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Pre-pulse inhibition (PPI) is a tool that may be used to identify how early life stress can result in a deficient adult nervous system (as represented by a deficit in sensorimotor gating). Since both animals and humans demonstrate a PPI, animal research on PPI can be used to model the relation of the early social environment to later susceptibility to maladaptive adult behavioral phenotypes. The current study examined the effect on adult PPI of early life stress in C57BL/6 offspring reared under four social conditions: Animal-Facility Reared (Control), Early Handling (EH, daily 15 min separation), Maternal Separation (MS, daily 4 hr. separation from dam) and Maternal Peer Separation (MPS, daily 4 hr. separation from dam and of littermates); and two post weaning housing conditions: Socially Housed (SH, 2-3 individuals/cage) and Social Isolation (IH, 1 individual/cage). Four different PPI types; 0, 76, 80, or 84 dB; each 20ms duration, and a startle stimulus of 120 dB, 40ms duration, were presented and the percentage reduction of the startle response that occurred with a prepulse in comparison to the startle response that occurred without a prepulse (i.e., 0 dB prepulse) was calculated. The results indicated that EH subjects displayed lower levels of PPI and ASR than AFR, MS & MPS offspring. The post weaning manipulation did not affect display of PPI or the ASR. Consistent with the human and animal literature, male mice displayed a greater ASR and PPI of the ASR than females.

MANIPULATION OF THE PRE- AND POST- WEANING SOCIAL ENVIRONMENT AND ITS EFFECTS ON PREPULSE INHIBITION OF THE ACOUSTIC STARTLE RESPONSE IN C57BL/6.

by

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Approved by

_____

Committee Chair

This thesis is dedicated to my mother who has always encouraged and believed in me. It is also dedicated to my father, the silent but present advocate. Lastly, I dedicate this work to my wife - without her, none of this would have been possible.

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of the Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Background

Sechenov (1863) was the first to describe inhibitory modification of a reflex. He found that presentation of midbrain stimulation (electrical or chemical), presented prior to a tactile stimulus, inhibited the cutaneous flexor reflex in the frog. He also found that the withdrawal response in humans to an acid bath was inhibited by a tactile pre-stimulation of "tickling".

In 1939, Peak demonstrated that inhibitory modulation of a startle reflex can occur in the auditory system of humans. She found that the presentation of two acoustic stimuli interspersed at an interval of 177ms inhibited reaction to the second stimuli by 25%. Hoffman and Fleshler (1963), using six male Wistar rats (tested around postnatal day (PND) 150), were first to report suppression of the startle response by pulsed background noises. They found that a constant background noise of 85dB doubled the startle amplitude (from 4mm to 8mm), whereas a pulsed background noise (500ms off, 500 ms on) was sufficient to suppress the startle amplitude by about 80% (from 8mm to 1.6mm). These changes were calculated by comparing the average response across individuals to a startle stimulus for the pulsed, constant and silence conditions. These

effects were specific to the temporal interval in which the stimuli were presented such that startle or inhibition of startle was only observed within the ten second interval between presentations of stimuli. However, whereas the effect of increased startle (pulsed background noise) or decreased startle (constant background noise) was present across the thirty respective trials, the overall effect (increased or decreased startle) diminished (albeit not statistically significant) as the number of trials increased. This indicated that some form of habituation may have occurred.

Finally, examination of whether the inhibition of the startle reflex by the pre-stimulation was specific to the acoustic modality revealed that the same effect was not observed when the prepulse stimuli were presented in the visual domain. Although Hoffman and Fleshler (1963) were the first to explore the effect of pulsed vs. steady acoustic stimuli on startle evoked in the same modality, the actual mechanism via which this phenomenon proceeds was not examined.

In 1965, Hoffman and Searle, using five male Wistar rats (tested around PND 200), demonstrated that attenuation of the startle response to acoustic stimuli can occur only if a preceding single noise pulse is presented 20-500ms before the startle stimulus onset. They posited that since this phenomenon appears to be dynamic and is sensitive to sound of similar levels as those found in the organism's life, it may reflect a general mechanism in all organisms that is more or less always operating, even though startle rarely occurs. Evidence that inhibition of the startle response does not require that the prepulse stimulus be acoustic came from both Buckland et al. (1969) (using four male, four female albino rats, tested around PND 130) and Pinckney (1976) (using 44 male

albino rats, tested around PND 120-150). These studies demonstrated that the acoustic startle response is also inhibited by tactile or visual prepulses. Ison and Hammond (1971) were the first to label this phenomenon as Prepulse Inhibition (PPI).

What is Prepulse Inhibition?

The startle response, also called startle or alarm reaction, can be defined as an unconditioned reflexive response of an organism to a sudden onset of a relatively intense environmental stimulus. This startle reflex can occur in response to a variety of environmental stimuli across multiple modalities, including auditory, tactile and visual. The reaction includes physical movement away from the stimulus, a contraction of the muscles of the arms and legs, and often blinking. It also includes changes in blood pressure, respiration and breathing. These reactions generally resolve themselves in a matter of seconds.

Prepulse Inhibition of the startle response is a neurological phenomenon that is "a measure of the inhibitory function of time-linked information processing" in neural functioning, in which a weaker sensory stimulus (the prepulse) inhibits the reaction of an organism to a subsequent strong and typically startling stimulus (the pulse). This "inhibition" is a direct function of the sequence, timing and duration of the presentation of the prepulse stimulus relative to the startling stimulus.

The reduction of the amplitude (size of the movement) of the startle response reflects the ability of the nervous system to temporarily alter processing of a strong sensory stimulus when a preceding weaker signal occurs. It has been proposed that this

inhibition can serve as an adaptive function by preventing an overflow of information to the brain. That is, the time taken for the brain to process the prepulse (30-500ms) will result in a diminution of processing the quickly followed pulse. However, it is not clear how such diminution is adaptive. Some propose that this diminution allows for attention to be directed to the more salient information of a stimulus laden environment. Graham et al. (1975), proposed a protection-of-processing theory of PPI. He argued that low-intensity changes in a sensory stimulation lead to a short-lived detection reaction which results in the triggering of a gating mechanism that prevents reactions to, or processing of, stimuli until the lead stimulus has been processed.

PPI is detected in numerous species ranging from mice to humans and is a relatively robust and stereotyped phenomenon. Although the PPI affects numerous neural systems such as the dopaminergic, glutamatergic and serotonergic systems, the most easily measured responses are the muscular reactions of the startle reflex, which are normally diminished as a result of the PPI.

PPI is often used as an operational measure of sensorimotor gating. Sensorimotor gating may be defined as "the state dependent regulation of the transmission of sensory information to the motor system" (Nausbaum & Contreras, 2004). Since the PPI inhibits the motor response to the intense stimulus, it is a form of sensorimotor gating. In humans, sensorimotor gating also describes the ability of the individual to "screen out" or "gate" from awareness excess or trivial stimuli so that attention can be focused on the more salient aspects of a rich stimulus environment (Braff, Geyer & Swerdlow, 2001).

Whether the ability to "efficiently gate" extraneous auditory stimuli confers any adaptive value to an organism is currently unknown. No research has been conducted that directly examines the ecological relevance of PPI. However, it is generally presumed that PPI must represent some aspect of the general mechanism that governs the neural processing relative to "attention" and the orientation to particular stimuli in the individual's environment. Therefore, PPI of startle in rodents is used as a model for human attention processes primarily because much of the underlying neural circuitry mediating this response is shared between both species.

The neural pathway of the startle response is well established. Acoustic startle stimuli are relayed to the cochlear nuclei; tactile stimuli are relayed to the trigeminal and dorsal columns; and vestibular startle stimuli are relayed to the vestibular nuclei. Acoustic and tactile stimuli signals are then relayed from the cochlear nuclei and the trigeminal and dorsal columns respectively, to the caudal pontine reticular nucleus (PnC) and then to spinal interneurons and motoneurons. This results in the elicitation of the startle response. Vestibular startle stimuli also follow this pathway, with the exception that there are projections *directly* from the vestibular nuclei to the caudal pontine reticular nucleus and to the spinal interneurons and motoneurons. It seems therefore that even though prepulse stimuli may come from various modalities, there is a convergence in the neural pathway such that the elicited startle reaction is mediated via signals from the caudal pontine reticular nucleus.

The neural pathway of PPI of the acoustic startle response is similarly well described. Carlson and Willot (1998) were one of the first to propose a neural model for

PPI of acoustic prepulses. They proposed that sensory signals from the acoustic prepulses are processed via the inferior colliculus nuclei (IC) and other auditory nuclei. These signals are then relayed to the caudal pontine reticular nucleus (PnC) which is a point of convergence of the PPI and startle pathways. These prepulses therefore inhibit processing of other stimuli, including startle. This occurs because the PnC becomes activated and processing of other stimuli is inhibited until the prepulse has been processed. Recall that the PnC facilitates reaction to startle as signals from the PnC are used to activate a motor response to startling stimuli. Since this circuit is actively processing the prepulse, it is unable to process extraneous stimuli and therefore "inhibition" of the processing of stimuli that quickly follow the prepulse occurs.

Fendt, Koch and colleagues (Fendt, Koch and Schnitzler, 1994; Koch and Schnitzler, 1997; Fendt & Fanselow, 1999; Koch 1999) added to this model by proposing that the IC also activates the superior colliculus (SC) which receives input from other modalities (visual and tactile). The anatomical connection between the SC and the pedunculopontine tegmental nucleus (PPTg) then activates an inhibitory cholinergic projection to the caudal pontine reticular nucleus to mediate PPI.

Contributions to PPI also include the laterodorsal tegmental nucleus (LDTg) and from the substantia nigra pars reticulate (SNR). Both the PPTg and the LDTg cholinergic neurons have ascending projections to the thalamus which lead to strong cortical activation. Furthermore, these neurons provide excitatory activation of the dopamine neurons in the SNR. The SNR plays an integral role in the inhibition of arousal and

exploratory behavior and the activation of the SNR via dopamine neurons results in the diminution of exploratory behavior.

The transient activation of these midbrain nuclei (SC, PPTg, LDTg and SNR) by PPI stimuli is converted into the longer lasting inhibition of the neurons of the caudal pontine reticular nucleus (PnC). Activation of these nuclei leads to cortical arousal and exploratory behavior. PPI is therefore mediated by a circuit involving contributions from the IC, SC, PPTg, LDTg, SNR & PNC. Disruption of any of these neural circuits by lesions has been shown to reduce or remove the response to startle and induces active exploration (approaching, sniffing) of novel and rewarding stimuli (Yeomans, 1995b).

Further evidence for the involvement of these nuclei (IC, SC, PPTg, LTDg and SNR) in the mediation of the startle response comes from electrical stimulation studies where it has been demonstrated that electrical stimulation of any one of these nuclei mimics the effects of a prepulse by reducing the startle response (Li et al., 1998b; Li and Yeomans, 2000; Saitoh, Tilson, Shaw and Dyer, 1987). Maximum diminution of startle using electrical stimulation is generally observed when the interstimulus interval (ISI) between electrical stimulation and the acoustic startle is between 12-30 ms in duration compared to acoustic prepulses which have longer latencies (4-40 ms).

In rodents, disruption in PPI of the startle response can also occur by stimulation of D_2 dopamine (DA) receptors with amphetamine, activation of the serotonergic systems by serotonin (5-HT) releasers or direct agonists at multiple serotonin receptors, and blocking of N-Methyl-D-aspartate (NMDA) receptors by drugs such as phencyclidine

(PCP). The most validated model of drug induced disruption of PPI is based on the effects of direct DA agonists (Geyer, Krebs-Thompson, Braff and Swerdlow, 2001).

PPI does not depend upon learning since it occurs on the first trial (Hoffman and Wible, 1970; Graham, 1975) and is clearly a neural reflex of unknown value for the system. Although PPI has been described as having no habituation because of its independence of the number and rate of prestimuli (Wu et al. 1984), habituation is observed if prepulses close to the background noise (2dB higher) are presented (Gewirtz and Davis, 1995). The amount of PPI is dependent on the intensity of and interval between prepulse and startle stimulus as well as the prepulse duration and modality, but is independent of the properties of the stimuli eliciting the startle (Stitt et al., 1976).

Whether prepulse inhibition invoked in the acoustic modality is subject to, or caused by, classical conditioning is one issue that has received little attention. As mentioned before, PPI occurs on the first trial and hence does not reflect learning (Graham, 1975). This suggests that conditioning is unnecessary as the display of PPI seems automatic and reflexive in nature. However, this does not preclude that the presentation of an acoustic prepulse stimuli within a fixed interval of the startling stimulus (generally between 20-500 ms) may result in a conditioned response.

Consider that in a typical acoustic PPI paradigm, a single startling stimulus and 3 to 5 prepulse stimuli are used. The interstimulus interval (ISI) and the intertrial interval (ITI) are randomly presented. Whereas the random ITI may prevent the acquisition of a conditioned response (due to variability and relatively long durations of time between trials), the ISI may not. The average ISI in a typical PPI paradigm is on the order of

milliseconds. If, for example, 3 acoustic prepulses and one startling stimulus are used with a maximum ISI of 1000 ms, then 3 out of any 4 trials (75%) will have a startling stimulus that is preceded within 1000 ms by an acoustic prepulse. If the PPI test consists of forty trials, then thirty of these trials will have this associative pairing. As such, over trials, the amount of PPI should diminish as the startle response should become a conditioned response. Direct evidence for this has been found in the human literature using the eyeblink PPI paradigm (Mordkoff and Barth, 2001) and from the animal literature (Gewirtz and Davis, 1995) in which there seems to be a diminution of PPI across trials. PPI therefore seems to be made up of two components; an initial automatic, reflexive one in the first few trials, and a conditioned one in the later trials. In particular, this conditioned response represents a form of classical conditioning known as trace conditioning, where the conditioned stimulus and the unconditioned stimulus are separated in time.

The magnitude of the acoustic startle response (ASR) can be increased using Pavlovian conditioning paradigms such as fear potentiation. Briefly, fear potentiation involves pairing of an initially neutral stimulus (e.g. light) with an aversive one (e.g. a footshock). After pairing, the light by itself is capable of eliciting the concert of behaviors (indicative of a "state of fear") that were formerly produced by the footshock. It has been demonstrated that presentation of the light (conditioned stimulus) with a loud acoustic stimulus elicits a greater ASR than would be present if a loud acoustic stimulus was presented by itself (Davis et al., 1993). Maximum potentiation of the ASR was observed at the interval in which the initial light shock pairings were conducted. For example, if

the light shock pairings were conducted using a 200ms interval, the greatest startle would be observed on testing at 200ms after the presentation of the conditioned light stimulus (Davis et al., 1993).

The observation that maximum potentiation is observed at the interval in which initial conditioning occurred has been interpreted as an "anticipatory response" of the animal to the aversive stimulus. The central nucleus of the amygdala has been demonstrated to be integral in the mediation of the potentiation of the ASR, and there are direct projections from this region to the caudal pontine reticular nucleus (PnC). Recall, transmission of sensory information to spinal motor and interneruons in the elicitation of the startle response occurs from the PnC. Lesions to the central nucleus block fear potentiated startle and electrical stimulation of the region markedly increases the startle amplitude (Davis et al., 1993).

If conditioning is occurring in the acoustic PPI paradigm, then a diminution of PPI or an increase in the acoustic startle response should be observed, similar to what is seen in other ASR paradigms using conditioning. As mentioned before, there is some evidence of this in the human literature using the eyeblink PPI paradigm (Mordkoff and Barth, 2001) and from the animal literature (Gewirtz and Davis, 1995) where there is a diminution of PPI and a small increase in startle across trials. No research has directly looked at conditioning in the acoustic PPI paradigm and its effects on startle. As such, any interpretations from these associations should be made with caution.

Why study Prepulse Inhibition?

PPI is highly conserved among vertebrates and is one of the few paradigms in which humans and animals are tested in similar fashion. Because it is argued that the PPI reflects sensorimotor gating and may be related to attention, animal models may be used to examine the pharmacology and neurology of attention. Moreover, because PPI is highly conserved across species, many inferences related to neural regulation of PPI that have emerged from animal studies have been supported by behavioral studies of PPI in humans.

Considerable evidence supports a high degree of homology between measures of PPI in rodents and humans. Deficits of PPI presumably represent an inability to filter out unnecessary information and abnormalities of sensorimotor gating. Similarly, deficiencies in latent inhibition are often observed in animals that display deficits in PPI. Latent inhibition refers to the process whereby pre-exposure to a stimulus disrupts later conditioning to that stimulus. This disruption is generally thought to represent deficiencies in selective attention or cognition. Interestingly, like PPI, disruption of latent inhibition is correlated with sensitivity to apomorphine such that as sensitivity to apomorphine increases, the degree of disruption increases (Ellenbroek and Cools, 1995). Such deficits in PPI and/or latent inhibition are noted in patients suffering from illnesses like Schizophrenia, Obsessive Compulsive Disorder, Huntington's disease, Attention Deficit Hyperactive Disorder, Tourette Syndrome, Post Traumatic Stress Disorder and Alzheimer's disease; and in people under the influence of drugs (Braff, Geyer & Swerdlow, 2001). Also, there is some evidence indicating that early life stress and

variations in maternal care can affect PPI (Geyer et al., 1993, Varty & Higgins (1995), Ellenbroek & Cools (1995).

Why might disruptions in parental care affect PPI?

A well investigated hypothesis posits that early life experiences, particularly exposure to early life stress, can exert an influence on later susceptibility to a variety of neuropsychiatric diseases, including but not limited to, schizophrenia (Cadenhead et al., 1993), obsessive compulsive disorder (Swerdlow et al., 1993a), Huntington's disease (Swerdlow et al., 1995c), Tourette's syndrome (Castellanos et al., 1996) and Alzheimer's disease (McCool et al., 2003). There is little understanding, however, of how early life stress may contribute to the neural abnormalities underlying these diseases. Of particular interest is that there is also a deficit in PPI observed in all of the aforementioned diseases. Models using rodents have proven to be highly useful in the elucidation of how these early life stressors can affect expressions of behaviors associated with these diseases, particularly schizophrenia. These behaviors include, but are not limited to, deficits in selective attention, arousal and exploration.

During post natal day (PND) 4 to 14, generally referred to as the stress hyporesponsive period (SHRP), rats show a reduced sensitivity to the activation of the Hypothalamic Pituitary Adrenal (HPA) axis by common stressors. This reduced sensitivity is characterized by decreased Adrenocorticotropic Hormone (ACTH) and corticosterone response to stress as well as adrenal insensitivity to ACTH (Rosenfeld et al., 1991; Suckecki et al., 1993; Suckecki et al., 1995, Sapolsky and Meaney, 1986). One

means of overcoming SHRP is the application of a severe stressor, such as maternal-offspring separation and early handling (Levine, 1994) (see next section for description). Hence, it is possible that disruption of maternal care may have an impact on the developing neural circuitry implicated in the manifestation of PPI in offspring.

It has also been demonstrated that rat offspring that are maternally deprived (one 24 hour separation of dam from offspring) during the SHRP period show a stronger, prolonged and enduring increase in plasma glucocorticoid levels when compared to control rats that are not separated from the dam but receive identical animal husbandry procedures (Rots et al., 1996). Also characteristic of these maternally deprived offspring is increased baseline levels of corticosterone (CORT), adrenocorticotrophin hormone (ACTH), and enlarged adrenals. Furthermore, there is evidence indicating that there is a reduction of mRNA for glucocorticoid receptors (GR) in the hippocampus as well as in the paraventricular nucleus and the pituitary (Rots et al., 1996).

Disruption of the dam offspring milieu has also been linked to modification of the developing neurotransmitter systems, particularly the dopaminergic system. For example, Ellenbroek & Cools (1995) have demonstrated that maternal deprivation of rats (for 24 hours on PND 3 or 9) enhances the behavioral response (enhanced sensitivity to apomorphine induced gnawing and disruption of latent inhibition) to dopamine agonists such as apomorphine (Ellenbroek and Cools, 1995). Furthermore, amphetamine reduces the behavioral response to the dopamine antagonist haloperidol (Zimmerberg and Shartrand, 1992; Gallegos et al., 1990). PPI can also be disrupted by dopaminergic

agonists (Ellenbroek and Cools, 1996) and maternal deprivation can lead to deficits in PPI in rat offspring as adults (Ellenbroek et al. (1998).

Socially isolated (SI) animals (housed singly/cage post weaning), similarly to offspring separated from the mother during the SHRP period, show elevated plasma glucocorticoid levels. Isolation housing has also been posited to contribute to mesoaccumbal dopamine hyperactivity (as evidenced by increased extracellular dompamine in the nucleus accumbens following corticosterone administration) (Deroche et al., 1994; Piaza et al., 1996). Interestingly, SI rats also show deficits in PPI when compared to socially housed rats (housed two-three same sex animals/cage). It is hypothesized therefore that maternally separated offspring that have high levels of circulating corticosterone will also have high levels of extracellular dopamine (Choy & Buuse, 2008). Also, as with socially isolated rats, maternally separated rats should display deficits in PPI since high circulating levels of corticosterone increases extracellular dopamine which disrupts PPI (Choy & Buuse, 2008; Eells, Misler & Nikodem, 2006).

The different types of disruptions of parental care

The effect of the manipulation of the mother-infant relationship in rodents is one that has been well studied and has been used as a model for understanding the development of emotional regulation in humans (e.g., Gunnar et. al, 1995; Diamond, 2001). The paradigms most commonly used to study the developmental effect of early life experiences are early handling (EH), maternal separation (MS) and maternal peer

separation (MPS). These three paradigms involve separation from the parents for different contiguous blocks of time and sometimes separation from the littermates as well.

Early handling (EH) refers to the procedure in which offspring are picked up by the experimenter and separated from the dam for approximately three to fifteen minutes during postnatal days one to fourteen. Maternal separation (MS) involves removal of the dam from the littermates for from three to six hours during postnatal days one to fourteen. Maternal peer separation (MPS) involves separation of the dam from the littermates, and the littermates from one another, for three to four hours during postnatal days one to fourteen. Control or comparison groups are animal facility reared groups (AFR) or Non-Handled (NH)/Early Deprivation (ED) groups. AFR groups do not undergo the separation procedure but experience cage changes weekly, similar to that of the other three manipulation groups previously mentioned, whilst the NH/ED groups neither undergo the separation procedure nor experience weekly cage changes. Some have argued that the NH/ED group is not a control group but rather an unusual "experimental" condition in which dam and offspring are never separated (Levine, 2000).

The literature demonstrates that both the early handling (EH), maternal separation (MS), and maternal peer separation (MPS) procedures have profound effects on the behavioral, hormonal, neuroanatomical and molecular development of the offspring (e.g., Fenoglio et al., 2004; Levine, 2000; Plotsky & Meaney, 1993; Sanchez, Ladd, & Plotsky, 2001) as compared to an Animal Facility Reared (AFR) or a Non-Handled (NH) control

group. For example, the brief handling experience (EH) makes rodents less reactive to stressors as adults.

The effects of disruption of parental care on "Anxiety-Like" behaviors

Rodents' reactions to stressors are generally determined by their behavioral response to novelty. They are classified as high anxious when they: 1) are immobile in a novel situation; 2) display increased latency to approach novel stimuli and to withdraw from familiar stimuli; 3) display decreased latency to approach familiar stimuli and to withdraw from novel stimuli. Conversely, low anxious mice exhibit the opposite reactions. The two most widely used behavioral tests of anxiety in rodents are "the open field" and "the elevated plus maze" tests. These tests have been pharmacologically validated and are considered both reliable and valid measures of anxiety and depression like behaviors in rodents (Rodgers et al., 1997; Sheperd et al., 1994; Pellow and File, 1986). Effects of early experience and the determination of anxiety are usually measured by comparison to the Animal Facility Reared or Non-Handled groups.

In the open field, the rodent is placed in the center of an open area (a 45 square inch base) (Levine, 1967) and the time spent in the center vs. the periphery is measured. More time spent in the center (more light and more open) of the field is indicative of less anxiety for a nocturnal animal. Increased defecation, grooming and more time spent in the periphery of the open field is indicative of greater anxiety.

The elevated plus maze consists of four arms, two of which are open platforms and two of which are enclosed with walls. Time spent in open and closed arms, as well as

entry into the arms, are usually recorded. Low anxiety is characterized by increased activity and entry into the open arms, whereas high anxiety is characterized by more time spent in the closed arms with low entry rates into the open arms and increased defecation and grooming (Carola et al., 2002).

The zero maze consists of an elevated annular platform with two opposite closed and open arms (no center area), allowing for uninterrupted exploration. It represents a modification of the elevated plus maze model of anxiety for rodents. This apparatus was developed to eliminate the ambiguity that was associated with time spent in the center square of the elevated plus maze, as it had been demonstrated that mice spend between 20-30% of the test period in the center square (Lee and Rodgers 1990; Rodgers et al. 1992b). Like the elevated plus maze, the elevated zero maze has been validated, and generates a clear and consistent behavioral profile in rats treated with antianxiolytics (Sheperd et al., 1994). As such, the zero maze arguably represents a better behavioral measure of "anxiety-like" behavior in rodents than the elevated plus maze.

Generally, studies have shown that EH results in rats that are more exploratory and less anxious (Levine, 1957; Levine, Haltmeyer, Karas & Denenberg, 1967). For example, Meerlo et al. (1999) demonstrated that EH rats that were handled from PND 1 to 21 for 15 minutes a day, with each pup isolated from the other, were less anxious (as defined by more time spent in the open arms of the zero maze and less time spent in the closed arms) than AFR rats.

However, studies of EH on anxiety in performance in the open field are more variable (Levine et al., 1967; Meerlo et al., 1999, Roy & Chapillion, 2004). Levine et al.

(1967) compared an EH group that was separated three minutes/day on PND 1 to 20, to a NH group and performed behavioral testing on the open field on PND 80. The NH group showed a decline in activity on days 2 to 4 of testing. On days 2 to 4, the EH animals were more active (significant) than the NH animals. By day 4, this significant difference disappeared.

Meerlo et al. (1999) found no difference between AFR and EH animals in total distance traveled over 5 minutes and time spent in the center of the field versus the periphery of the field between groups. Roy and Chapillion (2004) replicated Levine's study and found similar results. However, they found that EH animals spent more time motionless on day 3, which was not observed by Levine.

There is some evidence indicating that maternal separation (MS) produces effects that are opposite to EH (Huot et al., 2001). Huot et al. (2001) compared three groups; EH (15min/day separation), MS (3H/day separation) on PND 2 to 14, and a control group that was not handled (NH). It was observed that MS animals displayed more anxiety-like behaviors (as defined by more time spent in the closed arms of the zero maze) than EH and NH. There was no difference between the EH and NH animals. Generally, MS animals separated 3H/day for at least 10 days tend to be more anxious than NH and AFR groups. Shorter durations of parent-offspring separation associated with EH attenuates anxiety-like behaviors in response to novelty.

Some have argued (Lehmann, Stohr and Feldon, 2000) that the differences across research studies on the effects of these dam-offspring separation manipulations are a result of the variability in the methodology used. For example, when an AFR group is

used as a control (versus the use of a NH group) the effect of handling that occurs with AFR animals may affect the results. Levine et al. (1967) demonstrated that AFR and EH rats have similar outcomes (less anxiety) due to the effect of handling that both groups receive.

Post-weaning housing can also affect anxiety like behaviors. Generally, offspring are paired with a same sex conspecific from the litter. This again is variable. For example, Meerlo et al. (1999) chose to house their offspring 6/cage up until PND 90-120. Animals were separated one/cage one week before testing. The additive effects of social housing, followed by social isolation for one week, may have affected the results of testing.

Finally, there is some discrepancy in the literature pertaining to separation procedures. For example, EH generally occurs on PND 1-14 for 15 min/day. Some researchers (e.g., Meerlo et al., 1999) further isolate the pups from each other during the separation. Likewise, MS generally involves separation of the pups on PND 1-14 for 3-6 H/day. Millstein & Holmes (2006) in their procedure further separated the pups from each other during this time. This procedure has been coined MPS and has been shown to produce different results from the originally described MS group.

Interestingly, the effects of the separation procedures on adult emotionality appear to be mediated by their influence on parental behaviors. This indicates that the physical and social stimuli associated with parental care can directly modulate gene expression and protein synthesis in the nervous and endocrine systems of the offspring, and subsequently affect their neurobiological, physiological and behavioral phenotypes,

including gene expression (cf., Avishai-Eliner et al., 2001; Fenoglio et al., 2004; Meaney et al., 1996).

There is some evidence that longer periods of separation, as found in the maternal separation (MS) paradigm, results in effects that are opposite to those found in the EH paradigm. For example, Huot et al. (2001) found that MS rat offspring as adults were more anxious (as demonstrated by more time spent in the closed arms of the zero maze and more time spent in the periphery of the open field) when compared to EH offspring. Romeo et al. (2003) reported similar differences between MS mice and Animal Facility Reared (AFR) mice, where MS mice were more anxious in their exploratory behavior in the open field.

Variations in the maternal separation paradigm have also been conducted with similar results when the separated offspring are compared to offspring from AFR control group. For example, Barna et al. (2003) demonstrated that when rat pups are removed from the dam for twenty-four hours on PND 9 (also known as Single MS or Maternal Deprivation) and tested later as adults, they demonstrate more "anxious" behavior in the open field. When rat offspring are separated from the dam and also separated from each other repeatedly on PND 2-14 (Maternal Peer Separation) and tested as adults, they too display more anxious or reactive behavior in the open field (Daniels et al., 2004; Romeo et al., 2003).

The effects of disruption of parental care on HPA functioning

Any change in behavior has a corresponding change in physiology. It is no surprise therefore that the behavioral changes prompted by EH, MS and MPS manipulations are associated with a change in physiology. The activity of the Hypothalamic-Pituitary-Adrenal Axis (HPA Axis) is one of the most studied of these changes. Sometimes termed the "flight or fight response system", the HPA axis has high responsiveness to any situation that seems to be potentially dangerous to the individual. In these situations, the HPA axis is activated and corticosterone (CORT) is released from the adrenal glands. CORT circulates throughout the individual's system and affects the function of many organs and tissues. Many of the consequences of the actions of CORT increase the amount of available energy to the body to cope with a dangerous situation. In many instances, a situation is treated as dangerous although there is little likelihood of physical damage to the individual. These situations often are labeled as "stressful" because they elicit activity in the individual's HPA axis similar to that elicited by a potentially physically harmful situation. The specifics of the situation might be called a "stressor".

EH has been shown to attenuate the adult HPA response to such "stressful" situations. EH animals have low CORT secretions when placed in a novel environment like an open field (typically a stressful situation). This indicates that EH makes these situations less stressful by altering the thresholds of the nervous system. Conversely, it has been argued by Meaney et al. (1994) that MS has made the situation more stressful, thereby providing the animal with a nervous system with low thresholds for stimulation

of HPA activity. He demonstrated that handling of rat offspring for 15 minutes/day during PND 1-21 resulted in offspring with decreased levels of plasma CORT and ACTH response to restraint. Conversely, separation of dam from offspring for 3 hours or 6 hours daily during PND 1-14 resulted in offspring that displayed increased plasma CORT and ACTH response to restraint or novelty as adults when compared to Non-Handled Controls.

The effects of disruption of parental care on PPI

There is contrasting evidence in the literature which assesses the effects of disruption of parental care on development of adult PPI (Ellenbroek & Cools, 1995, 2000, 2002b; Ellenbroek et al., 2004; Finamore & Port, 2000; Lehmann, Pryce & Feldon, 2000; Varty & Geyer, 1998; Pryce, Bettschen, Bahr & Feldon, 2001). Some (Lehmann, Pryce & Feldon, 2000, Pryce, Bettschen, Bahr & Feldon, 2001) report no effects of disruption of parental care on PPI whereas others (Ellenbroek & Cools, 1995, 2002b; Ellenbroek et al., 2004; Finamore & Port, 2000) report that disruptions of parental care diminish PPI. Yet others (Varty & Geyer, 1998, Ellenbroek & Cools, 2000) demonstrate that the disruption of PPI is strain specific.

For example, Ellenbroek et al. (1998) looked at the effects of a 24 hour maternal separation (MS, also known as maternal deprivation or single MS) on either PND 3, 6, or 9 in Wistar rats. They found that regardless of the day on which the separation occurred, a reduction of PPI was observed in offspring tested as adults. The comparison control group received the same animal husbandry but no manipulation. Interestingly, a greater

deficit (although not significantly different) also was observed to result from separation for the oldest age pups. Lastly, they found that treatment of the subjects with either haloperidol or quetiapine (antipsychotics) fifteen minutes before testing was sufficient to eliminate the disruption in PPI (subjects showed similar levels of PPI as the control group).

Lehmann, Pryce & Feldon (2000) challenged the finding that a 24 hour maternal separation (on either PND 4, 9, or 18) results in deficits in PPI by replicating the previous study with one key difference – the offspring tested (separated by sex - see below) were from separate litters. They noted that the treatment of two pups per litter as independent almost triples the likelihood of obtaining a statistically significant effect. They bred forty-eight litters, repeated the maternal deprivation procedure by Ellenbroek et al. (1998), and randomly selected one male and one female offspring to test on PPI. It was found that the maternal separation manipulation did not have an effect on PPI of acoustic startle. These results are converse to those reported by Ellenbroek et al. (1998). However, since the Ellenbroek et al. (1998) study used several animals from the same litter (and did not control for litter effects), their results should be interpreted with caution.

The effect of maternal deprivation on PPI has also been demonstrated to vary based on the strain of rodent used. Ellenbroek and Cools (2000) extended the study published by Ellenbroek et al. (1998) by using three rat strains (Wistar, Fischer 344 and Lewis). Other than the use of two additional strains, the methodology employed was identical to their previous study (with the failure to control for litter effects). They found that the basal startle amplitude responses amongst these three strains were different (in

control animals). In addition, the startle response in Lewis rats (1666) was greater than twice that of both Fischer 344 (704) and Wistar rats (612). Recall that the units of startle amplitude are arbitrary, and represent the difference in movement of the chamber between the baseline (background noise) and the startle stimulus. With regards to prepulse inhibition, they replicated their previous findings that maternally deprived Wistar rats display deficits in PPI as adults. They found that maternal deprivation had no effects on PPI in Lewis rats which was consistent with previous findings (Varty & Geyer, 1998; Lipska & Weinberger, 1995). They also determined that Fischer 344 rats were susceptible to the effects of maternal deprivation, albeit to a lesser extent than Wistar rats. These results are again consistent with previous findings (Varty & Geyer, 1998; Lipska & Weinberger, 1995).

It seems, therefore, that disruption of PPI via application of disruption of dam offspring milieu does depend to some degree on the strain of rodent used. In 2006, Millstein et al. investigated the effects of repeated maternal peer separation in five inbred strains of mice (129S1/SvImJ, BALB/cByJ, C57BL/6, DBA/2J and FVB/NJ). In all strains, MPS, EH and AFR produced no significant effect on PPI. The different strains, however, did display differences in baseline levels of PPI. These baseline results of strain differences are consistent with the previous research of Paylor and Crawley (1997) who also investigated the effects of strain differences on PPI.

Of importance to note in the study by Millstein et al. (2006) was the type of manipulation used (in their paper, the maternal peer separation (MPS) procedure was termed maternal separation). It has been demonstrated that although MPS procedure may

seem to be more stressful to the HPA system than MS or EH conditions, it has been reported to be less detrimental (Pryce, Bettschen, Bahr & Feldon, 2001; Rees, Steiner, & Fleming, 2006). In fact, the literature demonstrates that offspring reared as EH, AFR & MPS are generally less "anxious" or reactive as adults and display a blunted CORT response to restraint and novelty. Therefore, it may not be surprising that in the study by Millstein et al. (2006) there was no significant effect of rearing condition on PPI as generally, these groups look quite similar with respect to their CORT response. Recall that the primary argument of why rearing condition would affect PPI lies in the fact that as CORT increases, dopamine secretion increases. This disrupts the neural circuitry of PPI and results in active exploration and arousal rather than "selective attention" to the stimulus.

Similarly in 2001, Pryce et al. (2001) compared the effects of EH (15min/day separation), MPS (Pryce et al. labeled this manipulation Early Isolation) (3H/day separation) on PND 1-21, and NH on PPI in rats. Not surprisingly, they found no effects of the pre-weaning rearing condition on PPI. Based on a literature review, no study has looked at the effects of repeated maternal separation (MS) on PPI. Research has been done involving only the examination of the effects of MPS, EH and AFR on PPI, and these studies show no effect of rearing condition on PPI. The present study will examine the effects of MS on PPI.

It has been demonstrated that maternally deprived (MD) offspring (single 24 hours separation performed during PND 1-14) look quite similar to MS offspring (repeated separation of dam from offspring for 3-6 hours/day on PND 2-14) with regards to their CORT response to novelty and stress – they are highly aroused and reactive.

Maternally deprived offspring show a deficit in PPI and it has been proposed that the elevated CORT response results in a cascade such that dopamine secretion increases, thus disrupting the neural circuitry of PPI. As such, it is hypothesized that Maternal Separation (MS) offspring will display deficits in PPI.

Confounds and Caveats in the Maternal Separation Literature

Although it has been demonstrated that separation of the dam from offspring can affect offspring development (with a "critical period" of PND 2-14 in which these separations produce differences in physiological and behavioral development), methodological confounds across laboratories prevent identification of the mediating mechanisms. For example, the longitudinal character of the pattern of responding of the dam upon reuniting with the pups has not been assessed. Rather, behavior sampled at intervals, which ignore sequence across the length of the application of the separation procedure, has been done (Boccia & Pedersen, 2001; Liu et al., 1997; Pryce, Bettschen, & Feldon, 2001). Recall that the main difference that is observed in these separation paradigms is that MS offspring display more anxiety-like behavior than EH and MPS offspring. The only difference between MPS and MS offspring is that the pups are separated from each other in MPS. Hence, differential maternal responding to these pups upon reuniting should mediate these patterns of responding. As such, the longitudinal character of this responding needs to be described.

In order to describe the longitudinal character of responding, care must be taken to remove other possible confounds which may change the pattern of responding other than the actual separation procedure. For example, pups that are separated from the dam but kept

together in a huddle (MS) are maintained at a higher temperature than pups that are separated from the dam and then further separated from each other (MPS). In the absence of a replacement (of the dam) thermoregulatory source, differential responding may in part be due to the temperature of the pups at reuniting rather than the application of the separation procedure. In fact, pups that are maintained at a higher temperature (e.g. MS offspring vs. MPS offspring) elicit differential behavior from the dam (Stern & Lonstein, 1996). There is a longer latency to begin crouching, and maintenance of quiescent nursing upon reuniting of separated pups maintained at a warmer temperature (39ºC) vs. nest temperature (34ºC) (Stern & Lonstein, 1996). In the literature, there is extensive variability in the temperature at which the pups are kept during separation from the dam, e.g., MS, 22-24ºC (Ellenbroek & Cools, 1995); Single MS, room temperature (Ellenbroek & Cools, 2000), EH, no temperature control (Avishai-Eliner et al., 2001), EH and MPS, 28-30ºC (Pryce et al., 2001).

Similarly, the time of day of behavioral testing of offspring as adults will affect behavior on these tests, particularly if this behavior is mediated by a hormone that follows a circadian rhythm, as is the case with corticosterone. The postnatal age of testing of offspring as adults will also affect behavioral responding. There is again extensive variability in these parameters in the literature. For example: MS, Open Field test at PND 16, 28 at 1600 hours (Zimmerberg & Shartrand, 1992); AFR, EH & MPS; Open-field test, elevated plus-maze test, light/dark exploration test, PPI, and forced swim test at PND56-84 – time of testing not specified, (Millstein & Holmes, 2006); EH, MS, AFR, defensive withdrawal test at PND 25-29 with time not specified, PPI at PND 42 and time not specified (Parfitt et al., 2004). Comparison of the effects of these separation

procedures on later behavioral outcome therefore becomes increasingly difficult as the test parameters differ widely across laboratories.

The role of the Post-Weaning Environment

Geyer et al. (1993) was one of the first to demonstrate that a post-weaning developmental manipulation can disrupt PPI. It was found that Sprague-Dawley and Lister Hooded pups reared in social isolation from weaning (PND 21) display deficits in PPI, as compared to socially reared rats, when both are tested as adults on PND 62.

The timing of this social isolation effect was investigated by Bakshi & Geyer (1999). They housed in isolation different groups of Sprague-Dawley and Lister Hooded rats for two weeks post weaning, four weeks post weaning or six-seven weeks post weaning. PPI testing occurred at the end of isolation housing for each group, respectively. Consequently, animals were tested for PPI at different levels of maturity. Comparison groups were socially housed (2-3 same sex/strain per cage) post weaning for equal durations of time. PPI disruption was observed in Sprague-Dawley and Lister rats that were isolated for four or six weeks but not two weeks.

These results may mean that the isolation rearing condition needs to be maintained continuously until puberty to produce an effect on PPI (Bakshi & Geyer, 1999). Once established, the effects are long lasting. This effect seems to be relatively specific to this age period since social isolation of adult rats for six weeks does not affect PPI.

Social isolation has also been shown to elevate extracellular dopamine in the nucleus accumbens, which has been linked to deficiencies in PPI. Increased locomotion is also observed in social isolation reared pups. Furthermore, Weiss et al. (2001) have reported that pups reared in social isolation have increased baseline levels of circulating corticosterone as compared to socially reared pups. Recall that an increase in circulating corticosterone leads to an increase in extracellular dopamine. This increase in extracellular dopamine has been linked to deficits that have been observed in PPI.

This disruption of PPI via social isolation post weaning has been demonstrated to occur in various strains of rat (F344, Lister, L-E, S-D & Wistar) and mice (C57BL/6, H1KO, ddY, 129T2) (Dai et al., 2004, 2005; Sakaue, Ago, Baba, & Matsuda, 2003; Varty, Powell, Lehmann-Masten, Buell, & Geyer, 2006; Varty & Geyer, 1998; Bakshi & Geyer, 1999; Weiss et al., 2001; Zimmerberg, Rosenthal & Stark, 2002). However, this association is not consistently reported across studies (Domeney & Feldon, 1998; Weiss, Di Iorio, Feldon, & Domeney, 2000; Weiss, Feldon, & Domeney, 1999).

As noted earlier, the effects of social isolation on later deficits in behavioral responding seem to be dependent on maintenance of the post weaning isolation condition to adulthood. The sensitive period ascribed for this effect occurs between PND 21 (weaning) and PND 49 in both rats and mice. Some have argued that this effect cannot be reversed by later resocialization as adults (Einon & Morgan, 1977) whilst others have reported the converse (Bakshi & Geyer, 1999). It seems therefore that social isolation post weaning and the associated deficits in PPI are variable at best and may be moderated by many factors including, but not limited to: the strain of rodent, the sex of the rodent,

the caging condition, husbandry procedures and the PPI parameters used at testing (cf. Weiss & Feldon, 2001).

There has been little or no research linking the pre-weaning and the post-weaning environment with respect to disruption of PPI. Furthermore, there has never been a study which simultaneously assessed the influence of the three main separation paradigms (MS, MPS, and EH) on the post-weaning social environment. This study will examine the influence of pre-weaning dam-offspring separation on the effect of post-weaning social isolation in the development of the adult PPI pattern.

C57BL/6 as a Model for the effects of disruption of Parental Care and Social Isolation on PPI

C57BL/6 has been used reliably as a model for investigation of the effects of early life stress (in the form of MS and EH) (cf., Romeo et al., 2003; Caldji et al., 2004; Parfitt et al. 2004; Millstein et al., 2006). Also, C57BL/6 generally has high levels of acoustic startle (1317 ± 121) and relatively low levels of PPI (36 ± 3) (Paylor & Crawley, 1997; Millstein & Holmes, 2006).

As noted above, there are no differences in PPI for C57BL/6 mice with respect to EH, MPS and AFR (Millstein & Holmes, 2006). This is consistent with other lines of evidence from work done with Wistar and Fisher rats, as reviewed previously. However, the effects of MS on PPI have not been examined. Furthermore, the simultaneous and sequential analysis of pre-weaning (MS, MPS, and EH) and post weaning (social housing - SH vs. social isolation - SI) manipulation on PPI has not been conducted for any strain

of rodent. Consequently, given the information with respect to baseline startle, PPI and expected outcome of three of our four manipulated groups, C57BL/6 represents a good strain for the investigation of the effects of disruption of maternal care followed by social isolation.

Hypotheses

It is hypothesized that:

1) MS offspring that are socially housed post-weaning should display a high level of startle and deficits in PPI when compared to socially housed AFR, EH and MPS offspring as adults.
2) Post-weaning socially isolated animals should display deficits in PPI regardless of the early rearing condition, with MS exhibiting the greatest disruption of PPI, and socially isolated AFR, EH and MPS offspring displaying similar levels of PPI disruption.

CHAPTER II

METHOD

Subjects

Subjects were C57BL/6 mice purchased from Harlan Laboratories. Animals were housed in 29x19x12cm polypropylene cages on a 14:10 light/dark cycle with lights on at 1400 hours. Temperature was kept at 21° C and humidity at 50%. All animals were provided with food, water and bedding of Harlan Aspen Sani-Chips approximately 1.3cm deep.

Sixty litters were bred and assigned via a pseudo-random manner to one of four groups described below (n = 15/group). Assignment was such that there was always a cohort of litters representing each of the four groups at any given time. The average litter size was six, with a minimum of four and a maximum of eight offspring. Four offspring (two male and two female) from each of the 60 litters were used on a different study - the consequence of the rearing environment on behavioral responses to novelty in the open field and zero maze - yielding a total of 120 males and 120 females. The **remaining** offspring of these litters were used in this project.

These offspring were assigned as follows: 9 Isolation Housed and 11 Socially Housed AFR Females; 5 Isolation Housed and 10 Socially Housed AFR Males; 3

Isolation Housed and 13 Socially Housed EH Females; 3 Isolation Housed and 11 Socially Housed EH Males; 11 Isolation Housed and 11 Socially Housed MPS Females; 6 Isolation Housed and 11 Socially Housed MPS Males; 10 Isolation Housed and 11 Socially Housed MS Females; and 11 Isolation Housed and 10 Socially Housed MS Males. Testing for pre-pulse inhibition occurred on PND 60-70, with testing beginning at 1400 hours (lights on) and completed by 1630 hours.

Early Experience Procedures

Dam-offspring separations occurred from postnatal day (PND) 2 to 14 (day of birth is PND 0). The dam was removed from the homecage and placed into a clean cage with bedding. Following this, pups were then removed from the homecage and placed into an adjacent clean cage with bedding. After pup removal, the dam was placed back into the home-cage for the duration of the separation.

Maternal Separation (MS) pups were separated from the dam for 240 minutes (between 0900 and 1300). Maternal and Peer Separation (MPS) pups were separated from the dam and their littermates for 240 minutes (between 0900 and 1300). Both MS and MPS pups were placed into a standard (29x19x12cm) polypropylene cage. For the MPS group, there were frosted plexiglass partitions within the cage to make 8 separate compartments, one for each pup (Millstein & Holmes, 2006). During the 240 minute separations (Groups MPS and MS), the pups cages were placed under an infrared heat lamp and maintained at 31°C (± 1°C) temperature in the nest to prevent cold stress. Pups

in the EH group were separated from the dam for 15 minutes in the same manner as the MS group (between 12:45-13:00) but were not placed under heating lamps.

For reunion, the dam was removed again from the homecage and placed into a clean cage with bedding (the same cage used previously), the pups were replaced into the homecage, and then the dam was replaced into the homecage. Holding cages and bedding for mothers and pups were not changed during the repeated separation period. An AFR control group was not separated from the dam, but received weekly cage changes like the other groups.

Weekly cage changes occurred when the pups were eight days old or on PND 07. The dam was removed and placed in a clean cage with bedding. A little soiled bedding from the home cage was sprinkled into a new cage and the nest from the homecage was relocated (same side/area) to this new cage. Pups were then individually placed in the relocated nest. The dam was then placed in the new homecage. This process took less than one minute.

Regular cage changes occurred on PND 7 and 14 between 1400 and 1500 hours. After PND 14, all litters were left undisturbed until weaning at PND 21. Upon weaning, subjects were either socially housed 2-3 subjects per cage with their same sex, same group siblings, or isolation housed. Cage changes continued to occur once per week thereafter.

Prepulse Inhibition Procedures

Startle response was measured using the SR-LAB (San Diego Instruments, San Diego, CA) startle response measurement system, including software (Blaszczyk, Tajchert, Lapo, & Sadowski, 2000; Bullock, Slobe, Vazquez, & Collins, 1997; Crawley, 1999; Crawley & Paylor, 1997; Logue, Owen, Rasmussen, & Wehner, 1997; Paylor & Crawley, 1997). In this system, a 3.9cm (outer diameter) acrylic cylinder for holding the mouse was mounted on a platform (20.4cm length x 12.7cm width x 0.4cm depth) with a piezoelectric accelerometer unit attached below the acrylic cylinder. The piezoelectric unit transduced vibrations created by mouse body movements into signals that were rectified and stored by a microcomputer and then converted into a signal proportional to response amplitude. The acrylic cylinder and platform were located in a sound-attenuated chamber with a loudspeaker (28 cm above the cylinder) and houselight.

Tested individually by one experimenter, each mouse was inserted into the SR-LAB test cylinder for a 5 min acclimation period, followed by blocks of startle response trials. When the prepulse (4 different PPI types; 0, 76, 80, or 84dB; each 20ms duration) were presented, the size of the startle response produced by the loud, startling stimulus (120dB; 40ms duration) was transduced and compared to when the startle stimulus occurred without a prepulse (i.e., 0dB prepulse).

The PPI test required 17.39min, which included a 5min acclimation period followed by 10 repetitions of the 5 different trial types (0, 76, 80, 84, and 120dB) and intertrial interval (average 15s) presented in pseudorandom order. The variable of interest was the difference in startle response amplitude with and without the prepulse or

amplitude reduction. This amplitude reduction is the percentage reduction of the maximum startle amplitude (MSA) for the prepulse trial expressed as the percentage reduction of the MSA for startle-alone trials: {[MSA (120dB) – Mean Prepulse (for either 76, 80 or 84 dB)] / MSA (120 dB)} x 100.

CHAPTER III

RESULTS

Exploratory Analyses

Exploratory analyses were conducted to determine whether there were any main or interactive effects that were not a part of the initial model and to identify any extraneous variables that might have affected the experiment. For example, the use of two PPI data collection chambers for testing subjects may have produced different results. Similarly, the use of multiple individuals from the same litter can create biased results because this can almost triple the likelihood of yielding a significant effect.

Number of Subjects

Table 1 provides a tally of the number of subjects used in the subsequent analysis for all subjects and for the mean composite score of individuals (used to correct for litter effects) across sex, early experience group and post weaning housing conditions. Table 2 provides a tally of the number of subjects that were in the subsequent analysis of the effects of sex, early experience group and post weaning housing conditions across testing chambers.

Table 1

Distribution of subjects according to sex, pre-weaning and post-weaning experience used for the analyses (IH = Individually housed post-weaning; SH = socially housed post-weaning)

	All Subjects					Number of Litters				
	Male IH	Male SH	Female IH	Female SH	Total	Male IH	Male SH	Female IH	Female SH	Total
AFR	5	10	9	11	**35**	2	4	3	4	**13**
EH	3	11	3	13	**30**	1	4	1	5	**11**
MS	11	10	10	11	**42**	4	4	4	6	**18**
MPS	6	11	11	11	**39**	4	4	4	4	**16**
Total	**25**	**42**	**33**	**46**	**146**	**11**	**16**	**12**	**19**	**58**

Table 2

Distribution of all subjects according to sex, pre-weaning, and post-weaning experience used for the analyses by chamber

	Chamber 1					Chamber 2				
	Male IH	Male SH	Female IH	Female SH	Total	Male IH	Male SH	Female IH	Female SH	Total
AFR	3	6	5	6	**20**	2	4	4	5	**15**
EH	1	6	2	7	**16**	2	5	1	6	**14**
MS	7	6	5	6	**24**	4	4	5	5	**18**
MPS	3	5	5	7	**20**	3	6	6	4	**19**
Total	**14**	**23**	**17**	**26**	**80**	**11**	**19**	**16**	**20**	**66**

An Exploratory Analysis of Litter Effects using a Nested ANOVA

Since multiple individuals from any given litter were used in this study, two three-factor nested ANOVAs (sex, early experience group and PPI; and sex, post weaning housing condition and PPI) were conducted on all subjects to determine whether prepulse inhibition varied as a function of litter even after removing effects of either membership in the four early experience groups or membership in the two post weaning housing conditions. This analysis indicated that prepulse inhibition does not vary as a function of litter even after controlling for early experience condition ($F_{32, 110} = 0.869$, $p > .05$) or for post weaning housing condition ($F_{35, 109} = 1.279$, $p > .05$). Similarly, two three-factor nested ANOVAs (sex, early experience group, startle response at 120 dB; and sex, post weaning housing condition, startle response at 120 dB) indicated that the acoustic startle response (at 120 dB) does not vary within litter even after controlling for early experience condition ($F_{32, 110} = .901$, $p > .05$) or for post weaning housing condition ($F_{35, 109} = 1.055$, $p > .05$)

An Exploratory Analysis of Acoustic Startle Response Amplitude

A 4 x 2 x 2 x 1 analysis of variance (ANOVA) was run to investigate whether there was a difference in startle response (at 120 dB) across early experience group (AFR, EH, MS, MPS), sex (male, female) or post weaning housing condition (socially housed, isolation housed) (See Appendix A). There was a significant effect of sex ($F_{1, 130} = 9.68$, $p < .05$). Male mice displayed a greater acoustic startle response amplitude ($M = 383.09$, $SE = 22.08$) than female mice ($M = 289.92$, $SE = 20.24$). There was no significant

difference in startle response amplitude due to early experience group ($F_{3, 130} = 1.80$, $p > .05$) or post weaning housing condition ($F_{1, 130} = 3.49$, $p > .05$).

Similarly, when a mean for each litter (a composite score of multiple individuals within litter across sex, housing and early experience group) was computed (in order to account for variability within litter) a similar sex difference was observed ($F_{1, 42} = 9.855$, $p < .05$) (See Appendix B). Male mice displayed a greater acoustic startle response amplitude ($M = 367.45$, $SE = 22.90$) than females ($M = 268.38$, $SE = 21.72$) and there was no main effect of group ($F_{3, 42} = 2.805$, $p > .05$) or housing condition ($F_{1, 42} = 2.085$, $p > .05$).

In order to assess whether testing the subjects across two chambers may have affected the observed effects of the treatments, the first analysis was repeated using chamber as an additional between-subjects factor (See Appendix C). A main effect of testing chamber was found ($F_{1, 114} = 39.42$, $p < .05$), with animals tested in Chamber 1 displaying a greater acoustic startle amplitude ($M = 410.19$, $SE = 17.86$) than Chamber 2 ($M = 245.58$, $SE = 19.19$). The sex difference was significant ($F_{1, 114} = 12.80$, $p < .05$) with male mice displaying a greater acoustic startle amplitude ($M = 374.79$, $SE = 19.34$) than females ($M = 280.98$, $SE = 17.70$). Lastly, a main effect of housing was observed ($F_{1, 114} = 4.25$, $p < .05$) with isolation housed animals displaying a greater acoustic startle amplitude ($M = 300.87$, $SE = 21.47$) than socially housed animals ($M = 374.79$, $SE = 19.34$). There was no effect of group ($F_{3, 114} = 2.01$, $p > .05$) and there were no significant interactions.

Lastly, in order to assess whether the observed sex difference in acoustic startle response was present within chambers, the analysis was rerun for each chamber individually (See Appendix D). This analysis demonstrated that regardless of the testing chamber, male mice displayed a greater acoustic startle response amplitude than female mice (Chamber 1 Males (M = 461.16, SE = 17.86) vs. Chamber 1 Females (M = 359.22, SE = 26.68); $F_{1, 64} = 5.94$, $p < .05$) and Chamber 2 Males (M = 288.41, SE = 19.68) vs. Chamber 2 Females (M = 202.75, SE = 19.61); $F_{1, 50} = 9.51$, $p < .05$). There was no main effect of group (Chamber 1; $F_{3, 64} = 1.30$, $p > .05$; Chamber 2; $F_{3, 50} = 1.87$, $p > .05$) or housing condition (Chamber 1, $F_{1, 64} = 2.73$, $p > .05$; Chamber 2, $F_{1, 50} = 1.96$, $p > .05$).

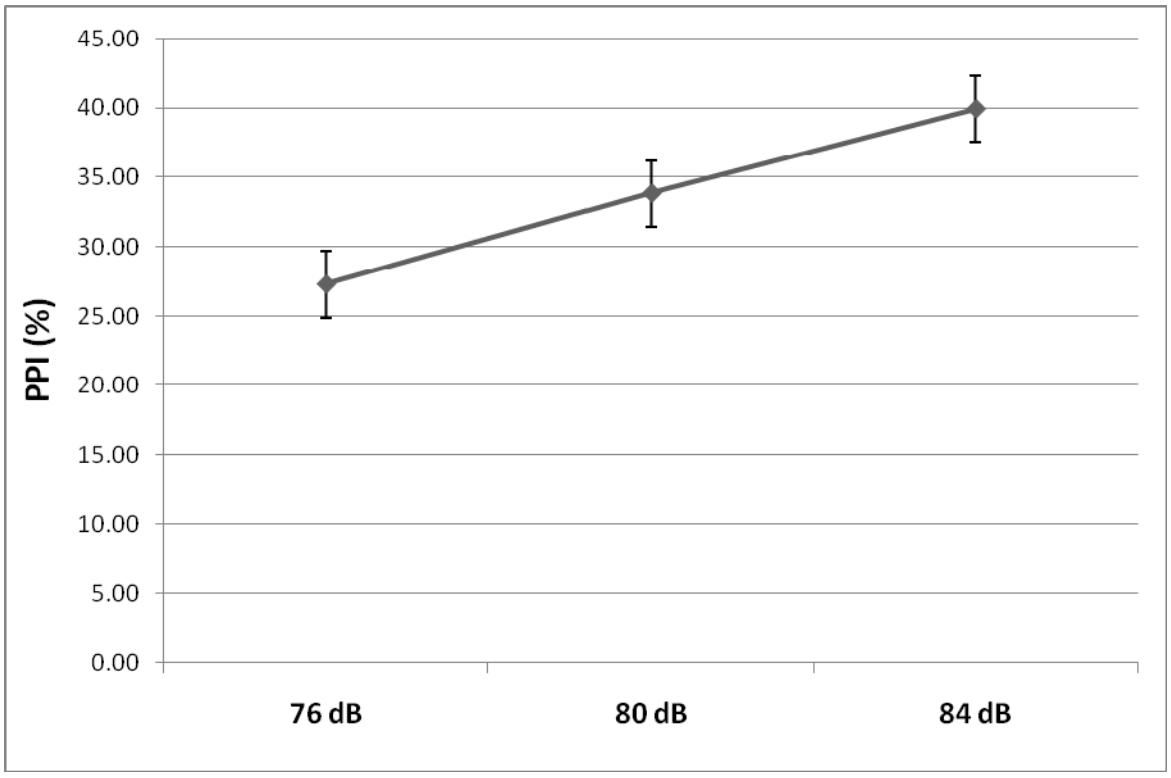
An Exploratory Analysis of Prepulse Inhibition of the Acoustic Startle Response

The effects of early life condition (AFR, EH, MS & MPS), post weaning housing (SH, IH), sex (male, female) and prepulse level on PPI was examined via a four-factor analysis of variance (3 Between-factors – Sex, Early Experience Group and Post Weaning Housing; and 1 Within-factor – Prepulse Level), with repeated measures for the prepulse level (See Appendix E). As expected, there was a main effect of PPI of the acoustic startle response ($F_{2, 260} = 60.26$, $p < .05$), indicating that as prepulse intensity increased, inhibition of the startle response also increased (See Figure 1). There was no main effect of group ($F_{3, 130} = 0.86$, $p > .05$), sex ($F_{1, 130} = 3.70$, $p > .05$) or housing condition ($F_{1, 130} = 0.19$, $p > .05$).

When a mean for each litter (a composite score of multiple individuals within litter across sex, housing and early experience group) was computed (in order to account

for variability within litter) the analysis revealed a similar result (See Appendix F). There was a main effect of PPI of the acoustic startle response ($F_{2,\ 84} = 34.90$, $p < .05$), indicating that as prepulse intensity increased, inhibition of the startle response also increased. No main effect of group ($F_{3,\ 42} = 0.26$, $p > .05$), sex ($F_{1,\ 42} = 0.36$, $p > .05$) or housing condition ($F_{1,\ 42} = 0.04$, $p > .05$) on prepulse inhibition was observed.

Figure 1. Change in PPI (%) as a function of prepulse level



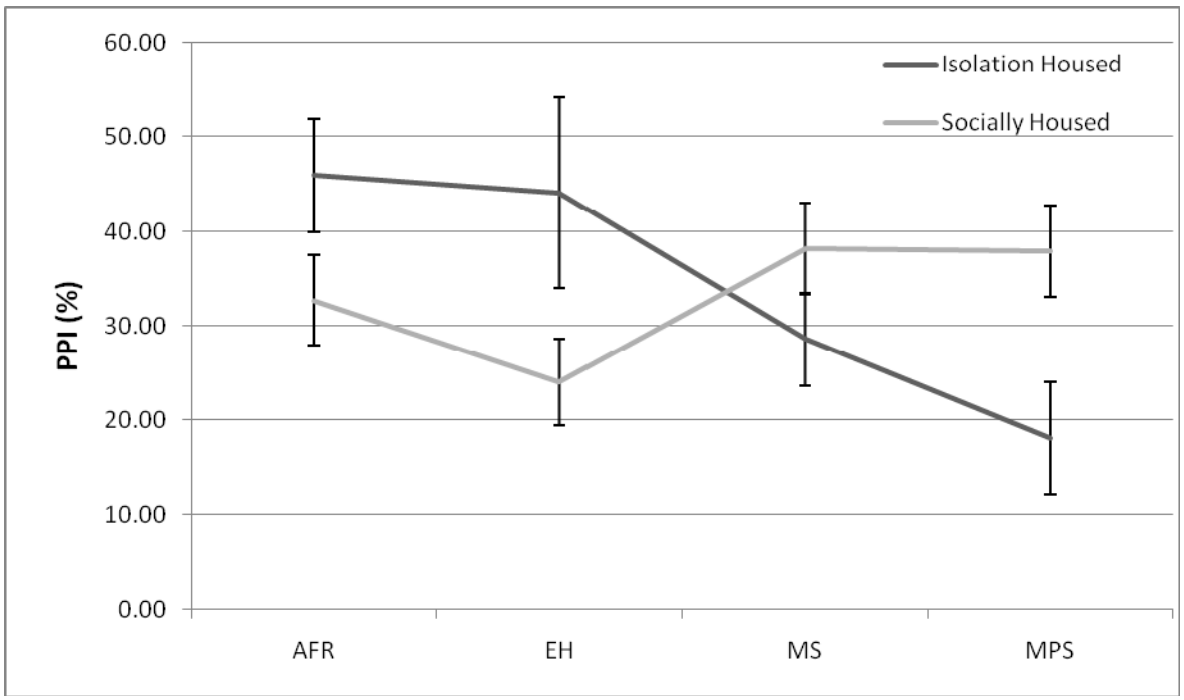
In order to assess whether testing the subjects across two chambers may have distorted the observed effects of the treatments, the initial analysis was repeated using chamber as a between subjects factor (See Appendix G). There was again a main effect of

PPI of the acoustic startle response ($F_{2, 228} = 58.39$, $p < .05$), indicating that as prepulse intensity increased, inhibition of the startle response also increased. There was no main effect of group ($F_{3, 114} = 0.71$, $p > .05$), sex ($F_{1, 114} = 2.66$, $p > .05$), housing condition ($F_{1, 114} = 0.27$, $p > .05$), or chamber tested ($F_{1, 114} = 2.00$, $p > .05$).

To investigate whether the observed effects were consistent across chambers, the analysis was rerun for each chamber individually (See Appendix H). A main effect of PPI of the acoustic startle response was found regardless of the chamber tested on (Chamber 1, $F_{2, 128} = 22.37$, $p < .05$; Chamber 2, $F_{2, 100} = 35.63$, $p < .05$). There was no main effect of group (Chamber 1; $F_{3, 64} = 1.46$, $p > .05$; Chamber 2; $F_{3, 50} = 2.03$, $p > .05$) or housing condition (Chamber 1, $F_{1, 64} = 0.05$, $p > .05$; Chamber 2, $F_{3, 50} = 0.75$, $p > .05$). Interestingly, an interaction between housing and group in Chamber 1 was present ($F_{3, 64} = 4.86$, $p < .05$) (See Figure 2).

Isolation housed animal facility reared ($M = 45.94$, $SE = 6.00$) and early handled ($M = 44.07$, $SE = 10.07$) subjects displayed higher levels of PPI compared to socially housed animal facility reared ($M = 32.71$, $SE = 4.75$) and early handled ($M = 24.07$, $SE = 4.57$) subjects. Furthermore, isolation housed maternal separated ($M = 38.22$, $SE = 4.75$) and maternal peer separated ($M = 37.90$, $SE = 4.81$) subjects displayed a diminution in PPI compared to isolation housed animal facility reared and early handled isolation housed subjects. Conversely, socially housed maternal separated ($M = 38.22$, $SE = 4.75$) and maternal peer separated ($M = 37.90$, $SE = 4.81$) subjects displayed an increase in PPI compared to animal facility reared and early handled socially housed subjects.

Figure 2. Interaction between Housing and Group as a function of PPI in Chamber 1



Post hoc pairwise comparisons of early experience group within housing condition (See Appendix I) revealed that isolation housed animal facility reared subjects were significantly different from maternal separated (*MD* = 17.43, $p < .05$) and maternal peer separated subjects (*MD* = 27.88, $p < .05$). Isolation housed early handled subjects were significantly different from maternal peer separated subjects (*MD* = 26.00, $p < .05$). Socially housed early handled subjects were significantly different from maternal separated (*MD* = -14.15, $p < .05$) and maternal peer separated subjects (*MD* = -13.83, $p < .05$). Similarly, post hoc pairwise comparisons of housing condition within early experience group indicated that isolation housed maternal peer separated subjects were

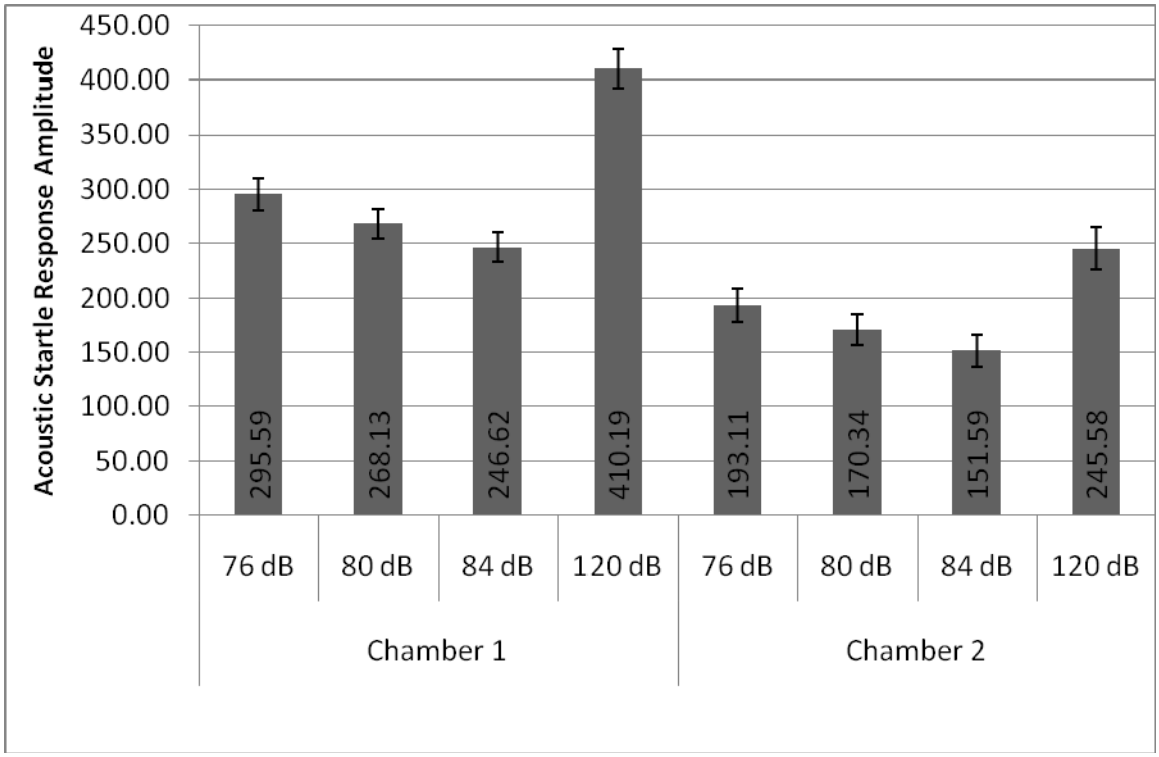
significantly different from socially housed maternal peer separated offspring (*MD* = -19.83, $p < .05$) (See Appendix J).

Since calculation of PPI involves transformation of the data it is possible that any difference in responding may have been "masked" as these scores were transformed into a proportional distribution. Recall that PPI was calculated as the percentage reduction of the maximum startle amplitude (MSA) for the prepulse trial expressed as the percentage reduction of the MSA for startle-alone trials: {[MSA (120dB) – Mean Prepulse (for either 76, 80 or 84 dB)] / MSA (120 dB)} x 100. If the output of Chamber 1 provided lower response values across all prepulse trial types regardless of all other factors, then this consistent lower output would be lost due to this transformation. To investigate this a five-factor analysis of variance (4 Between – Chamber of Testing, Sex, Early Experience Group and Post Weaning Housing; and 1 Within – Decibel Level (76, 80, 84 & 120 dB)) with repeated measures for the decibel level was conducted.

A main effect of decibel level was observed ($F_{3, 342} = 147.90$, $p < .05$) which indicated that responding was maximum at 120dB (*M* = 327.89, *SE* = 13.11) and that as prepulse level increased, responding diminished (at 76 dB, *M* = 244.35, *SE* = 10.77; at 80 dB, *M* = 219.24, *SE* = 9.68; and at 84 dB, *M* = 199.10, *SE* = 10.21). An interaction between decibel level and chamber was also observed ($F_{3, 342} = 12.74$, $p < .05$), indicating that across all decibel levels, individuals tested in Chamber 1 displayed lower levels of responding (See Figure 3). Interestingly, a main effect of sex was observed ($F_{1, 114} = 16.74$, $p < .05$), with males showing a greater level of responding (*M* = 289.61, *SE* =

15.13) than females (M = 205.68, SE = 13.85); this is similar to observed results in analysis of the acoustic startle response at 120 dB.

Figure 3. Comparison of startle response of individuals within chambers across decibel levels.



Lastly, in order to investigate whether the observed sex effect in the previous analysis may have been due in part to the inclusion of responding at 120 dB, the previous analysis was repeated using only the three prepulse decibel levels (76, 80 & 84 dB). Recall, it has already been demonstrated that a sex effect was observed when the startle decibel level (120 dB) was used. Consistent with the previous analysis, main effects of decibel level ($F_{2, 228} = 42.709$, $p < .05$), sex ($F_{1, 114} = 16.823$, $p < .05$) and chamber tested

($F_{1, 114} = 25.07$, $p < .05$) were observed. Converse to the previous analysis, no significant interaction between decibel level and chamber was observed ($F_{2, 228} = 0.294$, $p > .05$).

Within housing condition and sex, the AFR condition represents a non-manipulated condition and as such can serve as a baseline condition against which other manipulated groups can be compared. Therefore, for each individual score for members of the manipulated groups (EH, MS, MPS), a difference score from the mean AFR PPI was calculated (adjusted according to the prepulse decibel level, sex and post-weaning housing condition of the individual). This was then expressed as a percent proportion to AFR by dividing the difference by the mean AFR score used. A four factor ANOVA (sex, early experience group, housing condition and prepulse level) was run. A main effect of post-weaning housing was observed with isolation housed subjects displaying lower levels of PPI (M = -28.20, SE = 10.62) and socially housed subjects displaying higher levels of PPI (M = 8.78, SE = 7.39). However a main effect of PPI was not observed ($F_{2, 198} = 2.61$, $p > .05$) which indicates that as prepulse level increased the amount of PPI did not correspondingly increase. Since there was no significant difference in observed variability of PPI across prepulse level, the observed post-weaning housing effect, which is collapsed across PPI, may be an artifact of using the percent difference from mean PPI as the data. No other effects or interactions were observed.

Acoustic Startle Response Amplitude Multiple Regression Model

Exploratory analyses revealed a main effect of chamber tested and sex on the acoustic startle response, with no effect of group and no interactions. As such, the initial regression equation was:

$$1)\ \text{Startle at 120 dB} = \alpha + \beta*\text{Sex} + \beta*\text{Housing} + \beta*\text{Chamber}$$

Consistent with the ANOVA analysis, a main effect of sex (t = -4.207, p = 0.000) and chamber tested (t = -7.450, p = 0.000) as well as no effect of housing condition was observed (t = 1.605, p = 0.111). This model accounted for 35.3% of the observed variability (R^2).

No additional significant effects were observed if an interaction term of sex and chamber tested was added to the model, yielding a regression equation of:

$$2)\ \text{Startle at 120 dB} = \alpha + \beta*\text{Sex} + \beta*\text{Housing} + \beta*\text{Chamber} + \beta*\text{Sex}*\text{Chamber}$$

This model accounted for only an additional .4% of the observed variability (R^2 =35.7%).

Additionally, three effect coded *a priori* contrasts for early experience condition (AFR vs. EH, MS vs. MPS and AFR, EH vs. MS, MPS) were conducted. These yielded the following three regression equations:

$$3)\ \text{Startle at 120 dB} = \alpha + \beta*\text{Sex} + \beta*\text{Housing} + \beta*\text{Chamber} + \beta*\text{Sex}*\text{Chamber} + \beta*\text{Group01}$$

4) Startle at 120 dB = α + β*Sex + β*Housing + β*Chamber + β*Sex*Chamber + β*Group01 + β*Group23

5) Startle at 120 dB = α + β*Sex + β*Housing + β*Chamber + β*Sex*Chamber + β*Group01 + β*Group23 + β*Group01vs23

The contrast between AFR and EH subjects (equation 3) was significant (t = -2.752, p = 0.007), demonstrating that EH subjects displayed a diminution of startle by 94.5 units compared to AFR subjects. This model accounted for an additional 3.3% of the observed variability (R^2 =39%). The addition of the contrast between MS and MPS subjects (equation 4) to the model did not yield any additional significant effects and accounted for only an additional 0.1% of the observed variability (R^2 =39.1%). Similarly the contrast between AFR, EH vs. MS, MPS subjects to the model (equation 5) did not yield any additional significant effects and accounted for only an additional 0.5% of the observed variability (R^2 =39.6%). It should also be noted that the lowest observed tolerance across all equations was 0.975. This indicates that the predictors used in the regression equation do not suffer from issues of multicollinearity. That is, the predictors are not correlated and independently contribute to any of the observed variance predicted by the regression equation.

Since we observed only three significant effects, the final regression equation became:

Startle at 120 dB = α + β*Sex + β*Chamber + β*Group01

or

Startle at 120 dB = 327.65 + (-101.22)*Sex + (-172.83)*Chamber + (-86.87)*Group01

or

Startle at 120 dB = (-0.294)*Sex + (-0.501)*Chamber + (-0.169)*Group01

This model accounted for 37% of the observed variability (R^2), with a tolerance of .999.

Prepulse Inhibition of the Acoustic Startle Response Multiple Regression Model

Exploratory analyses revealed a main effect of PPI of the acoustic startle response irrespective of any other factors. This indicates that as prepulse acoustic level increased, prepulse inhibition on the acoustic startle response also increased. Similarly, when responding across decibel levels was assessed, a main effect of decibel level emerged, indicating that the maximum startle response was observed at 120dB and that as prepulse level increased, the startle response decreased. However, a main effect of sex also emerged when the data was analyzed in the "raw" rather than as a percent ratio transformation as in commonly done in literature. This indicated that the within subject/group variability diminishes significantly due to this transformation. Since the exploratory analysis indicated these effects, mean responding across decibel levels {(76 dB+ 80 dB + 84 dB+ 120 dB) /4} was used as the dependent measure, yielding a regression equation of:

1) Mean Response (dB) = α + β*Sex + β*Housing + β*Chamber

Consistent with the initial analysis, there was a main effect of sex (t = -5129, p = 0.000) and chamber tested on (t = -7.132, p = 0.000). No effect of housing was observed (t = 1.144, p = 0.255). This model accounted for 36% of the observed variability (R^2).

Three effect coded *a priori* contrasts for early experience condition (AFR vs. EH, MS vs. MPS and AFR, EH vs. MS, MPS) were then calculated via the following three regression equations:

2) Mean Response (dB) = α + β*Sex + β*Housing + β*Chamber + β*Sex*Chamber + β*Group01

3) Mean Response (dB) = α + β*Sex + β*Housing + β*Chamber + β*Sex*Chamber + β*Group01 + β*Group23

4) Mean Response (dB) = α + β*Sex + β*Housing + β*Chamber + β*Sex*Chamber + β*Group01 + β*Group23 + β*Group01vs23

The addition of the contrast between AFR and EH subjects (equation 2) was significant (t = -2.752, p = 0.027), demonstrating that EH subjects displayed a diminution of the mean level of responding by 209.43 units as compared to AFR subjects. This model accounted for an additional 2.1% of the observed variability (R^2 =38.1%). The addition of the contrast between MS and MPS subjects (equation 3) to the model did not yield any additional significant effects and accounted for only an additional 0.3% of the observed variability (R^2 =38.4%). Similarly, the contrast between AFR, EH vs. MS, MPS subjects to the model (equation 4) did not yield any additional significant effects and

accounted for only an additional 0.1% of the observed variability (R^2 =38.5%). It should also be noted that the lowest observed tolerance across all equations was 0.949 which indicates that the predictors used in the regression equation do not suffer from issues of multicollinearity.

Since we observed only three significant effects the final regression equation became:

$$\text{Mean Response (dB)} = \alpha + \beta*\text{Sex} + \beta*\text{Chamber} + \beta*\text{Group01}$$

or

$$\text{Mean Response (dB)} = 1013.39 + (-377.16)*\text{Sex} + (-513.08)*\text{Chamber} + (-193.58)*\text{Group01}$$

or

$$\text{Mean Response (dB)} = (-0.351)*\text{Sex} + (-0.477)*\text{Chamber} + (-0.121)*\text{Group01}$$

This model accounted for 36.9% of the observed variability (R^2), with a tolerance of .999.

Lastly, in order to assess the consistency of results when transformed PPI scores were used, the following regression model was run:

$$1)\ \text{Mean PPI} = \alpha + \beta*\text{Sex} + \beta*\text{Housing} + \beta*\text{Chamber}$$

Consistent with the former analysis, a main effect of sex (t = -2.482, p = 0.014) and no effect of housing condition was observed (t = 1.144, p = 0.255). In addition no

effect of chamber tested was found. Since it was demonstrated that Chamber 1 output higher raw scores than Chamber 2 in the former analysis, this analysis provided evidence that the lower output across chamber was consistently lower across prepulse levels (76, 80, 84 & 120 dB). This is evidenced in part because when the raw scores are expressed as a percent proportion score, the same main effects were observed as the raw scores, except for chamber tested. This model accounted for only 4.2% of the observed variability (R^2), compared to the 36% observed when mean responding across decibel levels was used.

Again, when the three effect coded *a priori* contrasts for early experience condition (AFR vs. EH, MS vs. MPS and AFR, EH vs. MS, MPS) were tested, a significant difference was observed ***only*** between AFR and EH subjects (t = -1.686, p = 0.045), indicating that EH subjects displayed a diminution of PPI by 5.6% as compared to AFR subjects. This model accounted for only 6% of the observed variability (R^2, tolerance = 0.999) as compared to 36.9% which is observed when mean responding across decibel levels is used.

Power Analysis

Since the number of subjects available per group was limited by the availability of "spare" offspring, the study may have suffered from low power to detect differences between groups. A post hoc analysis of the sample size needed to observe the hypothesized effects was performed in order to evaluate this. From a review of the literature, it was predicted that: 1) isolation housed animals should display a 10% reduction in PPI compared to socially housed animals; 2) females should display a 30%

reduction in PPI compared to males; and 3) AFR, EH and MPS offspring should display comparable levels of PPI. As no study has looked at the effects of MS on PPI in C57BL/6, a disruption of PPI by 15% was hypothesized. The hypothesized value for each group is reflected in Table 3.

Table 3

Expected mean values of PPI across groups

	AFR, EH, MPS				MS			
	Isolation Housed		Socially Housed		Isolation Housed		Socially Housed	
	Male	Female	Male	Female	Male	Female	Male	Female
PPI at 76 dB (%)	29	20	32	22	24	17	27	19
PPI at 80 dB (%)	32	23	36	25	28	19	31	21
PPI at 84 dB (%)	36	25	40	28	31	21	34	24

Given these values, the minimum required sample size needed to detect all main and interactive effects would be n = 88 / group ($\alpha = .05$, $1-\beta = .80$, $SD = .2$). As such, the lack of any observed effects in this study should be interpreted with caution, as this study suffers from low power.

CHAPTER IV

DISCUSSION

Investigation of the effects of the separation of dam and offspring on offspring development of maladaptive adult behavioral phenotypes arose in part as a consequence of a report by Bernstein (1952) in which daily handling (picked up by the experimenter for a few minutes) of infant rats led to better maze performance as adults when compared to non-handled rats. Not long after, Weininger (1953, 1956) reported that handled rats were less "emotional" (as measured by "fearful" behavior in the open field) and more likely to survive severe stress as adults. These studies opened the way for investigation into the mechanism by which this early handling phenomenon is achieved (including hormonal and neurobiological changes). In contrast, prolonged periods of maternal separation have been reported to produce the opposite behavioral, physiological and neuro-anatomical effects associated with the early handling phenomenon (Meaney et al., 1994). However, despite what seems to be a clear and predictable relationship between early life experience and adult behavioral characteristics, methodological variations have revealed paradoxical differences in observed outcomes across laboratories.

Post-weaning social isolation has also been demonstrated to affect adult behavior outcomes on a variety of measures, including PPI. However, like the dam-offspring separation literature, reports across laboratories are surprisingly variable, with some

laboratories reporting diminished PPI while others report no difference in PPI. As noted above, this variability in findings may be due to methodological differences across laboratories.

This study was primarily designed to make use of those extra offspring raised in four different early experience conditions (AFR, EH, MS, MPS) that were not tested on other behavioral measures. The overall goal was to assess the effects of early life experience, in the form of dam-offspring separation and subsequent post-weaning social housing, on the manifestation of adult prepulse inhibition using the inbred strain of mouse, C57BL/6. The three main aims of this study were:

1) To assess whether maternal-offspring separation (MS) differentially affects development of adult PPI. It was hypothesized that MS offspring as adults would have an exaggerated "stress" response. The presumed high circulating levels of CORT in these offspring as adults was posited to increase extracellular dopamine secretion in the nucleus accumbens which would lead to a diminished PPI.

2) To assess whether post-weaning social isolation in mice produces deficits in PPI. There was some evidence indicating that social isolation post-weaning increased circulating CORT levels and also increased extracellular dopamine secretion in the nucleus accumbens. This, coupled with reports that socially isolated rats and mice displayed deficits in PPI as adults, indicated that application of a persistent stressor (social isolation) post-weaning could be a necessary condition to induce disruption of PPI.

3) To assess whether there was an additive effect of the application of persistent stressors, in the form of dam-offspring separation followed by subsequent post-weaning social isolation, on the development of adult PPI.

Contrary to hypothesis 1, EH mice displayed lower levels of PPI and of the acoustic startle response than AFR, MS & MPS mice. The literature supports the notion that MS represents a more "stressful" condition to the developing pup's nervous system whereas AFR, EH and MPS are equitable in terms of the "stress levels". However, as discussed previously, the effects of MS on offspring adult behaviors are variable and can be affected by many factors, including but not limited to: the time of day of separation, the length of the separation, the caging condition on separation and the temperature of the pups at separation and reuniting. Indeed, a recent publication by Parfitt et al. (2007) indicates that applications of these separation procedures (which were originally validated in the rat) in mice may be insufficient or only marginally effective in producing the same response to stress-inducing conditions that was found in rats (i.e., EH = less "stressed" as adult, MS = "more "stressed" as adult").

The study by Parfitt et al. (2007) is the only published rodent study to simultaneously evaluate the effects of the three main separation groups (EH, MS, MPS) against an AFR control group using C57BL/6 mice. As in the present study, the study by Parfitt et al. (2007) also controlled for: the temperature of the pups at separation so that it was equivalent to the temperature of the natal nest; the procedures were applied at the same time for all groups; and the choice of time for application of the separation paradigms were validated by neuroendocrine data. Consequently, Parfitt et al. (2007)

demonstrated that EH was the only early experience condition that affected the stress response (marginal blunting) and that the MS procedures do not exacerbate the stress response in C57BL/6. Indeed, their study indicated that if MS did have an effect on the offspring, it is in the same direction (less "anxious") as that of EH! Perhaps, then, it is no surprise that in the present study the EH group was the only group that displayed a difference in PPI and the ASR. Of course, this result should be assessed by a replication study.

In regard to hypothesis 2, the results in general indicated that the post-weaning housing manipulation (SH, IH) had no effect on the development of either adult prepulse inhibition or the acoustic startle response in C57BL/6. However, when the mean AFR score was subtracted from each individual's score and the difference was divided by the mean AFR, social housing produced a greater PPI response. However, this result requires replication because this calculation of the PPI data did not reveal any evidence of the normally robust effect of prepulse level on PPI. Consistent with the human and animal literature, male mice displayed a greater acoustic startle response (ASR) and prepulse inhibition of the ASR than females (Swerdlow et al., 1997; Lehmann, Pryce & Feldon, 2000).

One possible reason for the observed lack of effect of the manipulation of the post-weaning environment on adult PPI may have been due to the fact that social isolation of offspring post-weaning has not been clearly demonstrated to produce a reliable effect on development of adult PPI in mice. For example, although Varty et al. (2006) demonstrated that socially isolated C57BL/6 mice post weaning (after PND 21)

display deficits in PPI when tested at PND 42 & 49, a recent study by Pietropaolo, Feldon & Yee (2008) found that social isolation of C57BL/6 offspring post-weaning (PND 21) did not affect circulating CORT levels, behavioral responses to novelty, or development of adult PPI and an ASR when tested as adults (PND 49).

Interestingly, these two studies differed in the source for their subjects. Varty et al. (2006) report "C57BL/6J (C57) mice arrived from the vendor (Jackson Laboratories, Bar Harbor, ME) as weaned 21-day-old pups (weighing approximately 10 g)", whilst subjects from Pietropaolo, Feldon & Yee (2008) were bred in the laboratory under standard animal husbandry procedures (similar to the present study). The subjects used in the study by Varty et al. (2006) bears scrutiny as disruption of the pre-weaning environment is evident. The effect of such a disruption may have confounded the purported disruption of PPI in response to social isolation and should be interpreted with caution. As such the observed lack of effect of post weaning social isolation on the development of adult PPI in this study is consistent with the more reliable report of Pietropaolo, Feldon & Yee (2008).

The present study found a main effect of testing chamber. Chamber 1 consistently provided higher raw response scores (regardless of sex, early experience condition, post weaning condition, and prepulse level) than Chamber 2. However, it was demonstrated that within chambers, the same main effect of sex was observed for both Prepulse Inhibtion and the ASR. Furthermore, when the raw scores were transformed to a proportion percent in the calculation of PPI, the same effects were observed as those

found the raw scores were used. This indicates that even though Chamber 1 output higher scores, the observed effects were robust as they were consistent across multiple analyses.

The interpretation of the observed result that male mice displayed higher levels of startle and PPI than female mice is difficult. This effect may have been due to a number of factors including weight and hormonal condition. In the present study, regardless of rearing condition, female mice weighed on average 19g whereas male mice weighed on average 24g. It has been suggested in research on Wistar and hooded rats (Blaszczyk & Tajchert, 1996) that the heavier the animal, the greater the muscle mass and the greater the associated motor strength. They posit that increased the motor strength is co-related with the display of a greater startle response and a deficiency in the ability to display PPI.

However, one cannot assume a correlation between body weight and PPI without adequate measurement of the factors which mediate this effect. Consider, for example, the study by Plappert, Rodenbucher & Pilz (2005) which reported the effect of estrous cycling in C57BL/6 and C3H strain on the display of PPI and the ASR. Both male and female C57BL/6 mice on average weighed more than male and female C3H mice. However, C3H mice displayed a greater ASR amplitude than C57BL/6 mice. This contradicts the notion that greater body weight is a necessary condition for an increased ASR or diminution of PPI. As such, any relationship that may be categorized in this study between weight and ASR or PPI is correlational at best and should be interpreted with caution.

Similarly, the hormonal state of female C57BL/6 has been demonstrated to have no influence on the display of PPI (Plappert, Rodenbucher and Pilz, 2005), contrary to

what has been reported in Sprague Dawley rats (Koch, 1998). As such, elevated estrogen levels which have been demonstrated to diminish PPI in female Sprague Dawley rats, seems to not play a homologous role in female C57BL/6 mice. Furthermore, as both the pre- and post-weaning manipulations applied in this experiments are sensitive to methodological influences, the estrous cycling of female C57BL/6 in this experiment was not evaluated because this would have created a different post-weaning manipulation for the male and female mice. Since the hormonal state associated with estrous cycling does not seem to affect the display of PPI in C57BL/6, sex-typical differences in hormonal state ars unlikely to be an explanation for the observed sex difference in this experiment. As such, interpretation of the observed sex difference in this experiment in display of the ASR and PPI requires further investigation.

In regards to hypothesis 3, only one interaction was observed between post weaning housing condition and early experience group (in Chamber 1) when exploratory analyses were conducted. Isolation housed MPS offspring were significantly different from socially housed MPS offspring. Similarly, isolation housed AFR were significantly different from isolation housed MS & MPS subjects and isolation housed EH were also observed to be significantly different from isolation housed MPS subjects. Socially housed EH were significantly different from socially housed MS & MPS subjects.

As these effects were limited to Chamber 1 (which output higher raw scores) they should be interpreted with caution. Even though the same main effects were observed across chambers, this interaction bears scrutiny as it may indicate that within chamber, the variability associated with group membership may have been inflated (if tested on

Chamber 1) or diminished (if tested on Chamber 2). As such, the mediating factors of this observed effect require investigation.

Regardless, it was predicted that irrespective of early experience condition, isolation housed subjects should display a deficit in PPI as compared to socially housed subjects. Furthermore, it was also predicted that MS isolation housed subjects should display the greatest disruption of PPI whilst AFR, EH & MPS isolation housed subjects should display similar levels of disruption. As such, there was no support for these predicted effects.

The results of the present study prompt the question: why do these pre- and post-weaning manipulations in C57BL/6 mice not produce the effects observed in rats? It is possible that the perception of stimuli associated with separation and isolation, and their corresponding effects on the neuroendocrine system in response to the application of these manipulations, may differ between a mouse and a rat. Some have argued that the mouse brain develops more quickly than a rat brain and is therefore less "plastic" and less affected by such developmental manipulations (Pellis and Iwaniuk, 2000).

In rats, dam-offspring separations have been demonstrated to increase the amount of maternal care that the pups receive upon reuniting. For EH offspring, maternal behavior almost doubles post reunion (Liu et al., 1997), and for MS pups an intense bout of maternal behavior for at least one hour post reuniting occurs (Pryce et al., 2001). There is also some evidence that maternal behavior in the mouse is already "high" compared to that of a rat (Priebe et al., 2005). Finally, C57BL/6 provide higher levels of maternal care (licking, grooming, retrieval and nest building) when compared to other mouse strains

(Priebe et al., 2005, Millstein and Holmes, 2007). As such description of post reunion behavior across the four separation groups in mice as well as the associated neuroendocrine effects is needed in order to evaluate whether application of these early experience procedures produces the same changes in maternal care as observed in rats.

A similar concern about the relative "plasticity" of rat and mice nervous systems is relevant to the effects of post-weaning social isolation. It has recently been demonstrated that post-weaning socially isolated C57BL/6 mice do not display any difference in PPI (Pietropaolo, Singer & Feldon, 2008). However, social isolation increases locomotor activity, decreases locomotor habituation and potentiates locomotion in response to amphetamine preferentially in male C57BL/6 (Pietropaolo, Singer & Feldon, 2008). Thus, differential behavioral phenotypes can emerge in response to this environmental manipulation. Consequently, the association of post-weaning social isolation with observed deficits in PPI may be weak or non-existent for mice. Developmental analysis of how disruption of PPI can emerge as a consequence of the isolation procedure needs further investigation.

Of primary concern in the present study is that the use of only those offspring that were available from a given litter may have contributed to the failure to observe clear effects of the pre- and post-weaning manipulations. Since this study was derived subsidiary to another one, the selection from the subject pool was limited. Such selection of subjects has been demonstrated to almost triple the likelihood of a significant finding (termed "litter effects"). However, the analyses using a two three-factor nested ANOVAs, showed that PPI does not vary within litter even after controlling for either the early

experience condition or the post weaning housing condition and provided support that the notion that "litter effects" were not contributing to the results.

Since C57BL/6 suffer from age related hearing loss, it is possible that failure to detect a significant effect in response to the administered treatments may stem in part from insensitivity to the applied prepulse levels at PND 60-70. Ouagazzal, Reiss and Romand (2006) report that the ASR and PPI due to weak auditory stimuli (70-90 dB) increase in C57BL/6 whilst the ASR and PPI due to intense auditory stimuli (90-120 dB) declines progressively between weeks PND 42 - 287. However as the study by Pietropaolo, Feldon & Yee (2008) did not find an effect of social isolation in C57BL/6 tested at PND 49 on PPI, there is some evidence that the lack of effect of post weaning housing condition on PPI in this study is valid. A developmental analysis of changes in PPI and the ASR associated with early experience condition requires further investigation.

One fundamental problem associated with the design of this study involves the presumed behavioral homology between rats and mice. Dam-offspring separation and its associated developmental effects on the offspring were first validated in rats and only later extended to certain strains of mice. A similar pattern of research also characterized the analysis of the effects of social isolation on PPI. Moreover, PPI was described initially for specific strains of rats and only subsequently described in certain strains of mice. However, it is unclear whether the developmental consequences for the offspring of dam-offspring separations in rat strains readily apply to mice strains. It is equally unclear whether PPI observed in specific strains of mice apply to C57 strains. Therefore, part of

the reason for the lack of influence of either preweaning stress or post-weaning housing on expressed PPI in the offspring may reflect differences in the way that C57BL/6 mice react to the separations and the way that PPI is manifested in this strain. A more extensive comparative analysis is warranted before drawing any conclusions from this study.

C57BL/6 is often used in research because it is the most common genetic background for targeted intervention. Furthermore, as an inbred strain, it is often thought to be useful for investigation into the effects of environmental manipulations. Since this strain appears to be " resilient" to these environmental manipulations, we need to determine what is it about the development of C57BL/6 that creates a nervous system that is so "resilient" to the effects of these early life experiences and post-weaning housing conditions.

Analysis of the longitudinal character of responding across these separation groups, comparison of this behavior across different mouse strains and translation of how this responding differentially affects the development of the nervous system is necessary to evaluate exactly why these paradigms do or do not reliably produce the same effects in mice as they do in rats. A similar analysis is needed to quantify how behaviors change as a consequence of post-weaning housing.

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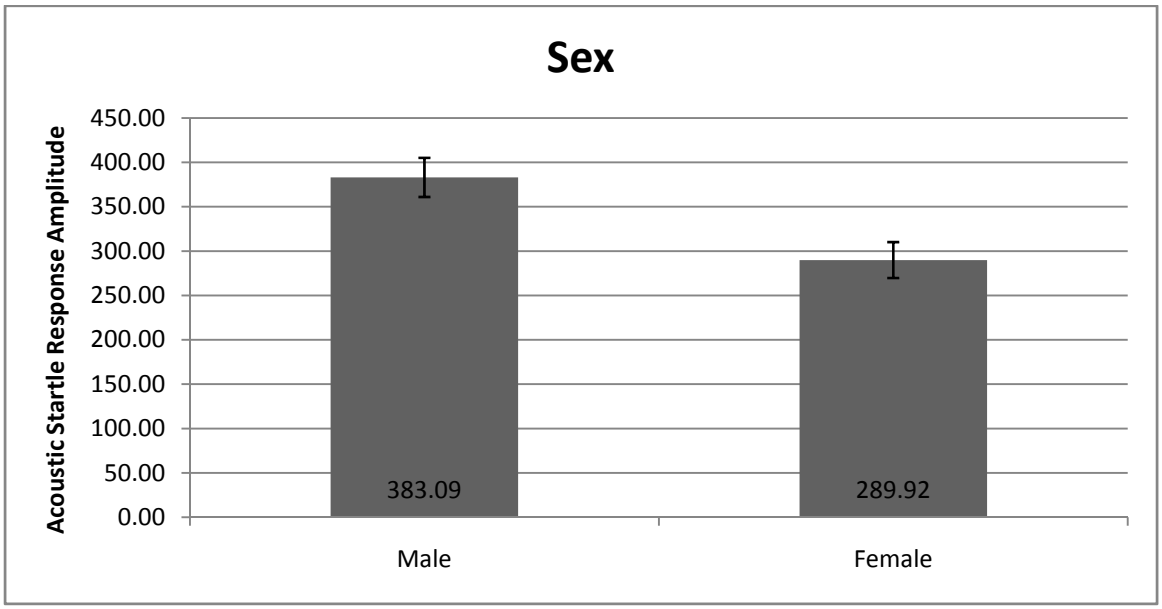
APPENDIX A. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 1 (120 dB) ANOVA for All Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	845775.24	15	56385.02	2.12	0.01
Intercept	13434539.52	1	13434539.52	504.91	0.00
Sex	257500.78	1	257500.78	9.68	0.00
Housing	92811.34	1	92811.34	3.49	0.06
Group	143998.55	3	47999.52	1.80	0.15
Sex * Housing	10525.50	1	10525.50	0.40	0.53
Sex * Group	128422.49	3	42807.50	1.61	0.19
Housing * Group	7213.92	3	2404.64	0.09	0.97
Sex * Housing * Group	54633.17	3	18211.06	0.68	0.56
Error	3458992.50	130	26607.63		
Total	21221755.14	146			
Corrected Total	4304767.74	145			

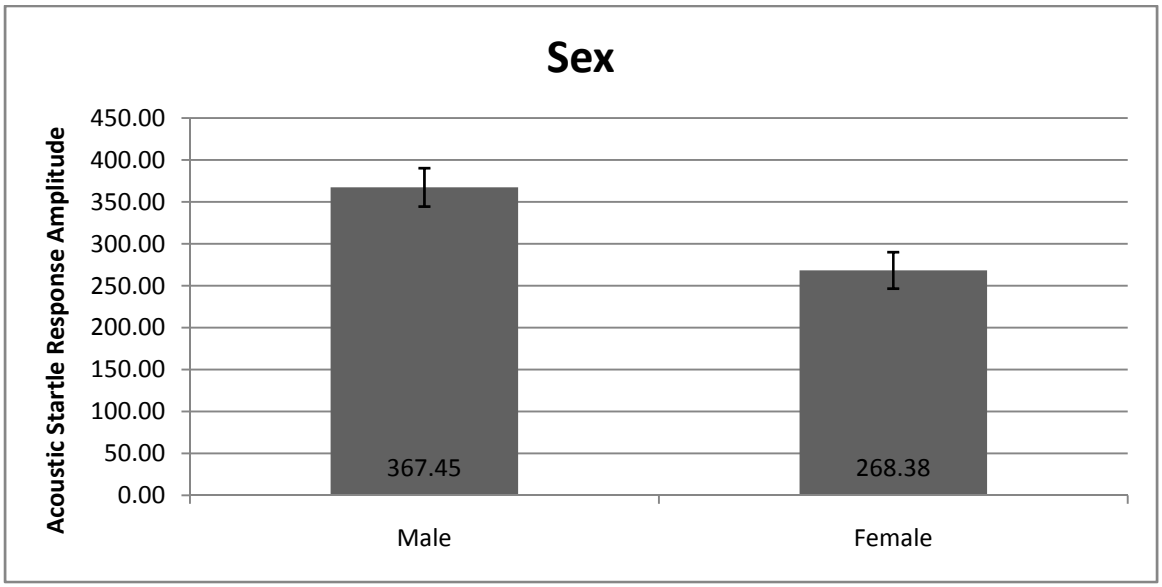
Sex	Mean	Std. Error
Male	383.09	22.08
Female	289.92	20.24



APPENDIX B. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 1 (120 dB) ANOVA for Individuals.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	378729.17	15	25248.61	2.26	0.02
Intercept	4539277.28	1	4539277.28	405.88	0.00
Sex	110215.76	1	110215.76	9.85	0.00
Housing	31366.81	1	31366.81	2.80	0.10
Group	69968.12	3	23322.71	2.09	0.12
Sex * Housing	9892.81	1	9892.81	0.88	0.35
Sex * Group	53575.24	3	17858.41	1.60	0.20
Housing * Group	2990.38	3	996.79	0.09	0.97
Sex * Housing * Group	11037.13	3	3679.04	0.33	0.80
Error	469724.54	42	11183.92		
Total	6671295.23	58			
Corrected Total	848453.71	57			

Sex	Mean	Std. Error
Male	367.45	22.90
Female	268.38	21.72



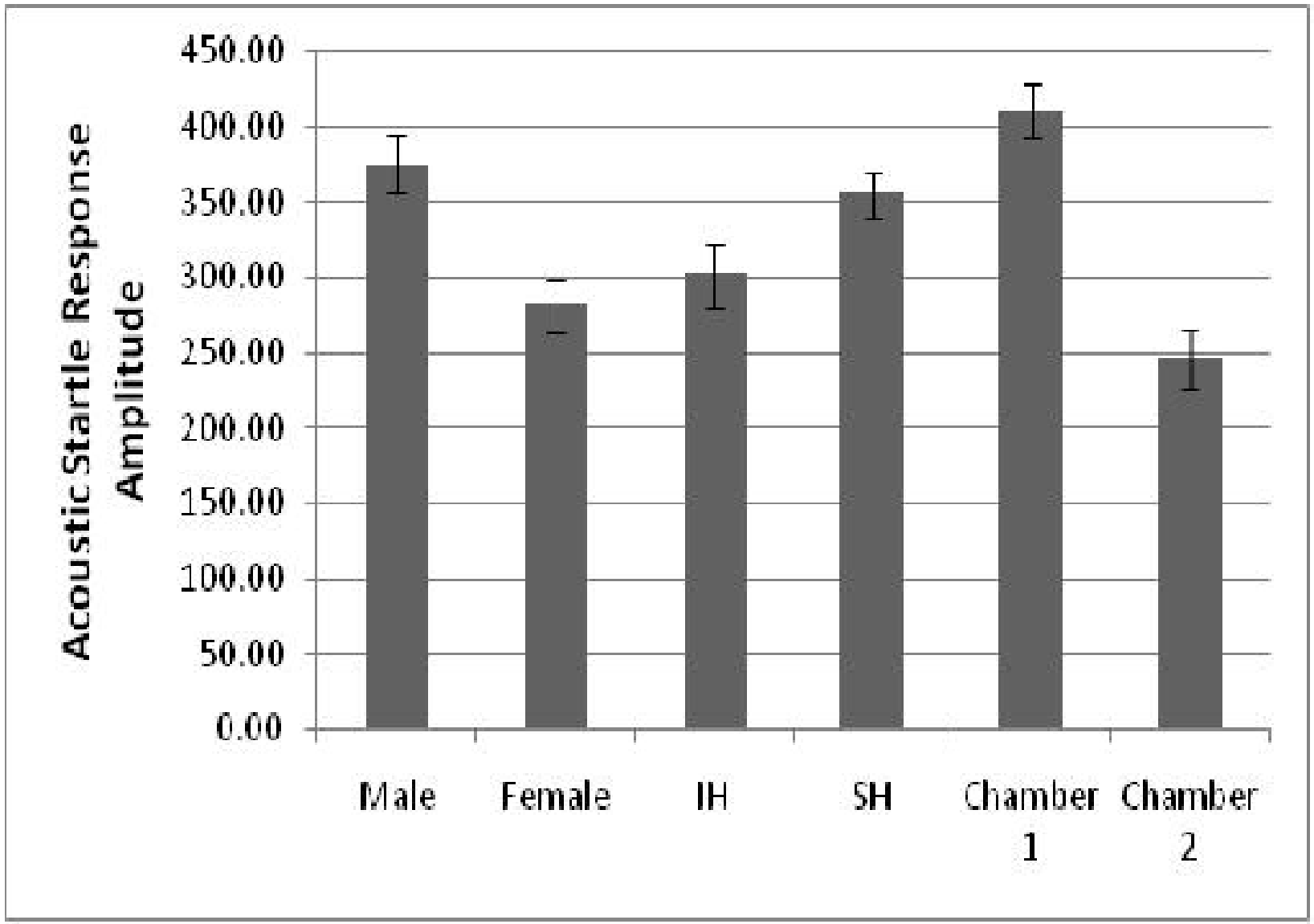
APPENDIX C. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 2 (Chamber 1, Chamber 2) x1 (120 dB) ANOVA for All Subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2103069.06	31	67840.94	3.51	0.00
Intercept	12081917.29	1	12081917.29	625.58	0.00
Sex	247227.23	1	247227.23	12.80	0.00
Housing	82020.12	1	82020.12	4.25	0.04
Group	116234.23	3	38744.74	2.01	0.12
Seq	761295.55	1	761295.55	39.42	0.00
Sex * Housing	13873.89	1	13873.89	0.72	0.40
Sex * Group	68704.95	3	22901.65	1.19	0.32
Housing * Group	6547.02	3	2182.34	0.11	0.95
Sex * Housing * Group	38882.44	3	12960.81	0.67	0.57
Sex * Seq	1861.10	1	1861.10	0.10	0.76
Housing * Seq	6454.47	1	6454.47	0.33	0.56
Sex * Housing * Seq	2162.49	1	2162.49	0.11	0.74
Group * Seq	45699.43	3	15233.14	0.79	0.50
Sex * Group * Seq	26330.68	3	8776.89	0.45	0.71
Housing * Group * Seq	30002.10	3	10000.70	0.52	0.67
Sex * Housing * Group * Seq	82140.45	3	27380.15	1.42	0.24
Error	2201698.68	114	19313.14634		
Total	21221755.14	146			
Corrected Total	4304767.74	145			

Sex	Mean	Std. Error
Male	374.79	19.34
Female	280.98	17.70

Housing	Mean	Std. Error
IH	300.87	21.47
SH	354.90	15.05

Seq	Mean	Std. Error
Chamber 1	410.19	17.86
Chamber 2	245.58	19.19

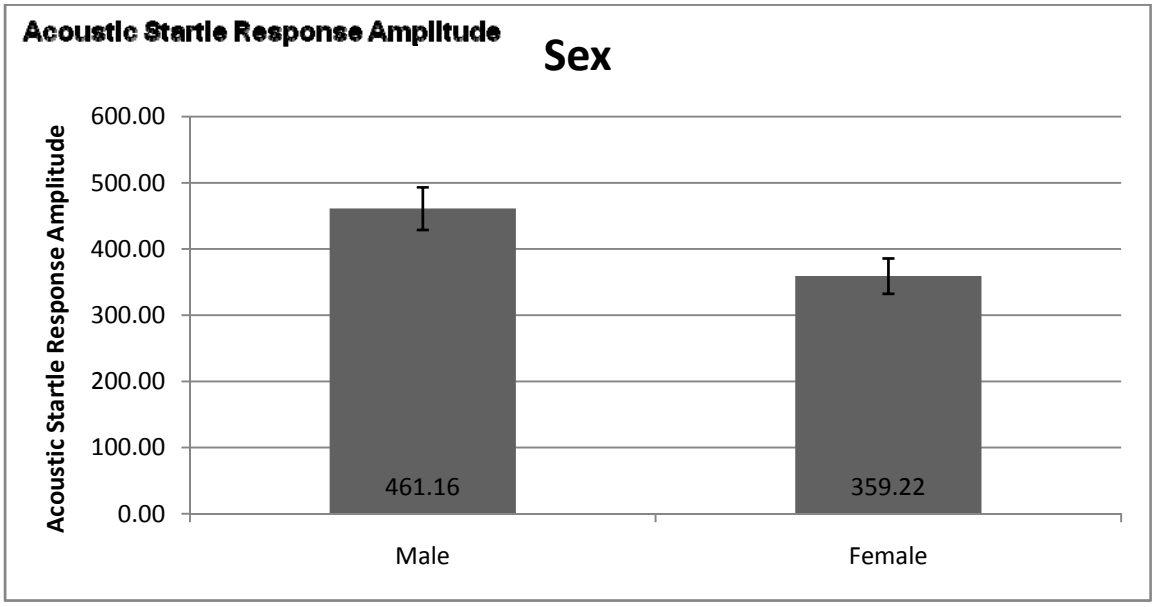

Acoustic Startle Response Amplitude
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Male
Female
IH
SH
Chamber 1
Chamber 2

APPENDIX D. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 1 (120 dB) ANOVA for All Subjects by Chamber.

Chamber 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	726775.81	15	48451.72	1.83	0.05
Intercept	10186383.69	1	10186383.69	384.38	0.00
Sex	157297.48	1	157297.48	5.94	0.02
Housing	72452.17	1	72452.17	2.73	0.10
Group	103102.01	3	34367.34	1.30	0.28
Sex * Housing	14540.46	1	14540.46	0.55	0.46
Sex * Group	58010.91	3	19336.97	0.73	0.54
Housing * Group	7911.04	3	2637.01	0.10	0.96
Sex * Housing * Group	131279.48	3	43759.83	1.65	0.19
Error	1696053.13	64	26500.83		
Total	16506022.32	80			
Corrected Total	2422828.94	79			

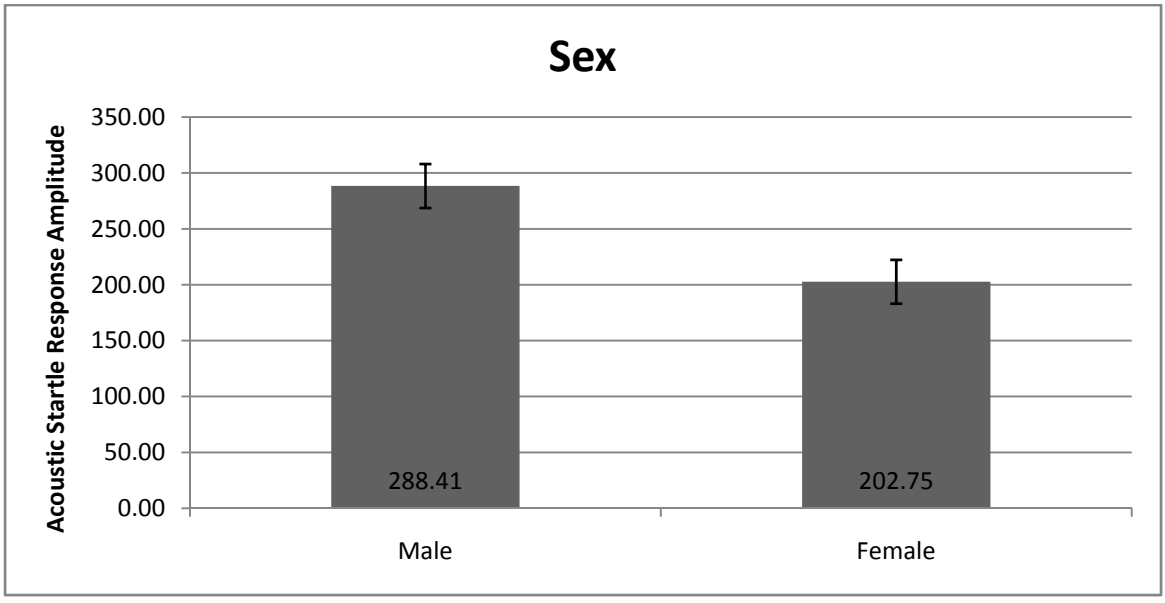
Sex	Mean	Std. Error
Male	461.16	32.24
Female	359.22	26.68



Chamber 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	266937.61	15	17795.84	1.76	0.07
Intercept	3161614.02	1	3161614.02	312.63	0.00
Sex	96182.44	1	96182.44	9.51	0.00
Housing	19805.50	1	19805.50	1.96	0.17
Group	56660.31	3	18886.77	1.87	0.15
Sex * Housing	2370.43	1	2370.43	0.23	0.63
Sex * Group	42185.83	3	14061.94	1.39	0.26
Housing * Group	26327.98	3	8775.99	0.87	0.46
Sex * Housing * Group	8588.60	3	2862.87	0.28	0.84
Error	505645.56	50	10112.91		
Total	4715732.82	66			
Corrected Total	772583.17	65			

Sex	Mean	Std. Error
Male	288.41	19.68
Female	202.75	19.61



APPENDIX E. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 3 (76, 80, 84 dB) ANOVA for All Subjects.

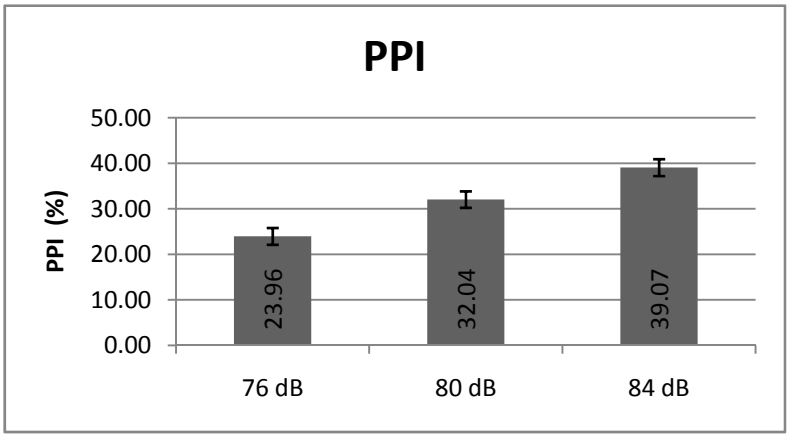
Test of Within Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PPI	13562.18	2	6781.09	60.26	0.00
PPI * Sex	316.58	2	158.29	1.41	0.25
PPI * Housing	94.94	2	47.47	0.42	0.66
PPI * Group	229.53	6	38.26	0.34	0.92
PPI * Sex * Housing	255.52	2	127.76	1.14	0.32
PPI * Sex * Group	430.00	6	71.67	0.64	0.70
PPI * Housing * Group	275.30	6	45.88	0.41	0.87
PPI * Sex * Housing * Group	467.24	6	77.87	0.69	0.66
Error(PPI)	29256.73	260	112.53		

Test of Between Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	357440.66	1	357440.66	365.66	0.00
Sex	3615.63	1	3615.63	3.70	0.06
Housing	182.04	1	182.04	0.19	0.67
Group	2514.05	3	838.02	0.86	0.47
Sex * Housing	522.99	1	522.99	0.54	0.47
Sex * Group	1397.99	3	466.00	0.48	0.70
Housing * Group	4994.15	3	1664.72	1.70	0.17
Sex * Housing * Group	263.67	3	87.89	0.09	0.97
Error	127076.72	130	977.51		

PPI	Mean	Std. Error
76 dB	23.96	1.85
80 dB	32.04	1.81
84 dB	39.07	1.86



APPENDIX F. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 3 (76, 80, 84 dB) ANOVA for Individuals.

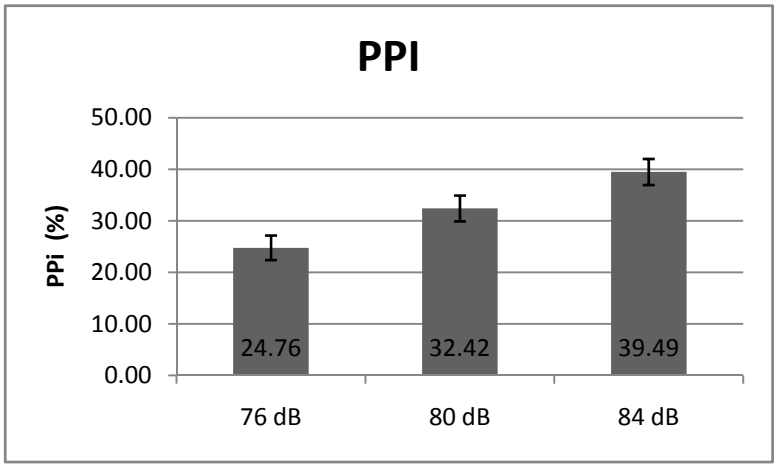
Test of Within Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PPI	4873.87	2	2436.94	34.90	0.00
PPI * Sex	232.21	2	116.11	1.66	0.20
PPI * Housing	64.55	2	32.27	0.46	0.63
PPI * Group	178.94	6	29.82	0.43	0.86
PPI * Sex * Housing	156.54	2	78.27	1.12	0.33
PPI * Sex * Group	450.94	6	75.16	1.08	0.38
PPI * Housing * Group	132.23	6	22.04	0.32	0.93
PPI * Sex * Housing * Group	571.46	6	95.24	1.36	0.24
Error(PPI)	5865.37	84	69.83		

Test of Between Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	139908.91	1	139908.91	203.58	0.00
Sex	250.35	1	250.35	0.36	0.55
Housing	29.12	1	29.12	0.04	0.84
Group	538.08	3	179.36	0.26	0.85
Sex * Housing	3.86	1	3.86	0.01	0.94
Sex * Group	1006.62	3	335.54	0.49	0.69
Housing * Group	1716.41	3	572.14	0.83	0.48
Sex * Housing * Group	251.89	3	83.96	0.12	0.95
Error	28864.81	42	687.26		

PPI	Mean	Std. Error
76 dB	24.76	2.39
80 dB	32.42	2.51
84 dB	39.49	2.54

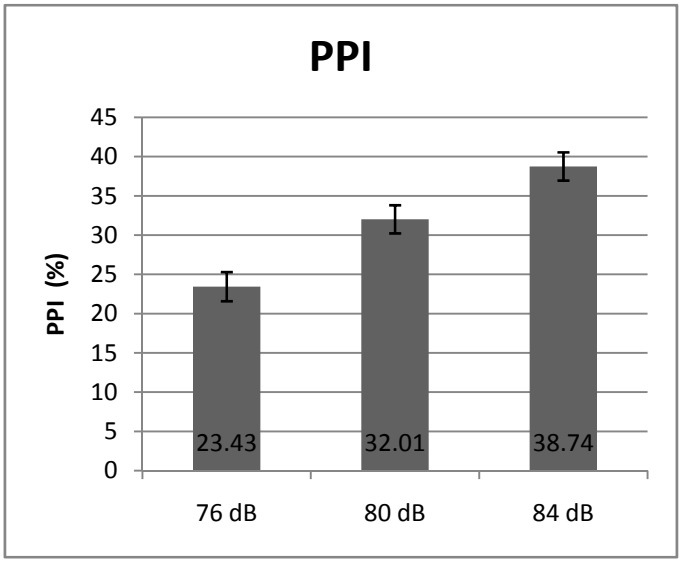


APPENDIX G. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 2 (Chamber 1, Chamber 2) x 3 (76, 80, 84 dB) ANOVA for All Subjects.

Test of Within Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PPI	13248.76	2	6624.38	58.39	0.00
PPI * Sex	283.04	2	141.52	1.25	0.29
PPI * Housing	80.68	2	40.34	0.36	0.70
PPI * Group	321.10	6	53.52	0.47	0.83
PPI * Seq	444.25	2	222.13	1.96	0.14
PPI * Sex * Housing	219.28	2	109.64	0.97	0.38
PPI * Sex * Group	393.80	6	65.63	0.58	0.75
PPI * Housing * Group	352.47	6	58.74	0.52	0.79
PPI * Sex * Housing * Group	601.25	6	100.21	0.88	0.51
PPI * Sex * Seq	75.12	2	37.56	0.33	0.72
PPI * Housing * Seq	51.37	2	25.68	0.23	0.80
PPI * Sex * Housing * Seq	126.03	2	63.01	0.56	0.57
PPI * Group * Seq	614.86	6	102.48	0.90	0.49
PPI * Sex * Group * Seq	210.73	6	35.12	0.31	0.93
PPI * Housing * Group * Seq	544.21	6	90.70	0.80	0.57
PPI * Sex * Housing * Group * Seq	732.06	6	122.01	1.08	0.38
Error(PPI)	25867.83	228	113.46		

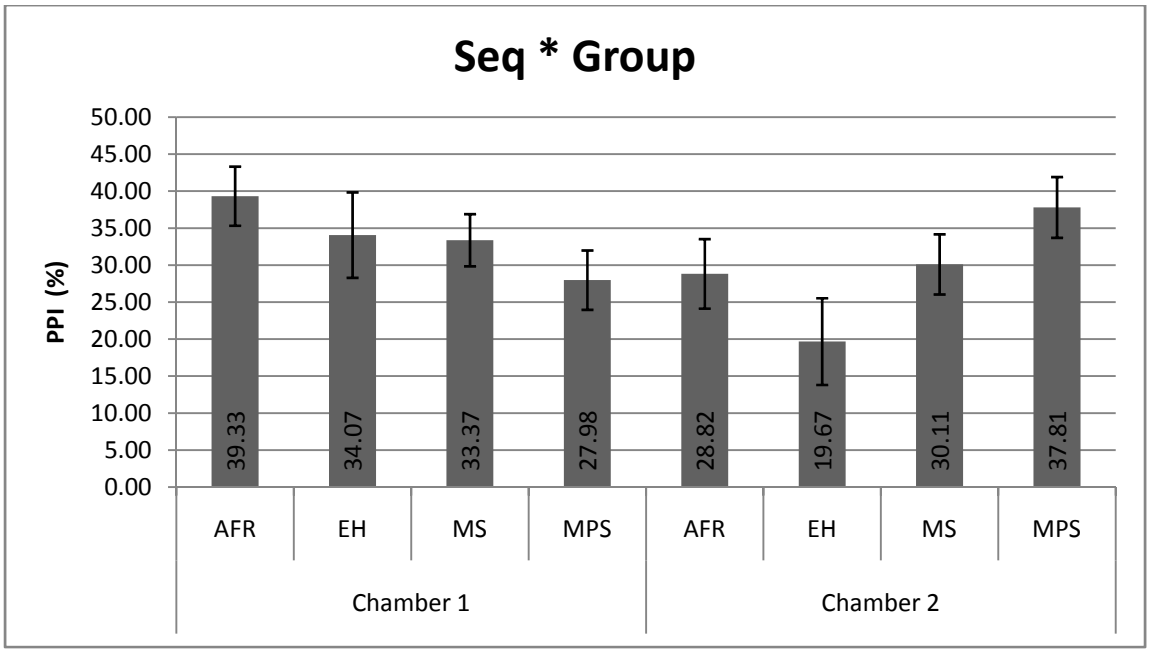
PPI	Mean	Std. Error
76 dB	23.43	1.86
80 dB	32.01	1.79
84 dB	38.74	1.8



Test of Between Subjects

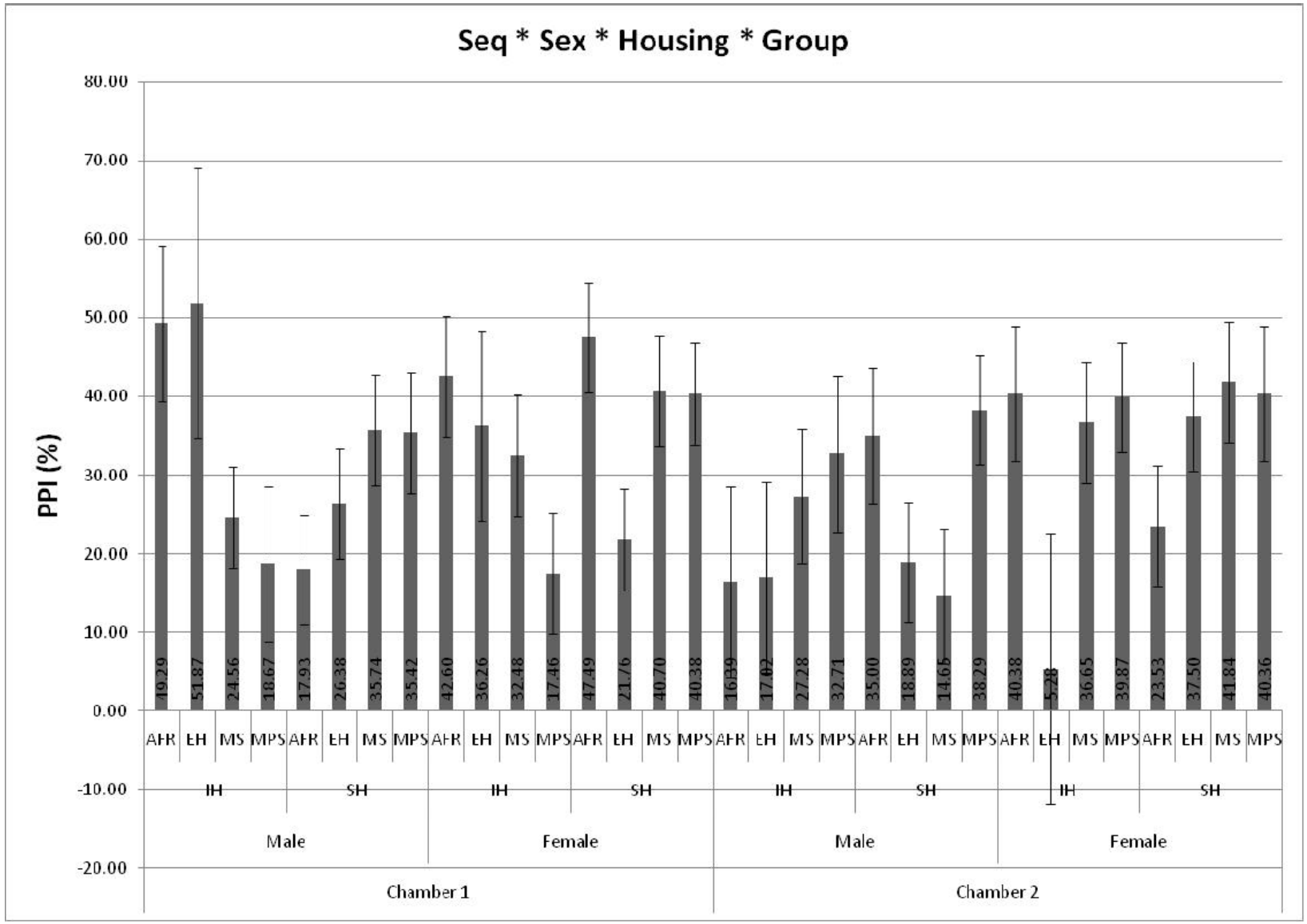
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	332296.89	1	332296.89	376.26	0.00
Sex	2349.28	1	2349.28	2.66	0.11
Housing	241.86	1	241.86	0.27	0.60
Group	1868.73	3	622.91	0.71	0.55
Seq	1770.71	1	1770.71	2.00	0.16
Sex * Housing	1109.89	1	1109.89	1.26	0.26
Sex * Group	2717.80	3	905.93	1.03	0.38
Housing * Group	4100.28	3	1366.76	1.55	0.21
Sex * Housing * Group	1096.66	3	365.55	0.41	0.74
Sex * Seq	693.59	1	693.59	0.79	0.38
Housing * Seq	577.49	1	577.49	0.65	0.42
Sex * Housing * Seq	604.22	1	604.22	0.68	0.41
Group * Seq	7408.99	3	2469.66	2.80	0.04
Sex * Group * Seq	1227.35	3	409.12	0.46	0.71
Housing * Group * Seq	8891.39	3	2963.80	3.36	0.02
Sex * Housing * Group * Seq	8020.53	3	2673.51	3.03	0.03
Error	100680.77	114	883.16		

Seq	**Group**	**Mean**	**Std. Error**
Chamber 1	AFR	39.33	3.99
	EH	34.07	5.77
	MS	33.37	3.53
	MPS	27.98	4.02
Chamber 2	AFR	28.82	4.70
	EH	19.67	5.86
	MS	30.11	4.07
	MPS	37.81	4.11



Seq	Housing	Group	Mean	Std. Error
Chamber 1	IH	AFR	45.94	6.27
		EH	44.07	10.51
		MS	28.52	5.02
		MPS	18.06	6.27
	SH	AFR	32.71	4.95
		EH	24.07	4.77
		MS	38.22	4.95
		MPS	37.90	5.02
Chamber 2	IH	AFR	28.39	7.43
		EH	11.15	10.51
		MS	31.97	5.75
		MPS	36.29	6.07
	SH	AFR	29.26	5.75
		EH	28.20	5.19
		MS	28.25	5.75
		MPS	39.33	5.54

Seq	Sex	Housing	Group	Mean	Std. Error
Chamber 1	Male	IH	AFR	49.29	9.91
			EH	51.87	17.16
			MS	24.56	6.49
			MPS	18.67	9.91
		SH	AFR	17.93	7.00
			EH	26.38	7.00
			MS	35.74	7.00
			MPS	35.42	7.67
	Female	IH	AFR	42.60	7.67
			EH	36.26	12.13
			MS	32.48	7.67
			MPS	17.46	7.67
		SH	AFR	47.49	7.00
			EH	21.76	6.49
			MS	40.70	7.00
			MPS	40.38	6.49
Chamber 2	Male	IH	AFR	16.39	12.13
			EH	17.02	12.13
			MS	27.28	8.58
			MPS	32.71	9.91
		SH	AFR	35.00	8.58
			EH	18.89	7.67
			MS	14.65	8.58
			MPS	38.29	7.00
	Female	IH	AFR	40.38	8.58
			EH	5.28	17.16
			MS	36.65	7.67
			MPS	39.87	7.00
		SH	AFR	23.53	7.67
			EH	37.50	7.00
			MS	41.84	7.67
			MPS	40.36	8.58


Seq * Sex * Housing * Group
PPI (%)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
-10.00
-20.00
19.29
51.87
24.56
18.67
17.93
26.38
35.74
35.42
42.60
36.26
32.48
17.46
47.49
21.76
40.70
40.38
16.39
17.02
27.28
32.71
35.00
18.89
14.65
38.29
40.38
5.28
36.65
39.87
23.53
37.50
41.84
40.36
AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS AFR EH MS MPS
IH
SH
IH
SH
IH
SH
IH
SH
Male
Female
Male
Female
Chamber 1
Chamber 2

APPENDIX H. 4 (AFR, EH, MS, MPS) x 2 (Male, Female) x 2 (IH, SH) x 3 (76, 80, 84 dB) ANOVA for All Subjects by Chamber.

Chamber 1 Test of Within Subjects

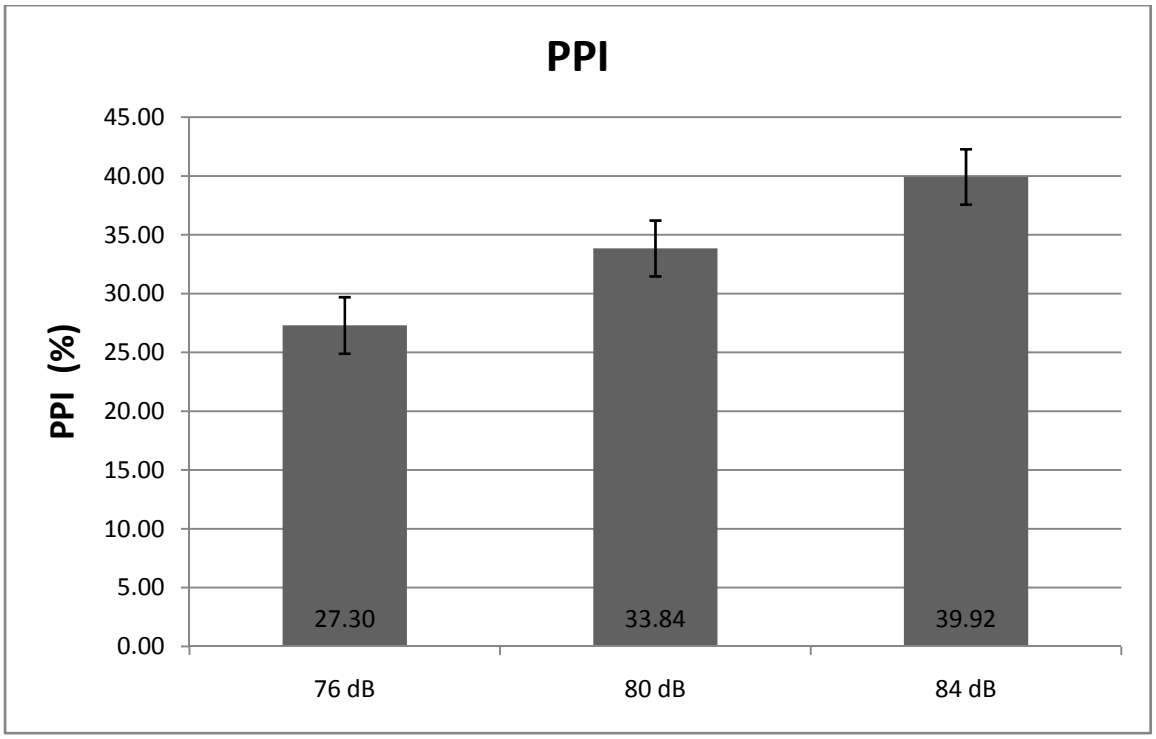
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PPI	4826.40	2	2413.20	22.37	0.00
PPI * Sex	234.57	2	117.28	1.09	0.34
PPI * Housing	126.10	2	63.05	0.58	0.56
PPI * Group	217.33	6	36.22	0.34	0.92
PPI * Sex * Housing	303.47	2	151.74	1.41	0.25
PPI * Sex * Group	193.51	6	32.25	0.30	0.94
PPI * Housing * Group	484.74	6	80.79	0.75	0.61
PPI * Sex * Housing * Group	649.88	6	108.31	1.00	0.43
Error(PPI)	13806.58	128	107.86		

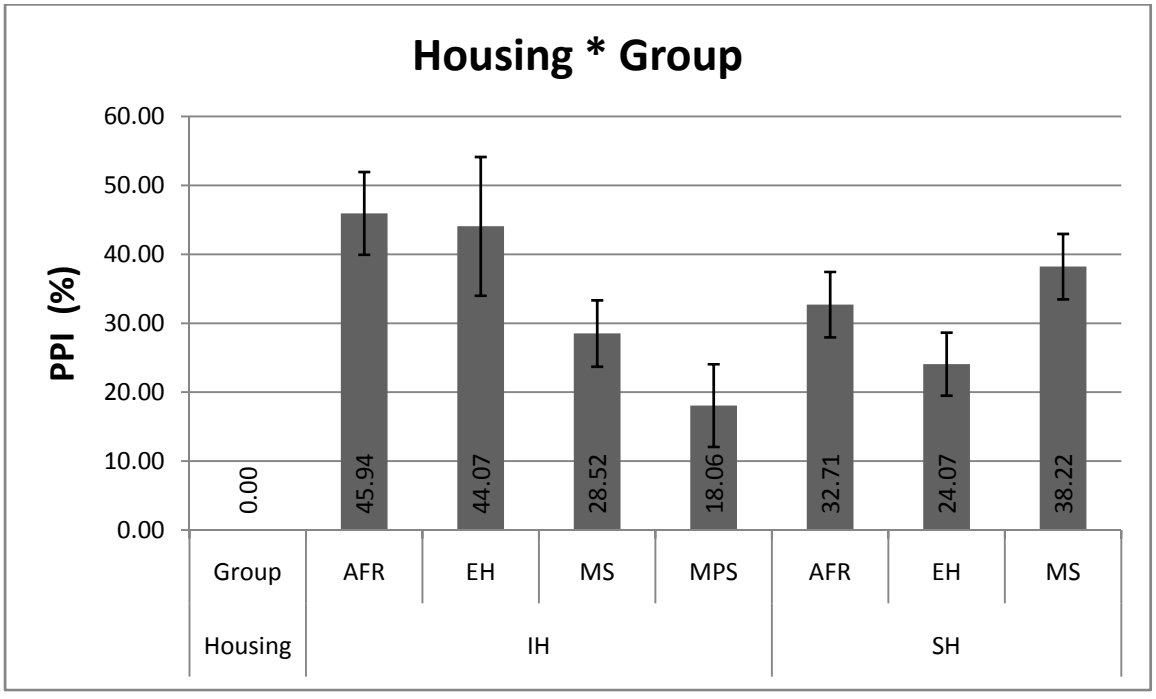
Chamber 1 Test of Between Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	206100.74	1	206100.74	254.26	0.00
Sex	263.90	1	263.90	0.33	0.57
Housing	38.73	1	38.73	0.05	0.83
Group	3555.29	3	1185.10	1.46	0.23
Sex * Housing	1805.72	1	1805.72	2.23	0.14
Sex * Group	2243.96	3	747.99	0.92	0.43
Housing * Group	11820.39	3	3940.13	4.86	0.00
Sex * Housing * Group	3141.87	3	1047.29	1.29	0.28
Error	51877.89	64	810.59		

PPI	Mean	Std. Error
76 dB	27.30	2.41
80 dB	33.84	2.37
84 dB	39.92	2.35

Housing	Group	Mean	Std. Error
IH	AFR	45.94	6.00
	EH	44.07	10.07
	MS	28.52	4.81
	MPS	18.06	6.00
SH	AFR	32.71	4.75
	EH	24.07	4.57
	MS	38.22	4.75
	MPS	37.90	4.81


PPI
PPI (%)
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
27.30
33.84
39.92
76 dB
80 dB
84 dB


Housing * Group
PPI (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
45.94
44.07
28.52
18.06
32.71
24.07
38.22
Group
AFR
EH
MS
MPS
AFR
EH
MS
Housing
IH
SH

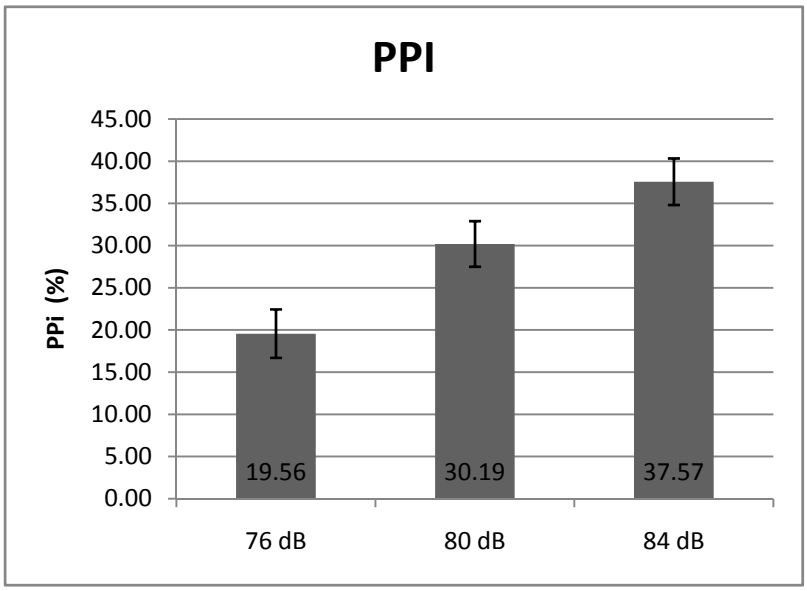
Chamber 2 Test of Within Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PPI	8595.75	2	4297.88	35.63	0.00
PPI * Sex	131.03	2	65.52	0.54	0.58
PPI * Housing	14.00	2	7.00	0.06	0.94
PPI * Group	655.10	6	109.18	0.91	0.49
PPI * Sex * Housing	59.38	2	29.69	0.25	0.78
PPI * Sex * Group	395.30	6	65.88	0.55	0.77
PPI * Housing * Group	418.09	6	69.68	0.58	0.75
PPI * Sex * Housing * Group	667.16	6	111.19	0.92	0.48
Error(PPI)	12061.25	100	120.61		

Chamber 2 Test of Between Subjects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	133204.99	1	133204.99	136.47	0.00
Sex	2610.36	1	2610.36	2.67	0.11
Housing	730.88	1	730.88	0.75	0.39
Group	5931.54	3	1977.18	2.03	0.12
Sex * Housing	35.59	1	35.59	0.04	0.85
Sex * Group	1671.71	3	557.24	0.57	0.64
Housing * Group	1907.12	3	635.71	0.65	0.59
Sex * Housing * Group	5767.96	3	1922.65	1.97	0.13
Error	48802.88	50	976.06		

PPI	Mean	Std. Error
76 dB	19.56	2.87
80 dB	30.19	2.71
84 dB	37.57	2.76



APPENDIX I. Pairwise comparisons of early experience group within housing conditions.

Housing	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Upper Bound	Lower Bound
Isolation Housed	AFR	EH	1.88	11.72	0.87	-21.53	25.29
		MS	17.43	7.69	0.03	2.06	32.80
		MPS	27.88	8.49	0.00	10.92	44.84
	EH	AFR	-1.88	11.72	0.87	-25.29	21.53
		MS	15.55	11.16	0.17	-6.74	37.84
		MPS	26.00	11.72	0.03	2.59	49.41
	MS	AFR	-17.43	7.69	0.03	-32.80	-2.06
		EH	-15.55	11.16	0.17	-37.84	6.74
		MPS	10.45	7.69	0.18	-4.92	25.82
	MPS	AFR	-27.88	8.49	0.00	-44.84	-10.92
		EH	-26.00	11.72	0.03	-49.41	-2.59
		MS	-10.45	7.69	0.18	-25.82	4.92
Socially Housed	AFR	EH	8.64	6.59	0.19	-4.53	21.80
		MS	-5.51	6.71	0.41	-18.92	7.89
		MPS	-5.19	6.76	0.45	-18.69	8.31
	EH	AFR	-8.64	6.59	0.19	-21.80	4.53
		MS	-14.15	6.59	0.04	-27.31	-0.99
		MPS	-13.83	6.64	0.04	-27.09	-0.57
	MS	AFR	5.51	6.71	0.41	-7.89	18.92
		EH	14.15	6.59	0.04	0.99	27.31
		MPS	0.32	6.76	0.96	-13.18	13.82
	MPS	AFR	5.19	6.76	0.45	-8.31	18.69
		EH	13.83	6.64	0.04	0.57	27.09
		MS	-0.32	6.76	0.96	-13.82	13.18

APPENDIX J. Pairwise comparisons of housing conditions within early experience group.

Group	(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Upper Bound	Lower Bound
AFR	IH	SH	13.24	7.65	0.09	-2.05	28.52
	SH	IH	-13.24	7.65	0.09	-28.52	2.05
EH	IH	SH	20.00	11.06	0.08	-2.09	42.08
	SH	IH	-20.00	11.06	0.08	-42.08	2.09
MS	IH	SH	-9.70	6.76	0.16	-23.20	3.80
	SH	IH	9.70	6.76	0.16	-3.80	23.20
MPS	IH	SH	-19.83	7.69	0.01*	-35.20	-4.47
	SH	IH	19.83	7.69	0.01*	4.47	35.20