher than males in the Amphetamine Condition regardless of Isolation Treatment.

B.) In the **EXP#4 v.CG3 study (Isolation Treatment Without Auditory),** original Trials, the greatest male v. female differences were seen in Rearing and in Stereotypy, as follows:

Rearing - Males had higher scores than females in the Isolation-Amphetamine Condition and lower than females in the No-Isolation-Amphetamine Condition; Females had higher scores in the No-Isolation-Saline Condition than males and approximately equal scores with males in the Isolation-Saline Condition.

Stereotypy - Females had equal scores with males in the Isolation-Amphetamine Condition and also in the Isolation-Saline Condition; Males scored lower than females in the No-Isolation-Amphetamine Condition and in the No-Isolation-Saline Condition.

Both Linecross and Locomotion scores were approximately equal between males and females within conditions.

(2.) As far as **activity levels** in general for the Main Inquiry Auditory Isolation Treatment study, as expected the subjects receiving amphetamine at trial, produced significantly *higher* activity scores across all measures than those that received saline at trials regardless of whether they received Isolation Treatment (or not). Across all measures the No-Isolation/amphetamine Condition scores *higher* than the Isolation Treatment/Amphetamine Condition And depending on which auditory exposure was experienced and which measure is examined, it is clear that there is a considerable difference in scores between Isolation vs. No-Isolation Condition receiving amphetamine, with the Isolation Treatment Condition producing ***less* activity**.

Also in the Main Inquiry Auditory Isolation Treatment conditions, and across all measures, the Isolation Treatment Condition receiving saline scored ***lower*** than the Main Inquiry No-Isolation Condition receiving saline. Unlike the Amphetamine groups however, the difference between the Saline groups' scores was less.

(3) As far as ***activity levels*** in general for the No-Auditory Isolation Treatment Group (EXP#4), as expected the subjects receiving amphetamine at trial, produced significantly higher activity scores across all measures than those that received saline at trial regardless of whether they received Isolation Treatment (or not). However, the effect was the reverse of the Main Inquiry amphetamine Condition in that the EXP#4 Isolation Treatment amphetamine Condition produced *greater scores* than the ***EXP#4* No-*Isolation Control Condition***, whereas the in the Main Inquiry the No-Isolation Amphetamine Condition -across all measures- produced higher scores than the Main Inquiry Isolation Amphetamine Condition Also, the difference between the scores produced by EXP#4 combined-condition groups for Locomotion, Rearing, Stereotypy, and the saline Linecross Condition, is considerably more than the score distribution within the Main Inquiry Group (See Figures 41, 42, 43, and 44; and mean scores for individual combined-condition groups are set out in Tables A for Main Inquiry Auditory

Isolation Treatment Groups; and Table C for EXP#4 v. CG3 No-Auditory Treatment Groups.)

On the other hand, as in the Main Inquiry Group, the No-Auditory Isolation Treatment Group(EXP#4) receiving **saline** scored ***lower*** than the No-Isolation Control Group receiving **saline**, however the degree of difference between the means for these two combined condition groups (No-Auditory Isolation/Saline v. No-Isolation/Saline) ; **reaches statistical significance and is clearly greater than the same comparison within the Main Inquiry *saline* Conditions which only approach statistical significance**. This result indicates a *greater (quantitative) effect* from type of Isolation Treatment in the No-Auditory Isolation Treatment group (EXP#4) when compared to the Main Inquiry Auditory Isolation Treatment Group; and an argument can also be made for a qualitative behavioral difference being produced by type of Isolation Treatment received (discussed below). Further, when compared to the *Postbaseline* measure scores, the Postbaseline performance is much more variable both within and between subjects, with the No-Isolation/Amphetamine (at trial) subjects ending with the highest scores and the Isolation/Saline subjects ending in the lowest scoring position across all measures..

(4.) As was stated in the analysis section, and can be seen in Table A, an interesting behavioral effect exists in the beginning and ending hierarchical ordering of scores between the combined-condition groups of the Main Inquiry Auditory Isolation Treatment Groups, and a time-dependant behavioral shift in the direction of the individual combined-condition group's scores. And with *time shift and directional change for these groups being the same for the particular group across all measures.* The time-dependant upward or downward shift (i.e., two separate factors - time of shift and direction of shift) in the individual combined-condition group's behavioral scores is directly dependant on:

(1) whether or not they received Isolation Treatment as pups;

(2) which Isolation Treatment procedure was received (Auditory v. No-Auditory Isolation Treatment); and

(3) whether or not they received saline or amphetamine at trial.

What could be a more interesting result is the *lack* of the above-described time-dependant behavioral shift for those combined-condition groups in the No-Auditory Isolation Treatment Experiment (EXP#4 v.CG3) for 14 of the 16 combined-condition group measures (see Table C), across measures, and also there is no shift after 15-20 minutes for any group Again, the hierarchical ordering of the measurement scores produced by these groups shows a remarkable pattern of regularity both at the beginning and at the end of the measurements.

Also notable is the fact that the *Postbaseline* measurements for the EXP#4 combined-condition groups **do** show time-dependant shifts for all but the Isolation Treatment group receiving Saline. This fact could argue against a novelty effect existing for those subjects receiving the No-Auditory Isolation Treatment effect in this group in particular, and this would be in line with several studies which show a reduced novelty effect for Isolation/Saline groups. The change in pattern of behavior for subjects in the other remaining combined-conditions of EXP#4 at Postbaseline could be interpreted (and supported in the drug/reward literature) by a Conditioned Drug effect. Also, this could be said to argue for the idea that this No-Auditory Isolation Treatment produces a *blunted novelty response*, (i.e., does not include overt freezing) and subjects could quite possibly be behaviorally exhibiting a **lower** glucocorticoid response, lower ACTH response and even an increased AVP to CRF ratio (should these hormone levels be measured) similar to the behavioral and physiological research findings of studies of Lewis Rats and stress-induced changes in that strain's HPA response to stress.

As a concluding remark, several brief conjectures could be made about possible underlying mechanisms which could be responsible for the differences seen in the scores between the No-Auditory Isolation Treatment and the Auditory Isolation Treatment subjects. As Meaney (2001) has pointed out in his article Nature, Nurture, and the Disunity of Knowledge, redundant developmental mechanisms underlie much of plasticity in the brain. Weighting one mechanism too heavily is always risky business.

As Munck and Seyle described, Glucocorticoid is the end product of the Hypothalamic cascade of stimulating hormones and, once released from the adrenals it has a number of regulating and modulating effects, both centrally and peripherally, in cell metabolism, including both increases and decreases in energy/metabolic processes and gene transcription. It is so important as a developmental regulator, that a strict buffering system in the form of the SHRP has evolved as a developmental process to carefully regulate the brain's exposure levels during the brain growth spurt period. However, it should also be remembered that production levels of Glucocorticoid (CORT) is not modulated only by the HPA. There are several other ways in which the production of Glucocorticoid in the adrenals can be stimulated without engaging the HPA cascade. One is via the noradrenergic stimulation of the adrenals, locally and by the Splanchnic Nerve via sympathetic excitation, and another is the vagal nerve (Dijkstra, Binnekade & Tilders, 1995; Tilders, 1990). This can be viewed as a redundant (or backup process) to initiate production and release of this critical hormone.

That said, it is also the case that if the timing of the presence of this hormone is disregulated, long-term changes in physiological, behavioral function ensues (Benesova & Pavlik, 1989; Sandman, et al, 1992; Vasquez, Oers, Levine & Akil, 1996; Vasquez, 1998, and Levine, Hutchton, Wiener & Rosenfeld, 1991). Kamphuis, Bakker, Broekhoven, Kunne, Croiset, Lentjes, Tilders, van Bel, & Wiegant, 2002, have reported a study where rats exposed to dexamethasone (synthetic glucocorticoid) during early life

show hyporesponsivity of the HPA, a blunted stress response as adults. McCormick, Kehoe & Kovacs (1997), have shown a cumulative increase in plasma corticosterone in neonates experiencing the 1-hour, 8-days Isolation Treatment. This mechanism could offer an explanation of why the Isolation Treatment animals receiving only saline prior to the test showed significantly lower scores on all behavior measures when compared to No-Isolation/Saline Controls.

Furthermore, Kehoe, et al, (1998) found a hyperresponse to amphetamine in Isolated neonates' Locomotor response, while Zimmerberg and Shartrand (1992) have demonstrated a temperature dependant effect on Isolated neonates' Locomotor response to amphetamine, with higher ambient temperatures producing reduced sensitivity to the drug while lower temperature producing increased drug sensitivity. Although the ambient temperature inside the chamber and pup container were tightly controlled, exogenous or ambient temperature *is not the only source of heat generation capable of inducing an upward change in brain core temperature.* Kiyatkin and Wise (2001) have shown that a variety of stimuli, all stressor related, can produce brain hyperthermia, including sound stimulation. There is a possibility that the difference between the types of Isolation Treatment produced subjects that were primed neonatally to have a differential response to amphetamine in adulthood. That remains to be seen, and tested..

END NOTES

1.) The experiment commenced on February 15, 2002 with the birth of the first litters and ended on September 30, 2002 with the collection of the last post-baseline. The last week of April pinworm eggs (but no adult worms) were found in the Sprague Dawley populations and the all colonies housed in the Facility were placed on a pinworm eradicating regimen that consisted of a medicated lab chow which substituted their regular Purina diet every other week; one week on regular diet and one week on the special food containing Fenbendazole (Purina Special Lab Diet). Listed in Paragraph "2." below is the drug specifications provided by the vendor and several cites for research papers on the studies on it. Fenbendazole was NOT given to dams, nursing dams or pre-weanling pups ever. Special diet began on May 14, 2002 and ran for 18 consecutive weeks, rotating off and on with regular lab pellets; that is, 9 of the 18 weeks included the special diet. Experimental groups were scheduled to be tested at the conclusion of an "off" (no Fenbendazole) week. This allowed for a more than adequate washout period-(twice as long as suggested by the vet and literature) prior to each group's behavioral testing (first group was tested beginning on May 19, 2002.) As a control for any possible pinworm feed effects, (although this was thoroughly researched in the veterinary literature and approved by the Animal Facility Veterinary consultant), stringent comparison by statistical analysis of within and between group behavioral test scores and weights were conducted to rule out any possible change-in-diet-effects. No statistically significant differences were found within the Main Inquiry Experimental Group (Litters No. 1, 3 and 5); and no statistically significant differences were found with the Control Group (Control Litters No. 1, 2 and 3). Moreover, Experimental Group Litter No. 1 (which received the ***least amount*** of time/exposure to the special feed) and Experimental Group Litter No. 5 (which received the ***most amount*** of time/exposure to the special feed) produced behavioral measurement scores that were not only **not**

significantly different, they were remarkably similar to each other. Total group body weights were also similar. Between-group tests done on the Control Group (Control Litters No. 1, 2, and 3) showed no significant differences and they were remarkably similar to each other on total body weight and on scores produced on the behavioral measurements. (See statistics section for details of which tests were run and actual scores and plots.)

2.) Bibliography for Treatment of Syphacia muris

Huerkamp, M.J., Benjamin, K.A., Zitzow, L.A., Pullium, J.K., Lloyd, J.A., Thompson, W.D., Webb, S.K., and Lehner, N.D. (2000). Fenbendazole treatment without environmental decontamination eradicates syphacia muris from all rats in a large, complex research institution, *Contemporary Topics 2000, American Association for Laboratory Animal Science, 39(5), 9-12.*

Coghlan, L.G., Lee, D.R., Psencik, B. and Weiss, D. (1999). Practical and effective eradication of pinworms (syphacia muris) in rats by use of fenbendazole, *Laboratory Animal Science*, 43(5)(Oct.1998).

Duwel, D. (1977). Fenbendazole, II. Biological properties and activity. *Pestic. Sci*, 8; 550-555.

Taffs, L.F. (1976) Pinworm infections in laboratory rodents. A review. *Lab. Animals*, 10:1-13.

Kirsch, R. (1978). In vitro an din vivo studies on the ovicidal activity of fenbendazole. *Rres. Vet Sci.*, 25:263-265.

REFERENCES

Agren, J., Zelenin, S., Hakansson, M., Eklof, A. C., Aperia, A., Nejsum, L. N., Nielsen, S., & Sedin, G. (2003). Transepidermal water loss in developing rats: role of aquaporins in the immature skin. *Pediatric Research*, **53(4)**, 558-65.

Ahmed, S.H., Stinus, L., LeMoal, M., & Cador, M. (1995). Social deprivation enhances the vulnerability of male wistar rats to stressor and amphetamine-induced behavioral sensitization. *Psychopharmacology*, **117**(1), 116-24.

Alberts, J. R. (1978). Huddling by rat pups: group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology*, **92(2)**, 231-246.

Angulo, J.A., & McEwen, B.S. (1994). Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens. *Brain Research Reviews*, **19**, 1-28.

Bachus, S.E., & Kleinman, J. E. (1996). The neuropathology of schizophrenia. *Journal of Clinical Psychiatry*, **57,** suppl. **11**, 72-83.x

Barbazangers, A., Piazza, P V., LeMoal, M., & Maccari, S. (1996). Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *Journal of Neuroscience,* **16(12)**, 3943-3944.

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, **153**, 31-43.

Bardo, M. T., Rowlett, J. K., & Harris, M. J. (1993). Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neuroscience and Biobehavioral Reviews,* **19(1)**, 39-51.

Benesova, O., & Pavlik, A. (1989). Perinatal treatment with glococorticoids and the risk of maldevelopment of the brain. *Neuropharmacology*, **28(1)**, 89-97.

Blumberg, M, Efimova, I. V., & Alberts, J. R. (1992). Ultrasonic vocalizations by rat pups: the primary importance of ambient temperature and the thermal significance of contact comfort. *Developmental Psychobiology*, **25(4)**, 229-250.

Buck, K.J., Lorang, D., & Amara, S. G. (1996). Discrete strctural domains and cell-specific expression determine functional selectivity of the dopamine and norepinephrine transporters. *NIDA Research Monograph*, **161**, 154-173.

Cador, M., Dumas, S., Cole, J., Mallet, Koob, G. F., LeMoal, M. & Stinus, L. (1992). Behavioral sensitization induced by psychostimulants or stress: search for a molecular basis and evidence for a CRF-dependent phenomenon. *Annals of the N.Y. Academy of Sciences*, **654,** 416-420.

Cador, M., Ahmed, S. H., Koob, G. F., LeMoal, M., &. Stinus, L. (1992). Corticotropin-releasing factor induces a place aversion independent of its neuroendocrine role. *Brain Research*, **597,** 304-309.

Callahan, J. B., & Rinaman, L. (1998). The postnatal emergence of dehydration anorexia is temporarily associated with the emergence of dehydration-induced inhibition of gastric emptying. *Physiological Behavior*, **64(5),** 683-7.

Calogero, A. E., Sternberg, E. M., Bagdy, G, Smith, C., Bernardini, R., Aksentijevich, S., Wilder, R. L., Gold, P W., & Chrousos, G. P (1992). Neurotransmitter-induced hypothalamic-pituitary-adrenal axis responsiveness is defective in inflammatory disease-susceptible lewis rats: in vivo and in vitro studies suggesting globally defective hypothalamic secretion of corticotropin-releasing hormone. *Neuroendocrinology*, **55(5),** 600-8.

Campbell, J., & Spear, L. P (1999). Effects of early handling on amphetamine-induced locomotor activation and conditioned place preference in the adult rat. *Psychopharmacology*, **143,** 183-189.

Carr, G. D., Fibiger, H. C., & Phillips, A. G. (1987). Independence of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference, *Psychopharmacology,* .

Champagne, F., & Meaney, M. J. (2001). Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. *Progress in Brain Research,* **133**, 287-302.

Changizi, McGhee & Hall, 2002; equate pup

Cho, A. K,. Melega, W. P , Kuczenski, R, Segal, D. S. (2001). Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. *Snynapse,* **39**(2); 161-6.

Chrousos, G.P , & Gold, P W. (1995). Introduction. Annals of the N.Y. Academy of Sciences, **771**, xv-xviii.

Cratty, M.S., Ward, H.E., Johnson, E.A., Azzaro, A. J., & Birkle, D. L. (1995). Prenatal stress increases corticotropin-releasing factor (CRF) content and release in rat amygdala minces. *Brain Research,* **27**, 675(1-2), 297-302.

Creese, I., & Iversen, D. (1974). The role of forebrain dopamine system in amphetamine induced stereotypic behaviors in the rat. *Psychopharmacology*, **39,** 345-357.

Culman, J., Itoi, K., & Unger, T. (1995). Hypothalamic tachykinins. *Annals of the N.Y. Academy of Sciences,* **771**, 204-217.

Darwin, C.(1859). The origin of species by means of natural selection:or the preservation of favored races in the struggle for life. Dolphin Books:Doubleday & Company, Inc., N.Y.

Daunais, J. B., & McGinty, J. F. (1995). Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. *Molecular Brain Research*, **29**, 201-210.

Deroche, V., Piazza, V., Casolini, P , LeMoal, M., & Simon, H. (1993). Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. *Brain Research*, **611**, 352-356.

DeSouza, E.B., & Nemeroff, C. (Eds.)(1990). Corticotropin-Releasing Factor:Basic and Clinical Studies of Neuropeptide. Florida:CRC Press.

Dhabhar, F. S., McEwen, B. S., & Spencer, R. L. (1997). Adaptation to prolonged or repeated stress–comparison between rat strains showing intrinsic differences in reactivity to acute stress. *Neuroendocrinology*, **65**, 360-368.

Dijkstra,, I., Binnekade, R., & Tilders, F. J. H. (1996). Diurnal variation in resting levels of corticosterone is not mediated by variation in adrenal responsiveness to adrencorticotropin but involves splanchnic nerve integrity. *Endocrinology*, **137(2)**, 540-547.

Dow-Edwards, D. (1996). Comparability of human and animal studies of developmental cocaine exposure. *NIDA Research Monographs*, **164**, 146-174.

Dunn, A.J., & Berridge, C. W. (1990). Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Research Review*, **15**, 1-100.

Dunn, A.J., & Berridge, C. W. (1973). Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems. *Pharmacology, Biochemistry & Behavior*, **27**, 685-691.

Ellinwood, Jr., H., & Balster, R. L. (1974). Rating the behavioral effects of amphetamine. *European Journal of Pharmacology*, **28**, 35-41.

Fleming, A. S., Kraemer, G. W., Gonzales, A., Lovic, V., Rees, S., & Melo, A. (2002). Mothering begets mothering: the transmission of behvior and its neurobiology across generations. *Pharmacology Biochemistry & Behavior*, **73(1)**, 61-75,

Fodor, J.A. (1985). Precis of the modularity of mind. *The Behavioral and Brain Sciences*, **8**, 1-42.

Francis, D.D., Caldji, C., Champagne, F., Plotsky, P M. Liu & Meaney, M. J.(1999). The role of corticotropin-releasing factor-norephrine systems in mediating the effects of early experiences on the development of behavioral and endocrine responses to stress. *Biological Psychiatry*, **46(9),** 1153-66.

Freeman, S., Khvoles, R., Cherny, L. & Sohmer, H., (1999). Effect of long-term noise exposure on the developing and developed ear in the rat. *Audiology Neuroontology*, **4(5)**, 207-18.

Gardner, J.M, Karmel, B.Z, Norton, K.I., Mangano, C.L., & Brown, E.G. (1990). Neurobehavioral Indicators of Early Brain Insult in High-Risk Neonates, *Developmental Psychology*, **26(4),** 563-575.

Gattaz, W.F., & Brunner, J. (1996). Phospholipase A2 and the hypofrontlity hypothesis of schizophrenia. *Prostaglandins, Leukotrines & Essential Fatty Acids*, **55(1-2)**, 109-13.

Glass, P (1999). The vulnerable neonate and the neonatal intensive care environment, In: *Avery, G., Fletcher, M. & MacDonald, M. Eds., Philadelphia: Lippincott, Inc., Neonatology: Pathophysiology and Managemente of the Newborn*, Chp 8, 91-108.

Glass, G. V., & Hopkins, K. D. (1996). Statistical methods in education and psychology.(3rd ed.). Boston: Allyn and Bacon.

Gold, P , Licinio, W. J., Wong, M. L., & Chrousos, G. P (1996). Corticotropin releasing hormone in the pathophysiology of melancholic and atypicl depression and in the mechanism of action of antidepressant drugs. *Annals of the N.Y. Academy of Sciences*, **801**, 716-729.

Goldstein, L. E., Rasmuson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine and prefrontal cortical monamine responses to psychological stress in the rat. *Journal of Neuroscience*, **16(15)**, 4787-98.

Goodlet, C.R., Leo, J., O'Callaghan, J., & Mahoney, J. (1993). Transient cortical astrogliosis induced by alcohol exposure during the neonatal brain growth spurt in rats. *Developmental Brain Research*, **72(1)**, 85-97.

Gottlieb, G. (1992). Individual development and evolution: the genesis of novel behavior. New York: Oxford University Press.

Gottlieb, G. (1983). *The psychobiological approach to developmental issues.* In P H. Mussen (Ed.) Handbook of child psychology, Vo. 2, (4th ed.), 1-26. New York: Wiley.

Gould, S.J. (1982). Darwinism and the expansion of evolutionary theory. *Science*, **216**, 380-387.

Gratton, A., & Wise, R. A. (1994). Drug and behavior- associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. *Journal of Neuroscience*, **14(7)**, 4130-46.

Gray, L., & Philbin, M. K. (2000). The acoustic environment of hospital nurseries. *Journal of Perinatology*, **20,** S100-S104.

Gray, T.S. (1993). Amygdaloid CRF pathways. *Annals of the N.Y. Academy of Sciences*, **697**, 53-59.

Grey, P (1994). Psychology, (2nd ed.). New York:Worth

Griffin, J.E., & Ojeda, E. E. (1992). Textbook of endocrine physiology. Oxford University Press, New York

Grilly, D.M. (1994). Drugs and Human Behavior, (2nd ed.). Boston:Allyn & Bacon.

Grino, M., Paulmyer-Lacroix, O., Anglade, G., & Oliver, C. (1995). Molecular aspects of the regulation of the hypothalamo-pituitary-adrenal axis during development in the rat. *Annals of the N.Y. Academy of Sciences*, **771**, 139-351.

Groenink, L., Compaan, J., VanDerGugten, J., Zethof, T., VanDerHeyden, J., & Oliver, B. (1995). Stress-induced hyperthermia in mice. *Annals N.Y. Academy of Sciences*, **771**, 271.

Hall, W. G. (1989). Neural systems for early independent ingestion: regional metabolic changes during ingestive responding and dehydration. *Behavioral Neuroscience*, **103**(2), 386-411.

Hall, F. S., Wilkinson, L. S., Humby, T., & Robbins, T. W. (1999). Maternal Deprivation of neonatal rats produces enduring changes in dopamine function. *Synapse*, **32**, 37-43.

Harlow, H. F. (1958). The evolution of learning. In A. Roe & G. G. Simpson (Eds.), Behavior and Evolution. New Haven: Yale University Press.

Heinrichs, S.C., Menzaghi, F., Pich, E. M., Baldwin, M. A., Rassnick, S., & Koob, G. F. (1994). Anti-stress action of corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology*, **11(3)**, 179-186.

Heinrichs, S.C., Menzaghi, F., Pich, E. M., Baldwin, M. A., Rassnick, S., & Koob, G. F (1995). The role of CRF in behavioral aspects of stress. *Annals of the N.Y. Academy of Science*, **771**, 92-104.

Henry, C., Guegant, G., Cador, M., Arnauld, E., Arsault, J., LeMoaland, M., & Demotes-Mainard, J. (1995). Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. *Brain Research*, **685(1-2)**, 179-186.

Hofer, M.A. (1981). The roots of human behavior: an introduction to the psychobiology of early development. New York:W.H. Freeman.

Hooks, M.S., Jones, G. H., Liem, B. J., & Justice, Jr., J. B. In: Kalivas, P , & Samson, H. (1992). Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intra-cranial amphetamine infusions. The Neurobiology of Drug and Alcohol Addiction. *Annanls of the N.Y. Academy of Science*, **654,** 444-447.

Hooks, M.S., Duffy, P , Striplin, C., & Kalivas, P W. (1994). Behavioral and neurochemical sensitization following cocaine self-administration. *Psychopharmacology*, **115(1-2)**, 265-72.

Hoyenga, K., & Hoyenga. T. (1993). Gender-Related Differences:Origins and Outcomes. Boston:Allen and Bacon.

Johnson, E.O., Kamilaris, T. C., Chrousos, G. P , & Gold, P W. (1992). Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neuroscience and Behavioral Reviews*, **16,** 115-130.x

Julien, R.M. (1995). A primer of drug action. New York: W.H. Freeman and Company.

Kagan, J., Snidman, N., & Arcus, D. (1995). The role of temperament in social development. *Annals of the N.Y. Academy of Sciences*, **771,** 485-89.

Kalat, J.W. (1994). Biological psychology, (5th ed.). California:Brooks/Cole Pub.

Kalin, N.H. (1985). The neurobiology of fear. *Scientific American*, 94-101.

Kalivas, P W., Striplin, C. D., Steketee, J. D., Klitenick, M. A. & Duffy, P (1992). Cellular mechanisms of behavioral sensitization to drugs of abuse. *Annals of the N.Y. Academy of Sciences*, **654**, 128-135.

Kamphuis, P J., Bakker, J. M., Broekhoven, M. H., Kunne, C., Croiset, G., Lentjes, E. G., Tilders, F. J., vanBel, F., & Wiegant, V. M. (2002). Enhanced glucocorticoid

feedback inhibition of hypothalamo-pituitary-adrenal responses to stress in adult rats neonatally treated with dexamethasone. *Neuroendocrinology*, **76(3),** 158-69.

Kandel, E., & Schwartz, J. H. (1985). Principles of neural science (2nd ed.). New York:Elsevier.

Kandel, E.R., Schwartz, J. H., & Jessel, T. M. (1996). Essentials of neural science and behavior. New York: Elsevier.

Kehoe, P , Shoemaker, W. J., Triano, L., Callahan, M., & Rappolt, G. (1998). Adult rats stressed as neonates show exaggerated behavioral responses to both pharmacological and environmental challenges. *Behavioral Neurosciences*, **112(1)**, 116-125.

Kehoe, P , Hoffman, J.H., Austin-LaFrance, R. J., & Bronmzino, J. D. (1995). Neonatal isolation enhances hippocampal dentate response to tetanization in freely moving juvenile male rats. *Experimental Neurology*, **136,** 89-97.

Kehoe, P , Shoemaker, W. J., Triano, L., Hoffman, J., & Aaron, C. (1996). Repeated isolation in the neonatal rat produces alterations in behavior and ventral striatal dopamine release in the juvenile after amphetamine challenge. *Behavioral Neuroscience*, **110(6),** 1435-1444.

Kelley, A. E. (1998). Measurement of Rodent Stereotyped Behavior. *Behavioral Neuroscience*, Supp **(4)**, 8.8.1-13.

Kenny, P A., & Turkewitz, G. (1982). Limitations on input as a basis for neural organization and perceptual development: a preliminary theoretical statement. *Developmental Psychobiology*, **15(4)**, 357-368.

Kim, J.A., &. Druse, M. J. (1996). Protective effects of maternal buspirone treatment on serotonin reuptake sites in ethanol-exposed offspring. *Developmental Brain Research*, **92(2),** 190-198.

Kiyatkin, E. A., & Wise, R. A. (2001). Brain temperature fluctuation: a reflection of functional neural activation. *European Journal of Neuroscience*, **16(1)**, 164-8.

Kosten, T. A., & Ambrosio, E. (2002). HPA axis function and drug addictive behaviors; insights from studies with lewis and fisher 344 inbred rats. *Psychoneuroendocrinology*, **27(1-2)**, 35-69.

Koob, G.F., S.C. Heinrichs, E.M. Pich, F. Menzaghi, H. Boldwin, K. Miczek and K.T. Britton (1993). The role of corticotropin-releasing factor in behavioral responses to stress. *Ciba Foundation Symposium*, **172**, 277-89.

Koob, G.F. (1992). Neural mechanisms of drug reinforcement. *Annals of the N.Y. Academy of Sciences*, **654**, 171-87.

Kuhn, T.S. (1970). The structure of scientific revolutions. Chicago:University of Chicago Press.

Kuhn, C., Ignar, D., & Windh, R. In: Kilbey, M., &. Asghar, K. (Eds.)(1991). Endocrine function as a target of perinatal drug effects: methodologic issues. Methodological issues in controlled studies on effects of prenatal exposure to drug abuse. *NIDA Research Monograph*, **114**, 206-232.

Kuhn, C.M., Pauk, J., & Schanberg, S. M. (1990). Endocrine responses to mother-infant separation in developing rats. *Developmental Psychobiology*, **23(5)**, 395-410.

Kumar, S., Bacci, A., Kharazia, V., & Huguenard, J. (2002). A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *Journal of Neuroscience*, **22(8)**, 3005-3015.

Kunovac, J.L., & Stahl, S. M. (1995). Future directions in anxiolytic pharmacotherapy. *Psychiatric Clinics of North America*, **18(4)**, 895-909.

Kuo, Z.Y. (1976). The dynamics of behavior development: An epigenetic view. New York:Plenum Press.

Lawson, K., Turkewitz, G., Platt, M., & McCarton, C. (1985). Infant state in relation to its environmental context. *Infant Behavior and Development*, **8,** 269-281.

Lawson, K., Daum, C., & Turkewitz, G. (1977). Environmental characteristics of a neonatal intensive-care unit. *Child Development*, **48,** 1633-39.

Lehmann, J., & Feldon, J. (2000). Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Reviews in the Neurosciences*, **11,** 383-408 .

Lehmann, J., Pryce, C. R., Bettschen, D., & Feldon, J. (1999). The maternal separation paradigm and adult emotionality and cognition in male and female rats. *Pharmacology Biochemistry and Behavior*, **64(4)**, 705-715.

Leon, M., Croskerry, P G., & Smith, G. K. (1977). Thermal control of mother-young contact in rats. *Physiology & Behavior*, **21**, 793-811.

Lerner, R.M., Perkins, D. F., & Jacobson. L. P (1993). Timing, process and the diversity of developmental trajectories in human live: A developmental contextual perspective. In: Turkewitz, G. and Devenny, D.(Eds.), Developmental time and timing, 41-59. New Jersey:Lawrence Erlbaum.

Levine, S. (1994). The ontogeny of the hypothalamic-pituitary-adrenal axis: the influence of maternal factors. *Annals New York Academy of Sciences*, 746, 275-288.

Levine, S. Cohen (1993). Effects of early unilateral lesions: changes over the course of development. In: Turkewitz, G. and D. Devenny (Eds.), Developmental time and timing, 143-165. New Jersey:Lawrence Erlbaum.

Levine, S., Huchton, D. M., Wiener, S. G., & Rosenfeld, P (1991). Time course of the effect of maternal deprivation on the hypothalmic-pituitary-adrenal axis in the infant rat. Developmental Psychobiology, **24(8),** 547-58.

Lewkowicz, D.J., & Turkewitz, G. (1980). Cross-modal equivalence in early infancy: Auditory visual intensity matching. *Developmental Psychology*, **16**, 597-607.

Lickliter, R. (1993). Timing and the development of perinatal organization. In: Turkewitz, G. and D. Devenny (Eds.), Developmental time and timing. New Jersey: Lawrence Erlbaum.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P M. & Meaney, M. J.. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, **277**(5332), 1659-62.

Luine, V.N., Martinez, C., Villegas, M. Magarinos, A.M. & McEwen, B.S. (1995). Restraint stress reversibly enhances spatial memory performance. *Physiology and Behavior*, **59**, 27-32.

Luine, V.N, Grattan, D.R. & Selmanoff, M. (1997). Gonadal hormones alter hypothalamic GABA and glutamate levels. *Brain Research*, **747**, 165-168.

Maccari, S., Piazza, P V., Deminiere, J. M., Lemaire, V., Mormede, P , Simon, H., Angelucci, L., &. LeMoal, M. (1991). Life events-induced decrease of corticosteroid type I receptors is associated with reduced corticosterone feedback and enhanced vulnerability to amphetamine self-administration. *Brain Research*, **547**, 7-12.

McCann, S.M., Lyson, K., Karantha, S., Gimeno, M., Belova, N., Kamat, A., &. Rettori, V. (1995). Mechanismm of action of cytokines to induce the pattern of pituitary hormone secretion in infection. *Annals of the N.Y. Academy of Sciences*, **771**, 386-395.

McCormick, C. M., Kehoe, P , & Kovacs, S. (1997). Corticosterone release in response to repeated, short episodes of neonatal isolation: evidence of sensitization. *Int. J. Devl Neuroscience*, **16(3/4)**, 175-185.

Morris, B. H., Philbin, M. K., & Bose, C. (2000). The full-term and premature newborn: physiological effects of sound on the newborn. *Journal of Perinatology*, **20**, S55-S60.

McEwen, B.S. (1987). Steroid hormones and brain development: some guidelines for understanding the actions of pseudohormones and other toxic agents. *Environ. Health Perspect.*, **74**, 177-184.

McEwen, B.S.(1987). Preface. *NIDA Research Monograph*, **78,** v-xii.

Meaney, M.J., Bhatnagar, S.,. Larocque, S., McCormick, C., Shanks, N., Sharma, S., Smythe, J., Viau, V., & Plotsky, P (1993). Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic CRF system. In: Tache, Y., and C. Rivier (Eds.), Corticotropin-releasing factor and cytokinese:role in the stress response, *Annals of the N.Y. Academy of Science*, **697**, 70-85.

Meaney, M. (2001). Nature, nurture, and the disunity of knowledge. *Annals of the New York Academy of Sciences*, **935(1)**, 50-61.

Menzaghi, F., Heinrichs, S. C., Pich, E. M., Weiss, F., & Koob, G. F. (1993). The role of limbic and hypothalamic corticotropin-releasing factor in behavioral responses to stress. *Annals of the N.Y. Academy of Sciences*, **697**, 142-154.

Menzaghi, F., Rassnick, S., Heinrichs, S., Baldwin, H., Pich, E. M., Weiss, F., & Koob, G. F. (1996). The role of corticotropin-releasing factor in the anxiogenic effects of ethanol withdrawal. *Annals of the N.Y. Academy of Sciences*, 176-184.

Meredith, G.E., Ypma, P , & Zahm, D. S. (1995). Effects of dopamine depletion on the morphology of medium spiny neurons the shell and core of the rat nucleus accumbens. *Journal of Neuroscience*, **15(5pt2)**, 3808-20.

Moghaddam, B., Bolinao, M. L., Stein Behrens, B., & Sapolsky, R. (1994). Glucocorticoid mediate the stress-induced extracellular accumulation of glutamate. *Brain Research*, **655(1-2)**, 251-254.

Monk, M. (1995). Epigenesis programming of differential gene expression in development and evolution. *Dev Genet*, **17(3)**, 188-97.

Munck, A., & Naray-Fejes-Toth, A. (1992). The ups and downs of glucocorticoid physiology; permissive and suppressive effects revisited. *Molecular and Cellular Endocrinology*, **90**, C1-C4.

Munck, A., Guyre, P M., & and Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews*, **5(1)**, 25-44.

Myers, K. P & Hall, W. G. (2001). Effects of prior experience with dehydration and water on the time course of dehydration-induced drinking in weanling rats. *Developmental Psychobiology*, **38(3)**, 145-53.

Nair, H. P , & Gonzalez-Lima, F. (1999). Extinction of behavior in infant rats: development of functional coupling between septal, hippocampal, and ventral tegemental regions. *Journal of Neuroscience*, **19(19)**, 8646-8665.

Paulson, P E., & Robinson, T. E. (1995). Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. *Synapse*, **19**, 56-65.

Purchowicz, M., Tonelli, L. H., & Sternberg, E. M. (2003). Differential expression of class I MHC mRNA in the hypothalmus of lewis and fisher rats. Journal of Neuroimmunol **134(1-2)**, 35-43.

Penke, Z., Felszeghy, K., Fernette, B., Sage, D., Nyakas, C., & Burlet, A. (2001). Postnatal maternal deprivation produces long-lasting modifications of the stress

response, feeding and stress-related behavior in the rat. *Eur J. Neurosci*, **14**(4), 747-55.

Philbin, M.K., Ballweg, D.D., & Gray, L.(1996). The effect of intensive care unit sounds on the development of habituation in healthy avian neonates.

Philbin, M. K., & Klaas, P (2000). The full-term and premature newborn: Evaluating studies of the behavioral effects of sound on newborns. *Journal of Perinatology*, **20,** S61-S67.

Philbin, M.K., Lickliter, R., & Graven, S.N. (2000). Sensory experience and the developing organism: a history of ideas and view to the future, *Journal of Perinatology*, **20**, S2-S5.

Piazza, P V., Deroche, V., Deminiere, J. M., Maccari, S., LeMoal, M., & Simon, H. (1993). Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviors. *Proc. Nat'l. Academy of Sci.* USA, **90**, 11738-11742.

Pick, T.P , and R. Howden (Eds.)(1977). Gray's Anatomy. New York:Gramercy.

Pihoker, C., Owens, M. J., Kuhn, C. M., Schanberg, S. M., &. Nemeroff, C. B. (1993). Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor. *Psychoneuroendocrinology*, **18(7)**, 485-493.

Pitch, E.M., Lorang, M., Yeganeh, M., Rodriguez de Fonseca, F., Raber, J., Koob, G. F., & Weiss, F. (1995). Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *The Journal of Neurosciences*, **15(8)**, 5439-5447.

Popper, K.R. (1965). Conjectures and refutations: the growth of scientific knowledge. New York:Harper & Row.

Post, R.M., Weiss, S.R.B., Smith, M., Rosen, J., & Frye, M. (1989). Stress, conditioning, and the temporal aspects of affective disorders. *Annals of the N.Y. Academy of Sciences*, **771**, 677-686.

Prasad, B.M., Sorg, B. A., Ulibarri, C., & Kalivas, P W. (1995). Sensitization to stress and psychostimulants. Involvement on dopamine transmission versus the HPA axis. *Annals of the N.Y. Academy of Sciences*, **771**, 617-25.

Pryce, C. R., Bettschen, D., & Feldon, J. (2000). Comparison of the effects of early handling and early maternal deprivation on maternal care in the rat. *Developmental Psychobiology*, **38(4)**, 240-251.

Pryce, C. R. and Feldon, J. (2003). Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. *Neuroscience & Biobehavioral Reviews*, **27(1-2)**, 57-71.

Purves, D., & Lichtman, J. W. (1985). Principles of Neural Development. Massachusetts:Sinauer.

Putnam, F.W. (1995). Traumatic stress and pathological dissociation. *Annals of the N.Y. Academy of Sciences*, **771**, 708-715.

Rang, H.P , Dale, M. M., Ritter, J. M., & Gardner, P (1995). Pharmacology. New York:Churchill Livingstone.

Reid, M.S., Hsu, K., Tolliver, B. K., Crawford, C. A., & Berger, S. P (1996). Evidence of the involvement of phospholipase A2 mechanisms in the development of stimulant sensitization. *Journal of Pharmacology & Experimental Therapeutics*, **276(3)**, 1244-56.

Rosenfeld, P , D. Suchecki, and S. Levine (1992). Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neuroscience and Biobehavioral Reviews*, **16**, 553-568.

Rouille, Y., S.J. Duguay, K. Lund, M. Furuta, Q. Gond, G. Lipkind, A.A. Oliva, S.J. Chan, and D.F. Steiner (1995). Proteolytic processing mechanisms in the biosynthesis of neuroendocrine peptides; the subtilisin like proprotein convertase. *Frontiers in Neuroendocrinology*, **16(4)**, 322-61.

Ryan, A. F., Axelsson, G. A., & Woolf, N. K. (1992). Central auditory metabolic activity induced by intense noise exposure. *Hearing Research*, **61(1-2)**:,24-30.

Sandman, C. A., Wadhwa, P , Chicz-DeMet, A., Dunkel-Schetter, C. & Porto, M. (1997). Maternal stress, HPA activity, and fetal/infant outcome. *Annals New York Academy of Sciences*, **814**, 266-274.

Sapolsky, R.M., & Meaney, M.J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews*, **11**, 65-76.

Sapolsky, R.M. (1995). Social subordinance as a marker of hypercortisolism. *Annals of the N.Y. Academy of Sciences*, **771**, 626-639.

Schulkin, J., Gold, P W., & McEwen, B. S. (1998). Induction of corticotropin-releasing hormone gene expression by glucocorticoid: implication for for understanding the states of fear and anxiety and allostatic load. *Psychoneroendocrinology*, **23(3)**, 219-243.

Siegel, G.J., Agranoff, B. W., Albers, R. W., & Molinoff, P B. (1996). *Basic Neurochemistry*. Fifth Ed. Raven Press, New York.

Simar, M R., Saphier, D. & Goeders, N. E. (1996). Differential neuroendocrine and behavioral responses to cocaine in lewis and fisher rats. *Neuroendocrinology*, **63**, 93-100.

Simonds, P E.(1974). The Social Primates. Eds: Dethier, V.G., and H. P Zeigler. Harper & Rowe Pubs.: New York, Chp 7, 151-68.

Sircar, R. & Kim, D. (1999). Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in fisher, lewis, and sprague-dawley rats. *The Journal of Pharmacology and Experimental Therapeutics*, **289(1)**, 54-65.

Smagin, G.N., Zhou, J., Harris, R. B. S., &. Ryan, D. H. (1996). Corticotropin-releasing factor antagonist infused into the locus coeruleus attenuates immobilization stress-induced increase of norepinephrine in the prefrontal cortex in rats. *Society for Neuroscience*, **22**, 464(#184.1).

Sokoloff, G, & Blumberg, M. S. (2001). Competition and cooperation among huddling infant rats. *Developmental Psycholobiolgy*, **39(2)**, 65-75.

Sorg, B.A., & Kalivas, P W. (1993). Behavioral sensitization to stress and psychostimulants: role of dopamine and excitatory amino acids in the mesocorticolimbic system. *Neurosciences*, **5**, 343-350.

Sorg, B.A. (1992). Mesocorticolimbic dopamine systems: cross-sensitization between stress and cocaine. *Annals of the N.Y. Academy of Sciences*, **654**, 136-143.

Spear, L.P (1994). In: Weiss, B., & O'Donoghue, J. (Eds.)
Friedman, D., and D.H. Clouet (Eds.) Issues in developmental toxicology. *NIDA Research Monograph*, **164,** *59-69 (1994).*

Spear, L.P (1987). In: Friedman, D., & Clouet, D.H. (Eds.) Drug-vunerable events in neuronal development: implications for the selection of test methodologies. The role of neuroplasticity in the response to drugs. *NIDA Research Monograph* **78**, 30-43.

Stanton, M.E., & Levine, S. (1988). Maternal modulation of infant glucocorticoid stress responses: role of age and maternal deprivation. *Psychobiology*, **16(3)**, 223-228.

Sternberg, E.M., & Licinio, J. (1995). Overview of neuroimmune stress interactions. *Annals of the N.Y. Academy of Sciences*, **771**, 371.

Stenzel-Poore, M.P , Heinrichs, S. C., Riveist, S., Koob, G. F., & Vale, W. W. (1994). Over-production of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *Journal of Neuroscience*, **14(5;1)**, 2579-2584.

Storrs, E., & Williams, R. J. (1968). A study of monozygous quadruplet armadillos in relation to mammalian inheritance. *Proc. Nat. Acad. Sci.*, **60**, 910-914.

Stohr, T., Szuran, T., Welzl, H., Pliska, V., Feldon J., & Pryce, C. R.(2000). Lewis/fisher rat strain differences in endocrine and behavioural responses to environmental challenge. *Pharmacology Biochemestry and Behavior*, **67(4)**, 809-19.

Stratakis, C.A., & Chrousos, G. P (1995). Neuroendocrinology and pathophysoilogy of the stress system. *Annals of the N.Y. Academy of Sciences*, **771**, 1-17.

Suchecki, D., Nelson, D. Y., VanOers, H., & Levine, S. (1995). Activation and inhibition of the hypothalamic-pituitary-adrenal axis of the neonatal rat: effects of maternal deprivation. *Psychoneuroendocrinology*, **20(2)**, 169-182.

Suchecki, D., Rosenfeld, P & Levine, S. (1993). Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: the roles of feeding and stroking. *Developmental Brain Research*, **75,** 185-192.

Sun, N., Yi , H., & Cassel, M. D. (1994). The distribution of GABAergic interface between cortical afferents and brainstem projection neurons in the rat central extended amygdala. *Journal of Comparative Neurology*, **340(1)**, 43-64.

Tache, Y., & Rivier, C. (Eds.) (1995). Corticotropin-releasing factor and cytokinese:role in the stress response. In: The hans selye symposium on neuroendocrinology and stress. *New York:Academy Press.*

Takahashi, L.K., &. Kim, H. (1994). Intracranial action of corticosterone facilitates the development of behavioral inhibition in the adrenalectomized preweanling rat. *Neuroscience Letters*, **176**, 272-276.

Takahashi, L.K. (1994). Organizing action of corticosterone on the development of behavioral inhibition in the preweanling rat. *Developmental Brain Research*, **81**, 121-127.

Tan, X. X., & Costa, L. G. (1995). Long-lasting microencephaly following exposure to cocaine during the brain growth spurt in the rat. *Brain Research: Developmental Brain Research*, **84(2)**, 179-184.

Tilders, F.J.H., Schmidt, E. D., & De Goeij, D. C. E. (1993). Phenotypic plasticity of CRF neurons during stress. *Annals of the N.Y. Academy of Sciences*, **697**, 39-51.

Trent, F., Nakamura, S., & Tepper, J. M. (1991). Amphetamine exerts anomalous effects on dopaminergic neurons in neonatal rats in vivo. *European Journal of Pharmacology*, **204**, 265-272.

Turkewitz, G. (Ed.) (1992). Developmental psychobiology. In: Developmental Psychobiology, *Annals of the N.Y. Academy of Sciences*, 662, vii-ix.

Turkewitz, G., & Kenny, P A. (1985). The role of developmental limitations of sensory input on sensory/perceptual organization. *Journal of Developmental and Behavioral Pediatrics*, **6**, 302-306.

Turkewitz, G., & Devenny, D.A. (1993). Timing and the shape of development. In: Turkewitz, G. and Devenny, D.A. (Eds.), Developmental time and timing, (1-12). New Jersey:Lawrence Erlbaum

Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: a comprehensive reviw of drug effects, recent progress and new issues. *Progress in Neurobiology*, **56,** 613-672.

Valle, F. P (1970). Effects of strain, sex, and illumination on open-field behavior of rats. *American Journal of Psychology*, **83,**103-111.

Vanderschuren, L.J., Stein, E. A., Wiegant, V. M., & VanRee, J. M. (1995). Social isolation and social interaction alter regional brain opioid receptor binding in rats. *European Neuropsycholpharmacology*, **5(2)**, 119-27.

Vanderschuren, L. J., Schnmidt, E.D., De Vries, T. J., Van Moorsel, C. A., Tilders, F. J., & Schoffelmeer, A. N. (1999). A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. *Journal of Neuroscience*, **19(21)**, 9579-86.

Vasquez, D. M. (1998). Stress and the developing limbic hypothalamic-pituitary-adrenal axis. *Psychoneuronendocrinology*, **23(7)**, 663-700.

Vasquez, D. M., Eskandari, R., Phelka, A. & Lopez, J. F. (2003). Impact of maternal deprivation on brain corticotropin-releasing hormone circuits: prevention of CRH receptor-2 mRNA changes by desipramine treatment. *Neuropsychopharmacology*, **28(5)**, 898-909.

Walker, C.D., M. Perrin, W. Vale and C. Rivier (1986). Ontogeny of the stress response in the rat: role of the pituitary and the hypothalamus. *The Endocrine Society*, **118(4)**, 1445-1451.

Walker, C.D., Scribner, K. A., Cascio, C. S., &. Dallman, M. F. (1991). The pituitary-adrenocortical system of neonatal rats is responsive to stress throughout development in a time-dependent and stressor-specific fashion. *Endocrinology*, **128(3),** 1385-1395.

Walsh, R. N., & Cummins, R. A. (1976). The open field test: a critical review. *Psychological Bulletin*, **83(3)**, 482-504.

Wei, R., Listwak, S. J., & Sternberg, E. M. (2003). Lewis hypothalamic cells constitutively and upon stimulation express higher levels of mRNA for pro-inflammatory cytokines and related molecules: comparision with inflammatory

resistant Fisher rat hypothalamic cells. *Journal of Neuroimmunol.*, **135(1-2)**,10-28.

Weiner, H. (1985). Perturbing the organism: the biology of stressful experience.

Weinstock, M. (1997). Does prenatal stress impair coping and regulation of hypotholomic-pituitary-adrenal axis?. *Neuroscience and Biobehavioral Review*, **21(1)**, 1-10.

Whitnall, M. H., & Smallridge, R. C. (1997). Altered thyroid axis function in lewis rats with genetically defective hypothalmic crh/vp neurosecretory cells. *Endocrine Research*, **23(4)**, 365-376.

Widmaier, E .P (1990). Changes in responsiveness of the hypothalamic-pituitary-adrenocortical axis to 2-deoxy-d-glucose in developing rats. *Endocrinology*, **126(6)**, 3116-3123.

Wolffgram, J., & Heyne, A. (1995). From controlled drug intake to loss of control: the irreversaible development of drug addiction in the rat. *Behavioral Brain Research*, **70(1)**, 77-94.

Zimmerberg, B., & Shartrand, A. M. (1992). Temperature-dependent effects of maternal separation on growth, activity, and amphetamine sensitivity in the rat. *Developmental Psychobiology*, **24(3)**, 213-226.