ANIMAL WELFARE AND POULTRY SLAUGHTER: DETERMINING IF CHICKENS CAN DEVELOP AN AVERSION TO ELECTRICAL STUNNING

by

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(Under the direction of Daniel L. Fletcher)

ABSTRACT

Two studies were conducted to determine if chickens can associate an unpleasant experience with electrical stunning. In the first study, association between a discriminative cue and electrical shock or electrical stunning was examined. A motivational conflict experiment was set up using electrical stimulation as a potential negative reinforcement and food as reward to prompt avoidance and approach motivations, respectively, in the laying hens. Because the birds were not shackled, electricity was applied through clips to the cloaca and comb. However, other research has shown that the electrodes misplaced on the bird may not simulate stunning as it would occur in the processing plant. The second study used an electroencephalogram to record brain wave patterns in White Leghorn hens and a commercial strain of chickens following different sources of electrical stimulation.

Index words: Chickens, Stunning, Aversion Conditioning, Electroencephalogram

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CHAPTER 1

INTRODUCTION

Animal welfare is a controversial issue. Many consumers are concerned with the practices involved in the humane production and processing of food animals. Depending on national origin, ethic background, religious or economic status, people have differing opinions on what is considered humane and ethical. However, most people feel that animals have the right to a comfortable existence, proper treatment, and a humane death.

There are many different slaughter methods, including ventricular fibrillation, decapitation, gas killing, and electrical stunning followed by ensanguination. The major difference between stunning and killing is that with stunning the animal's heart continues to have the ablility to pump fresh blood to the brain. Killing either stops the brain from signaling the heart to beat or stops blood from reaching the brain. Stunning impairs brain function but continues to allow the heart to pump. In the US poultry industry the preferred method of slaughter is electrical stunning followed by neck cutting and exanguination. Stunning provides two major benefits: it is economical and is deemed to be humane. Effective stunning first renders the bird unconscious, and therefore insensible to pain, before neck cutting.

In the U.S., most broilers are electrically stunned and then followed by an automated bilateral neck cut (to sever both the carotid arteries and jugular veins). The loss of blood is so rapid that brain ischemia quickly ensues and the bird in most cases is prevented from regaining consciousness. If the cut or bleed loss is insufficient, it is possible that some birds could regain consciousness if inadequately stunned. For this reason it is important that stunning be sufficient to render the animal unconscious for a time sufficient to allow death to occur without the bird regaining consciousness.

The limiting electrical conditions (voltage, current, frequency and time) of application necessary to render and keep a bird unconscious have not been clearly defined. Some animal welfare advocates are concerned that birds may only be electrically immobilized and therefore still sensible to pain, rather than being made unconscious and insensible to pain. To determine if birds are conscious following stunning, experiments were conducted to determine if the bird can associate a discriminative cue with an unpleasant experience, such as electrical shock or electrical stunning. If the bird associates an electrical stimulation with a cue, one may be able to elicit a behavior that will show the birds' reaction to the cue when the stimulation is not present. For example, a bird that finds a situation aversive may avoid that situation. The test hypothesis for this experiment was to determine if chickens can associate a discriminative cue with an unpleasant experience and with electrical stunning.

CHAPTER 2

LITERATURE REVIEW

Animal welfare standards are becoming increasingly important in regard to the care, handling and processing of animals. There is ongoing debate over animal welfare in regard to in regard to research, livestock production, companion animals, etc. This paper will focus on the welfare of poultry during slaughter.

In a recent article in Consumer Reports (Guest, 2004), it was pointed out that many labeling ploys involving words like "cruelty free" and "no animal testing" have no government or industry wide definition of terms and that manufacturers may label their products with these phrases to play on the consumer's emotions. While this paper is not concerned with ploys of manufacturers and their advertisers, the problem is similar. The terminology involved in animal welfare with regards to poultry slaughter, words like poultry, welfare, humane, stunning, and consciousness must first be clarified before meaningful guidelines can be established.

The U.S. Code of Federal Regulations (CFR) document "Title 9-Animal and Animal Products" addresses the humane handling, care, treatment, transportation and slaughter of agricultural animals. However, under the definition of "farm animal" poultry is absent.

> "Farm animal means any domestic species of cattle, sheep, swine, goats, llamas, or horses, which are normally and have historically, been kept and raised on farms in the United States, and used or intended for use as food or fiber, or for improving animal nutrition, breeding, management, or product efficiency, or

for improving the quality of food or fiber. This term also includes animals such as rabbits, mink, and chinchilla, when they are used solely for purposes of meat or fur and animals such as horses and llamas when used solely for work and pack animals" (9CFR1.1).

The definition for poultry can be found in part 145 of Title 9, entitled "National Poultry Improvement Plan," which has voluntary participation. The definition reads "*Poultry*. Domesticated fowl, including chickens, turkeys, ostriches, emus, rheas, cassowaries, waterfowl, and game birds, except doves and pigeons, which are bred for the primary purpose of producing eggs or meat." Part 145, however, does not address the slaughter of poultry.

Neither Section 313 nor 145 of the CFR give a definition for "welfare" or "humane" as related to agricultural practices. One can build upon the most basic definition of the words to extract a definition from the CFR. The word "welfare" is defined as "the state of doing well esp. in respect to good fortune, happiness, well-being, or prosperity" (Merriam Webster's Colligate Dictionary, 1993). The same dictionary defines humane as "marked by compassion, sympathy or consideration for humans or animals." Keeping these descriptions in mind with respect to animals, one could argue that more precise definitions of welfare and humane are based on what they are not, i.e. animal cruelty. Animal cruelty is "a cruel act; a deliberate infliction of pain and suffering" (Merriam-Webster, 1993), in other words animal cruelty is a deliberate painful procedure inflicted on an animal. Using this definition, the CFR defines painful procedure as;

> "painful procedure as applied to any animal means any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being which that

procedure was applied, that is, pain in excess of that caused by injections or other minor procedures" (9CFR1.1).

While the CFR addresses the humane slaughter of livestock, it does not include poultry in its definition as stated earlier. Using the definitions provided by Merriam-Webster for welfare and humane, we can find related definitions in the CFR which would suffice for the purpose of this paper. However because slaughter is such a broad topic and because there has been so much research in the area, it is necessary to review poultry slaughter before defining "stunning" or consciousness."

STUNNING

Historically in the meat processing industry, stunning was used to immobilize a large animal to ensure the safety of the operator during slaughter (Fletcher, pers. comm.). In the case of poultry, stunning was used to allow automation of the neck cutting procedure. Currently, stunning is used to render the animal unconscious without feeling pain or distress prior to neck cutting and exsanguinations. The most prevalent stunning method, in chickens, is to pass an electric current through the brain prior to bleed out.

Other stunning methods exist. Captive-bolt stunning is used with large animals. It involves a bolt fired from a gun at a specific point on the animal's forehead with sufficient force to cause a concussion. A percussion bolt works similarly; however, the bolt doesn't enter the skull. Neither of these are useful for stunning poultry due to the high rate labor that would be required and the small target area. Lastly, stunning can occur via assorted mixtures of inert gases, oxygen or carbon dioxide. Gas stunning reduces handling stress and increases the ease of shackling. However, before any method can be labeled more humane than the others, well planned research is needed on these methods.

TONIC IMMOBILITY and STUNNING

Alarms calls, tonic immobility (TI), flight responses (such as jumping and flapping), and avoidance are all examples of behavioral responses of fearful birds. Fear behavior is influenced by genetics, novelty of a stimulus, learning, intensity of a stimulus or interaction with conspecifics (Gray, 1979). While some behaviors may seem contradictory to one another, they can be grouped into one of general categories: active defense, active avoidance, or immobility/movement inhibition (Boissy, 1995).

A somewhat unique behavior of the chicken is a tendency to become completely immobile, a condition termed tonic immobility, when put into a fearful situation. The function of such a behavior is to increase the probability of escape or reduce injury from a predator.

> Tonic immobility is "characterized by inhibition of righting reflexes and a state of catatonic-like reduced responsiveness following a brief exposure to physical restraint, which may last from a few minutes to over several hours. Other characteristics [of tonic immobility] include suppressed vocal behavior, intermittent periods of eye closure, mydriasis, waxy flexibility and muscle tremors in the extremities." (Nash *et al.*, 1976).

Researchers cannot agree upon physiological responses during tonic immobility, such as the direction or significance in heart rate or respiration. A clear understanding of the mechanisms underlying tonic immobility also continue to be poorly understood; however the duration of the response has proven to be directionally related to factors which affect fear (Gallup, 1974).

Under natural conditions tonic immobility is elicited by predator prey interactions. In the laboratory, tonic immobility has been elicited via exposure to loud noise (Gallup *et al.*, 1970), suspension over a visual cliff (Gallup and Willamson, 1972), injections of adrenaline (Braud and Ginsburg, 1973), conditioned aversive stimuli and electric shock (Gallup, 1973).

Shock induced tonic immobility is similar to electroimmobilization, a muscular immobilization caused by an electrical current, in the fact that a mild electrical stimulus can elicit a tonic immobility reaction. Electroimmobilization is caused by low currents, often at low frequencies (Gregory, 1998). During water bath stunning, it has been estimated that only 10 to 28% of the electrical current flows through the brain, while the majority of the current flows through the muscle. The route of current controls the muscle movement making it hard to judge if the animal is unconscious (Wooly *et al.,* 1986a, 1986b). The possibility that electro-immobilization does not always cause unconsciousness is one argument against stunning. Some believe that the stunning process should be such that the bird is rendered irreversibly unconscious. However, the definition of unconsciousness in poultry has yet to be clarified.

STUNNING and UNCONSCIOUSNESS

One proposition for a "stun" is given by Raj and Tserveni-Gousi (2000), "It is assumed that the 'near threshold' depolarized state of neurons in the brain, especially in the thalamus and cerebral cortex, is necessary for the perceptual processes. Electrical stunning therefore needs to disrupt the depolarized state of the neurons in the brain and render the animals or birds unconscious and insensible."

Another definition of stunned, based upon behavior and physiological state, is given by N.B. Gregory (1998),

> "A useful behavioral event to watch for when inspecting electrical stunning is the return of rhythmic breathing movements. This coincides with the end epileptiform activity in the brain. Often it coincides with the end of carcass kicking. It indicates that hypersynchrony emanating from the reticular formation has ended, and some of the normal medullary function has been reinstated. This means that other features of brain function will probably be recovering and so in this situation the return of brain functions will probably be recovering and so in this situation the return of normal rhythmic breathing should be regarded as a prelude to the resumption of consciousness.
>
> Running movements can occur during the clonic convulsive phase of electrically-induced epilepsy. These are due to the activation of subthalamic locomotor regions. They take over once the tonic phase of electrical stunning has ended. The behavior arising from this activity should be distinguished from escape behavior in otherwise unstunned animals when evaluating the effectiveness of electrical stunning. This can be done by watching for simultaneous breathing and righting activity."

In this definition clonic is defined as "alternate muscular contraction and relaxation in rapid succession" and tonic is "characterized by continuous tension" (Dorland's Medical Dictionary, 1994).

Minimum currents necessary to achieve an effective stun can be derived in the laboratory using either the spontaneous EEG or loss of brain responsiveness determined using somato-sensory evoked potentials (SEPs). The current required to lose either in the brain of these is 100mA (Gregory, 1989). However, a sustained loss of SEPs (until death supervenes) requires the same as that for ventricular fibrillation, i.e. irreversible stunning methods, which is 120mA (Gregory, 1990). This research is focused on the behavior and EEG waveform patterns as a method for determining unconsciousness.

No clear brain pattern can be identified on an EEG that establishes when unconsciousness occurs, though several theories exist on the correct waveform. In humans, a grand mal seizure (epilepsy) is associated with the loss of consciousness. Persons who have undergone such seizures report a lack memory during episodes. On an EEG, epileptic seizures show high amplitude, around 100µV, and low frequency waveforms ranging from 8 to 13 Hz. Similar waveforms have been found in large animal species, such as cattle, swine, veal, and sheep (Gregory, 1998). However, no such waveforms have been consistently found in avian species. Instead, some researchers (Gregory and Wotton, 1987) choose to use the formation of polyspike activity found in avian species' EEGs to determine stunning. These polyspikes which are comparable to petit mal seizures in humans, but are not always associated with unconsciousness. However, this wave formation requires high current stun around 120 mA, which is likely to kill the bird and cause carcass damage.

Gregory and Wotton's (1987) conducted an experiment attempting to determine an electrical threshold (current and frequency) and a mode of electrical application at which the disruption in a chicken's brain activity was similar to the grand mal waveforms found in humans. The result was an electrically induced waveform recorded by EEG. Basically, after stimulation an epileptic waveform should appear followed by an electrically quiet period. Methods notwithstanding, the low frequency, polyspike activity was defined as a waveform of 3.1 Hz and the high polyspike activity was defined as a waveform of approximately 6 Hz. Voltage varied between 50 and 250 V. The polyspike activity ended abruptly, within a range of 8 to 36 seconds after the start of stunning. The results indicate that when very high currents were used the epileptiform phase dissipated in frequency, rather than an increased. It was also found that at low currents, between 30-60 mA, less than 5% of the birds experienced fibrillation, while 99% of birds subjected to 148mA experienced fibrillation. The authors noted that the higher currents (over 100mA) eliminated the expression of the epileptiform phase rather than increasing frequency in the waveform as would be expected, but only increased the likelihood of fibrillation. Based upon these results, their conclusion was that for a 50 Hz sinusoidal waveform the current must be set at 148 mA. However, higher frequency waveforms (grand mal) were not as likely to occur as low frequency waveforms (petit mal). In fact of the 18 birds tested, only two gave the higher frequency waveforms.

In humans electroconvulsive therapy (for drug resistant psychiatric patients) induces seizures. A common, effective method for conduction uses a pulsed DC waveform which requires less electrical energy than the sine wave (Liberson, 1948). Though conventional DC waveforms are capable of inducing seizure, new ultrabrief pulsed DC devices are reported to be more effective (Sackiem *et al*., 1994). The ultrabrief pulse DC causes high amplitude, low frequency waveform, similar to that of polyspike activity, followed by a drastically suppressed EEG (Krystal and Weiner, 1999). This criterion would seem to justify the effectiveness of the high voltage and low frequency electrical stunning method in chickens using the formation of polyspike activity in the EEG as indicative of unconsciousness. But it does not go without criticism, Raj (2003) states that although "…a prolonged application of such a low current delivered with this waveform and frequency may render the chickens unconscious, it would involve a potentially painful induction of unconsciousness or immobilization."

Therefore, strength and type of stunning current are the main attributes affecting the level of consciousness. "The duration of consciousness depends on the current that is used and the duration for which the current is applied" (Gregory, 1998). More specifically, it has been observed that as the frequency of the stunning current increases, the time taken to resume consciousness decreases (Mouchoneire *et al.,* 1999).

STUNNING REQUIREMENTS FOR HUMANE SLAUGHTER

Though, the US government has placed no mandatory regulations on the stunning or slaughter processes in the poultry industry, however, most companies comply with stunning prior to slaughter for automation and welfare purposes. For example, the National Chicken Council (NCC), a membership based organization, produces animal welfare guidelines for all aspects of a vertical integration. The council has the right to audit members to ensure chicken companies are complying. The NCC requirements for proper stunning and killing during processing are

> "Stunning and killing equipment should be constantly monitored to insure proper functioning for humane processing. Birds should be insensible to pain when killed. A post-stun posture that includes arched neck and wings tucked in is visual evidence of an effective stun. Backup personnel should be

> employed at the killing station to euthanize manually any bird not
> properly killed by the equipment."

The typical manner for stunning in a poultry processing plant uses 25 to 45 mA/bird stun current applied for 8 to 12 seconds followed by a deep ventral cut (both carotid and jugular veins are severed). The US bleed out time is a relatively short 80 to 90 seconds and the rapid blood loss makes it almost impossible for birds to regain consciousness.

In comparison, the EU has formed regulations with regards to slaughter. The regulations with regard to killing or slaughter are found in 93/119/EEC (1993) and specify the procedure. It generally states that any avoidable excitement, pain or suffering should be prevented; however, shackling by the feet is allowable. If stunning is used following shackling, it should cause immediate unconsciousness and applied until death supervenes. Should a water bath stunner be used, there are specific regulations on the electrical current and on operation. Shackles must be wetted to increase conductivity. Birds must be stunned at a minimum current of 100mA per bird. The current's duration must last long enough to induce unconsciousness. In a multiple bird stunner, the voltage must be high enough to insure sufficient stunning for each bird. By regulation, slaughter must follow stunning as closely as possible. At least one of the arteries in the neck must be cut during slaughter and no further electrical stimulation can be applied. This one-sided neck cut is the reason for a longer bleed out time in the European system. It is important to mention that "type of cut gives the birds an opportunity to regain consciousness when the cut or bleeding is incomplete" (Gregory, 1992). "This potential for regaining consciousness has been the major reason from the humane standpoint that current levels of 120 to 150 mA are used in Europe to insure an instantaneous and irreversible stun" (Bilgili, 1999).

These two qualifications make the European method a "stun-to-kill," where the bird actually dies from anoxia and ventricular fibrillations (Fletcher, 1993).

QUALITY vs WELFARE

This leads to the current debate between low current/high frequency methods and high current/ low frequency methods for a humane slaughter: a conflict between quality and welfare. Stunning standards also have cost and quality ramifications. The kind of stun affects the quality of poultry meat. Electrical stunning considered humane in the European community, tends to be detrimental to carcass and meat quality. For example, hemorrhages on deep breast muscles of broilers have been shown to increase with high stunning currents of 130 to 190 mA (Gregory and Wilkins, 1990). High stunning voltages are associated with increased incidences of red wing tips and tails (Veerkamp and de Vries, 1983) and broken bones (Walther, 1991). Research shows that low frequency waveform stunning is highly correlated with breast meat hemorrhages and that high frequency stunning can reduce hemorrhages by as much as 70% (Gregory, 1998). Other stunning problems associated with low frequency/high current stunning are wing and shoulder hemorrhages, breast skin hemorrhages, bleeding efficiency (poor bleed out increases the incidence of red feather tracts and red neck skins), and decrease in breast meat pH (increases toughness).

The European poultry industry has placed greater emphasis on welfare standards and opted for high current stunning at the cosumers' bidding, resulting in a higher cost and lower quality product. It is thought by some that European's have a societal perspective, or a social consciousness and accountability, of farming practices (Jameson, 2004, personal communications). They see animals as sentient beings; an object to be protected rather than a subject to be manipulated. European consumers believe they have the right to influence practices via government regulations and non-government organizations. Many changes have been made in Europe as a result. For example, in the egg layer industry there is no beak trimming, no male dubbing, more cage space, housing enrichment (dust baths, nesting, and scratch areas) and less aggressive, lower population strains of hens are used. Some European broiler producers give birds access to a screened in area where they have access to outdoors and dust bathing areas.

However, these practices come at a cost to the farmer, the government and ultimately the consumer. Compared to U.S. farms, European farms are small, less efficient and less competitive. Jameson states "cultural supplementation" must be used to sustain inefficiencies, i.e. the farmers are subsidized because the consumer feels "cultural values are worth defending." In the U.S. customers prefer cheaper, higher quality meat and have only just begun to place an emphasis on animal welfare(Jameson, 2004, personal communications).

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CHAPTER 3

EVALUATION OF AVERSION OF CHICKENS TO ELECTRICAL STUNNING[1]

[1] J. A. Chamier, D. L. Fletcher, R. J. Buhr, A. B. Webster prepared for submission to Poultry Science

ABSTRACT Electrical stunning is the most common method for rendering poultry unconscious prior to slaughter. It has been suggested that some methods of stunning may not completely render the bird insensible to pain during slaughter. Research was conducted to determine if birds trained to approach a feeder would show signs of aversion after receiving an electrical stimulus. Feed deprived laying hens were first trained to approach feed at the opposite end of a straight run alley. During the test period, when the bird reached the feeder, one of four treatments was applied. The treatments included a mild shock treatment (3.3V pulsed DC for 10 seconds), a commercial stun (16V pulsed DC at 500 Hz for 10 seconds), a delayed commercial stun after allowing the birds 10 seconds of feed (reward) or a 0V stimulus control. Bird behavior and vocalizations were recorded. Latency to forward movement and the time taken to approach the feed (180 seconds maximum) were measured. The two electrical stun and mild shock groups showed increases in latencies for both behaviors, while the control group showed no difference from the pre-test training period. The immediate stun and delayed stun treatments showed no differences between treatments; however, both had greater latencies than the mild shock treatment. These results suggest the stimulus treatments perceived the electrical application as an aversive event.

Key Words: Electrical stunning, welfare, aversion, elicited behavior

INTRODUCTION

Stunning of poultry, originally employed to immobilize an animal for slaughter, has become a focus in the concern for animal welfare in agriculture. The primary welfare issue regarding low current high frequency electrical stunning is whether it is a sufficient method to render the animal unconscious and insensible to pain prior to slaughter. Much research has been done to determine the proper amount of current which induces unconsciousness using physiological indicators of unconsciousness, such as electroencephalograms, somatosensory evoked potentials, muscle tension, or neurological transmitters, and therefore insensibility to pain (Cook et al., 1995); (Gregory and Wotton, 1986, 1987, 1989, 1990, 1994). The reason for using physiological indicators are that unconsciousness and perceived pain are mental states of the animal, which are not readily accessible for direct investigation, much less for determining the level of such states in relation to welfare.

There are three major views in the assessment of animal welfare. Duncan and Petherick (1991) claimed that the emotion state of the animal and the extent of which that state is compromised (suffering) determines the welfare status of an animal. Moberg (1993) argued for a risk assessment view of animal welfare on the basis of physical condition. If the animal's physical condition included such "risks" as illness, poor performance, reduced reproductive performance or death, then the welfare of the animal was compromised. Lastly, Broom (1996) maintains that a coping strategy be used to determine an animal's welfare. If an animal is unable to cope with its environment, its welfare is compromised. Though the three approaches cannot agree on the causes of suffering, they do agree that the continuum, or the extent, of the animal's suffering is the acceptable criterion for judging animal welfare. Also, it is important to note the general acceptance of the use of behavior as the indicating element for the welfare criterion of suffering.

Because of the nature of electrical stunning little experimentation has been done on its effect on behavior. Shackling prior to stunning limits the amount of behaviors a bird will exhibit and also confounds any behaviors caused by stunning alone. If shackling is removed from the equation, then an experiment may be set up to elicit behaviors from the animal that will be cluea to the bird's mental state during stunning. Rushen (1996) promotes the use of aversion-learning techniques to give insight to animal's mental state through its behaviors. "When an animal is [exposed to a stimulus] in a way that causes it some distress, we cannot directly perceive the suffering it is experiencing. However, animals can learn to predict, from certain cues or signals, how they are to be [exposed] and then show some aversion to these signals." He also stated that these types of methods created an objective tool to measure the degree of the responses and, therefore "infer the extent of the suffering caused to the animal by the handling method" (Rushen, 1986). The ability to associate and predict cues assumes that an animal retains some memory of the event and the consequences of particular behaviors related to the event. Poultry have been shown to have distinct short-term memories (Rose, 1991) and long-term memories (Rose, 1991; Rosta *et al.*, 1991) and passive avoidance experiments have shown the use of both in day old chicks (Stewart and Rusakov, 1995). It is important to note that "the outcome of experiments in aversion learning can be affected by factors influencing the learning ability and memory of animals" (Rushen, 1996).

This experiment was conducted to determine if a bird can remember over repeated trials an unpleasant event paired with feed. Specifically in this case, can the bird associate feed with electrical stunning? If association is learned, then a behavior might be elicited from the bird showing aversion to the feed. "In avoidance learning, the animal must perform a behavior in order to avoid the treatment" (Rushen, 1996). The learned behavior in this method, avoidance of the feed, allows for the inference of an underlying motivational conflict between hunger and pain. In this study, the different birds receiving no electric pulse, a shock and a stun are compared for differences and similarities in behavioral responses to the electrical stimulus.

MATERIALS AND METHODS

Source of Birds

Single Comb White Leghorn laying hens (Hy-Line W36[2]) were obtained at 54 weeks of age. The hens were caged individually in a single level layer house with free access to water and a standard layer diet. Leg bands were used to identify individual birds to their cage.

Electrical Attachment

A total of 30 birds were fitted with electrodes immediately behind the comb (head electrode) and on the underside of the tail above the cloaca (cloacal electrode). The electrodes consisted of standard safety pins subcutaneously inserted immediately behind the comb and the underside of the tail. All electrodes were inserted under the supervision of an attending veterinarian. During training and experimental trials, the electrical conduction wires by which the current was applied to the birds were connected to the electrodes using alligator clips. The wiring from the head electrode was aligned along the neck, back and under the wing to prevent the wires distracting the bird's vision and to allow the bird unhindered movement. The wires were attached to a commercial stunner to allow a head to tail application similar to commercial stunning via head contact with the electrical plate and grounding via caudal rub bars and shackles. When the wiring harness was attached, both head and cloacal electrodes were

[2] Hy-Line International, West Des Moines, Iowa

dampened with salt water solution to enhance electrical contact.

Training

For 30 days over a six week period hens were trained to approach a feed source placed in a test alley. The birds were deprived of feed for 7 hours prior to training and testing to ensure they were hungry and motivated to approach the feeder. The test alley was a black-plastic covered wire mesh pen 0.61 m wide by 1.32 m long by 0.61 m high. The black plastic was used to reduce visual stimulation and distraction from outside the test alley. When necessary, a flexible plastic mesh cover was used to prevent birds from jumping out of the alley during early training sessions. The test alley was located in a separate room isolated from other birds and free from outside distractions. A section of layer cage feed trough filled with feed served as the feed source and was placed at one end of the test alley.

During training, each bird was removed from its home cage and hand carried from the layer house to the laboratory containing the test alley (approximately 50 meters). Following the training session, the bird was hand carried back to its home cage. Access to feed was restored approximately 30 m later to avoid the bird perceiving an immediate reward.

The birds were trained using a shaping exercise conducted in four phases. In phase 1, the bird was actually placed on the feed and allowed to eat for 3 min. This was continued until 70% of the birds began to eat immediately upon placement. Those birds that did not eat immediately were removed from the experiment. In phase 2, the birds were placed directly in front of, but not on the feeder. Phase 2 continued until 90 % of the birds began to eat immediately upon placement. Again, those birds that did not eat immediately were removed from the experiment. In phase 3, the birds were placed in the middle of the alley facing the feeder until 90% immediately approached the feeder and ate. Once, again, those that did not eat were removed from the experiment. In phase 4, the birds were placed at the opposite end of the alley from the feeder until 90% immediately traversed the alley and ate. Following the final phase, 15 hens had been successfully trained to immediately approach the feeder and eat upon placement in the test alley.

During the training session and subsequent trial periods, individual bird reactions to handling were subjectively scored as follows: a score of 1indicated no struggle during removal from the cage or handling (a docile bird), a score of 2 indicated the bird exhibited some escape behavior during catching, but calmed immediately and was docile during removal from the cage and handling, and a score of 3 was used to identify a bird that exhibited escape behavior during removal from the cage and continued struggle during handling. Also, during the training and subsequent trial periods, egg production of the hens was noted.

Experiment

The 15 trained birds were randomly assigned to one of four treatment groups with 4, 4, 4 and 3 birds in each group respectively: Group 1, the Control birds were allowed to approach the feeder and eat for the entire 180 s test period. Group 2, the Immediate Stun birds were allowed to approach the feeder at which point they were immediately stunned using a commercial stunner[3] at 14 V pulsed DC, 500 Hz, for 10 s. Group 3, the Mild Shock birds, were allowed to approach the feeder at which point they were subjected to a mild shock by setting the stunner to its lowest setting approximately 3.1 V for 10 s. Group 4, the Delayed Stun birds were allowed to approach the feeder and were allowed to eat for 10 seconds before applying the identical stun as Group 2.

In each of the 14 test days conducted over a 3 week period, individual birds were removed from the cage house, subjected to their treatment and replaced in its cage in the same

[3] Simmons model SF-7001, Simmons Engineering Co., Dallas GA 30132

order. Birds were tested in the order described by group. Bird activity during removal from the cage and handling was scored as previously described.

A timer was started as each bird was placed in the test alley. The time was recorded until the bird first made a move toward the opposite end of the alley with the feed. This time delay (latency period) between placement and movement was recorded in seconds. The time period beginning after the latency period until the bird started to eat was measured as the "approach delay." The maximum allowable time was 180 s. Therefore it was possible for a bird to never begin to approach the feed (reported as 180 s latency and 0 s approach delay) or to start to approach the feeder, but never get close enough to actually eat (reported with a latency of less than 180 s but with an approach delay of 180 s).

The experiment was terminated after 14 trials at such time as the treatments clearly showed a learning plateau and no further trials were deemed necessary. The electrodes (safety pins) were removed and the laying hens returned to the general laying house population. The data were analyzed using ANOVA, Duncan's Multiple Range Test, and descriptive statistics (mean and standard error of the mean) using the GLM procedure (SAS Insitute, 1998). Treatments were tested by trial and reported by means and standard error of the means.

RESULTS AND DISCUSSION

During the trials, the hens appeared to react physically similarly to stunning through the electrodes as they do to stunning on a commercial line. However, since the birds were standing and not hung on shackles, the first reaction was to immediately go rigid and fall to one side. Once the treatment was over, the immobile birds were returned to their cages where they recovered from the stunning treatment. The Mild Shock treatment birds exhibited vocalization, wing flapping and physical agitation during the treatment, but returned to a docile state immediately once the treatment ceased. No birds exhibited any noticeable physical injury or trauma during the 14 test trials.

There were no observed differences in activity scores of the test birds during the 14 trials. All birds received a handling score of 1, indicating that the treatments did not affect the birds responses to handling. Egg production records were maintained on the test birds throughout the training and testing and no change was noted compared to non experimental birds in the flock. (Data not presented.)

The results for the latency test (time delay between placement in the alley and initial movement towards the feed reward) are presented in Table 1 and illustrated in Figure 1. The control birds were highly consistent showing almost no latency throughout the experiment. Significant treatment effects appeared in Trials 5, 7, 9, 10, 12, 13, and 14 (Table 1). The Immediate Stun and Delayed Stun treatments resulted in similar patterns of delaying the birds' initial movement toward the feed by the 6^{th} or 7^{th} trial and were consistent thereafter. The Mild Shock treatment birds appeared to have less inhibition than the Stun treatments on initial latency and were never significantly different from the Control treatment.

The control birds started moving forward quickly after being placed in the alley. The latency to forward movement did not show such dramatic decrease in the three stimulus treatments over the course of the experiment. The stimulus treatment birds increased the time taken until first forward movement. The bird's behavior, increased latency, clearly showed a developed aversion to moving forward, presumably moving forward toward the feeder where treatment was applied. The birds that did not show increased latency, mainly exhibited an escape behavior, such as jumping out of the alley, vocalizing, or circling (walking in circles at the front of the alley). Latency alone though is not enough to establish a direct connection between the electrical stimulus and the feed; however, it does indicate a general aversion was learned.

Since latency to forward movement was the time between placement in the test alley and the first movement toward the feed, it was difficult to determine how to assess birds that moved latterly or only took a step and stopped. This resulted in scoring inconsistencies and may have contributed to variation between birds within and between trials. However, an examination of the three stimulus treatments compared to the Control clearly show that by the 7th trial those birds were reacting differently than the control birds.

The results for the Approach Delay (the total time, including latency, between placement in the alley, approaching the feed and actually eating) are presented in Table 2 and illustrated in Figure 2. The Control treatment resulted in a consistent approach delay averaging 17 s from the time they were placed in the alley until they approached the feed and began eating. For all stimulus treatments there was a dramatic increase in approach delays during Trials 2 through 7. Except for Trial 6, the Immediate Stun and Delayed Stun treatments were significantly greater than the Control in Trials 3 through 14. The Mild Shock treatment resulted in more variable results. Although the approach delay for the Mild Shock treatment were consistently greater than the Control, the differences were significant only in Trials 7, 8, and 10 through 14. It is clear from Figure 2 that although some variation occurred between trials, there was a pronounced difference in bird behavior between the three stimuli treatments compared to the control.

The dramatic increased approach delay in treatment groups would suggest those birds avoided a negative experience associated with the feeder. The Immediate and Delayed Stun treatments were very similar and were the most pronounced and consistent difference compared to the Control, non-stimulus treatments. The Mild Shock treatment appeared to have resulted in a behavior that was closer to the stun treatments than to the Control. These results would suggest that stunning caused a more pronounced behavioral response than did the electrical shock.

If in fact birds remained conscious during the application of the stunning treatments, it would make sense the learning delay was shorter in the stunned treatments due to a more aversive stimulus than the shocked treatment (i.e. the more unpleasant the situation, the faster one learns to avoid it). However, fatigue and residual soreness could have affected the outcome in a similar manner. Since stunning used a higher current (amperage) than the shock treatment, the birds may have suffered more muscular fatigue and possible soreness following the treatment. Therefore, the shorter learning delay in the stunned treatments could be due to the harsher effects after the stun, rather than the stun itself. If this is the case, the fatigue or residual soreness would serve as negative reinforcements and an association between the fatigue or residual soreness and the feeder could mask any behavior indicative of an association with the stun and the feeder. On the other hand, reduction of possible fatigue and residual soreness could also function as positive reinforcement for reducing the approach behavior. The positive reinforcement would be the delaying or eliminating residual soreness and fatigue in association with a delayed or eliminated approach towards the feeder. This positive reinforcement between the reduction of aversive after effects the stun and the delayed approach could again, mask any behavior indicative of an association with the stun and the feeder.

Another concern is of motivational conflict. It is a possibility that the reward associated with the stimulus treatments was not significant enough when compared to the reward of the Control treatment. If the treatment bird did not view the feed as “enough payment” to outweigh the costs effects of the stun during or following treatment, then the reduced motivation to approach the feeder would in turn delay or eliminate the approach behavior. In future experiments, the reward should be equal between groups by allocating a specific amount of time to eat to all groups or by reducing the Control group's time spent with the feeder. The latter may provide for an extinction effect to better compare with the extinction seen with the stun treatments. If reducing the time spend eating, i.e. reducing the reward, for the Control group causes the control group to stop approaching the feeder, i.e. extinction, it would imply the birds' lack of motivation to approach the feeder. This would be comparable to the stun treatments' lack of motivation to approach the feeder when there is an association between the feeder and a negative outcome.

The recognition of the test environment could have also affected the trials. Due to the fact that birds were not tested in their home cage and were transported to a testing environment, it is possible that there were environmental associations between either the stun or the unpleasant feelings after the stun. However, the behavior exhibited by the birds clearly demonstrated that the association was with feeder at opposing of the alley, therefore diminishing any effect the environment had over the stun in association with the feeder.

We were concerned with the possibility of an induced electrical amnesia with the amount of current flowing through the bird to produce a stun. The inclusion of a delayed stun treatment was used to determine if immediate stunning may have resulted in amnesic or short term memory loss. Given that the Stun group and the Delay Stun group of birds reacted similarly to the electrical experiences, but the behavioral responses between the Stunned birds and the Mild Shocked birds were different, it can not be assumed that there is a simple relationship between the stimuli treatments. Although these data are not conclusive, they do suggest that electrical stunning may not result in an amnesic effect that would overcome the events that occur immediately prior to slaughter, such as stunning.

Behavior is a variable between birds which allows researchers to make inferences about internal states of the bird, but it is just that-a variable. Not all birds will react the same way to the same experience. All of the above data promotes the use of pairing behavioral methodology with a more reliable methodology before any conclusive evaluation can be made about the effects of stunning on the bird. It is doubtful that a single method for determining the experiences perceived by the bird during stunning is comprehensive enough to make totally accurate inferences.

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Table 1. Latency (seconds) of first forward movement by test birds towards the feed reward

located at the opposite end of the test alley. Treatments were Control (n = 4 birds per trial), Immediate Stun (n = 4 birds per trial), Mild Shock (n = 4 birds per trial), and Delayed Stun (n = 3 birds per trial).

Latency to First Forward Movement

Trial	Control	Delay	Shock	Stun	p
1	27 ± 23^{a}	8 ± 7^{a}	2 ± 2^{a}	23 ± 17^{a}	0.6318
2	7 ± 7^{a}	10 ± 6^{a}	0 ± 0^{a}	5 ± 3^{a}	0.5108
3	19 ± 14^{a}	5 ± 4^{a}	3 ± 3^{a}	48 ± 44^{a}	0.5710
4	4 ± 2^{a}	11 ± 3^{a}	2 ± 2^{a}	91 ± 5^{a}	0.1106
5	5 ± 2^{b}	1 ± 1^{b}	3 ± 1^{b}	93 ± 50^{a}	0.0866
6	9 ± 6^{a}	67 ± 57^{a}	4 ± 0^{a}	7 ± 4^{a}	0.2932
7	4 ± 2^{b}	121 ± 59^{b}	15 ± 10^{a}	137 ± 44^{a}	0.0283
8	7 ± 2^{a}	122 ± 58^{a}	70 ± 40^{a}	94 ± 50^{a}	0.2965
9	9 ± 3^{b}	180 ± 0^{a}	16 ± 8^{b}	$95 \pm 49^{a,b}$	0.0076
10	7 ± 2^{b}	150 ± 3^{a}	10 ± 7^{b}	$78 \pm 37^{a,b}$	0.0054
11	5 ± 2^{b}	134 ± 46^{a}	66 ± 39^{a}	66 ± 41^{a}	0.1670
12	7 ± 3^{b}	180 ± 0^{a}	51 ± 34^{b}	$92 \pm 51^{a,b}$	0.0269
13	9 ± 2^{b}	180 ± 0^{a}	$113 \pm 40^{a,b}$	140 ± 23^{a}	0.0671
14	14 ± 4^{b}	$180 \pm 0^{a,b}$	$63 \pm 32^{a,b}$	144 ± 36^{a}	0.0783

*p=0.05
**Within each row a different superscript letter was significantly different at least at p=0.05 using Duncan's Multiple Range Test.

Table 2. Approach Delay (seconds) of test birds to approach and eat feed located at the opposite end of the test alley. Treatments were Control (n = 4 birds per trial), Immediate Stun (n = 4 birds per trial), Mild Shock (n = 4 birds per trial), and Delayed Stun (n = 3 birds per trial).

Approach Delay

Trials	Control	Delay	Shock	Stun	p
1	34 ± 22^{a}	13 ± 6^{a}	6 ± 3^{a}	46 ± 35^{a}	0.5796
2	20 ± 15^{a}	72 ± 54^{a}	53 ± 42^{a}	52 ± 53^{a}	0.8319
3	11 ± 1^{b}	131 ± 49^{a}	$65 \pm 39^{a,b}$	145 ± 36^{a}	0.0576
4	12 ± 1^{b}	131 ± 49^{a}	$65 \pm 39^{a,b}$	145 ± 36^{a}	0.0586
5	15 ± 2^{b}	160 ± 10^{a}	68 ± 40^{b}	180 ± 0^{a}	0.0006
6	14 ± 5^{a}	133 ± 47^{a}	135 ± 45^{a}	107 ± 43^{a}	0.1175
7	12 ± 3^{c}	180 ± 0^{a}	94 ± 32^{b}	180 ± 0^{a}	<.0001
8	13 ± 4^{b}	168 ± 12^{a}	159 ± 22^{a}	180 ± 0^{a}	<.0001
9	16 ± 2^{b}	180 ± 0^{a}	24 ± 8^{b}	180 ± 0^{a}	<.0001
10	25 ± 10^{c}	180 ± 0^{b}	102 ± 33^{a}	180 ± 0^{a}	<.0003
11	13 ± 1^{b}	180 ± 0^{a}	132 ± 28^{a}	180 ± 0^{a}	<.0001
12	16 ± 2^{b}	180 ± 0^{a}	175 ± 5^{a}	180 ± 0^{a}	<.0001
13	17 ± 1^{b}	180 ± 0^{a}	180 ± 0^{a}	180 ± 0^{a}	<.0001
14	21 ± 4^{b}	180 ± 0^{a}	180 ± 0^{a}	180 ± 0^{a}	<.0001

*p=0.05

**Within each row a different superscript letter was significantly different at least at p=0.05 using Duncan's Multiple Range Test.

Figure 1. Latency (seconds) of first forward movement by test birds towards the feed reward located at the opposite end of the test alley. Treatments were Control (n = 4 birds per trial), Immediate Stun (n = 4 birds per trial), Mild Shock (n = 4 birds per trial), and Delayed Stun (n = 3 birds per trial).

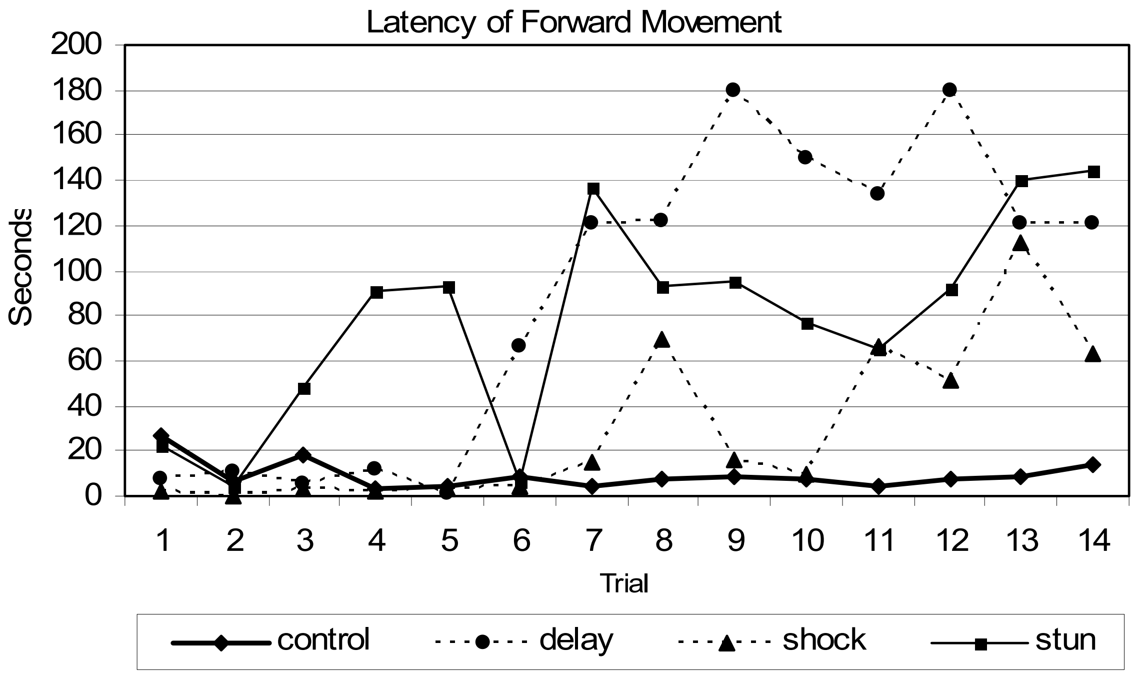
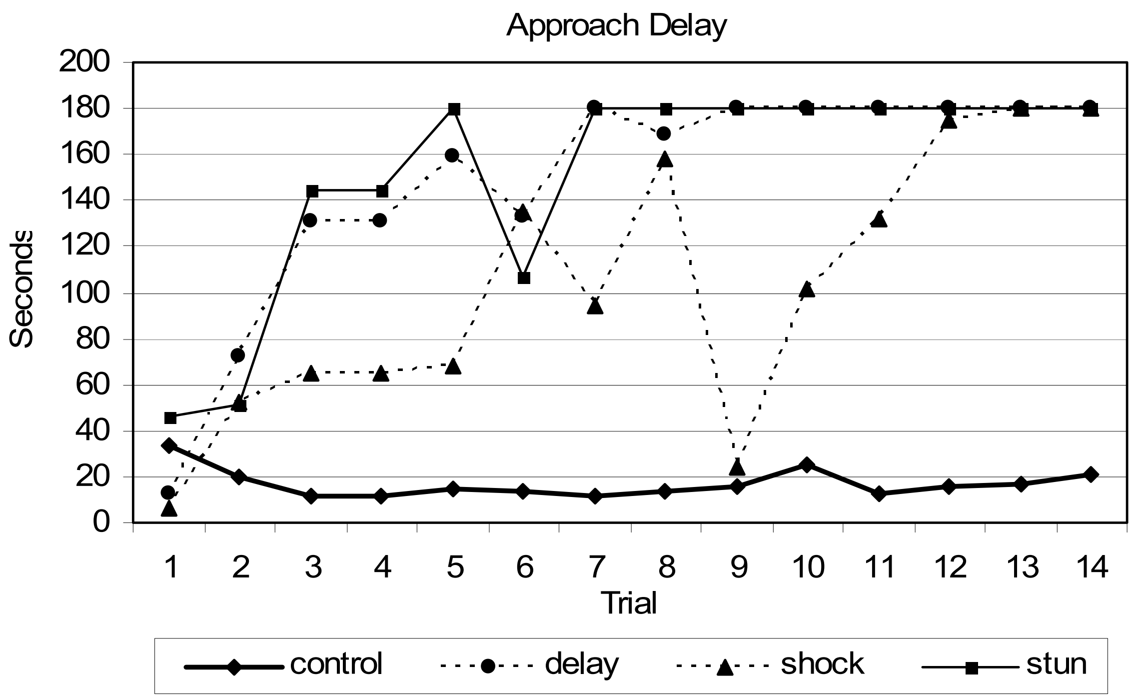


Figure 2. Approach Delay (seconds) of test birds to approach and eat feed located at the opposite end of the test alley. Treatments were Control (n = 5 birds per trial), Immediate Stun (n = 4 birds per trial), Mild Shock (n = 4 birds per trial), and Delayed Stun (n = 4 birds per trial).



CHAPTER 4

ELECTROENCEPHALOGRAM RESPONSE OF LAYING HENS AND BROILERS TO ELECTRICAL TREATMENTS[1]

[1] J. A. Chamier, D. L. Fletcher, R. J. Buhr, A. B. Webster to be submitted to Poultry Science

ABSTRACT Electroencephalograms (EEGs) are commonly used to record the electrical impulses of the brain. Two experiments were conducted to determine if the brain wave patterns of chickens changed when treated with varying degrees of electrical stimulation. The first experiment was conducted with Single Comb White Leghorn laying hens and the second with commercial broilers (Rock x Cornish cross). Both experiments consisted of five treatments: 1) 120V sinusoidal AC at 60 Hz applied subcutaneously. 2) 120V sinusoidal AC at 60 Hz applied through external clips. 3) 16V pulsed DC at 500 Hz applied subcutaneously. 4) 16V pulsed DC at 500 Hz applied through clips. 5) 3.1V pulsed DC at 500 Hz applied subcutaneously. External electrodes on the bird recorded a baseline EEG following by a 10 s electrical stimulus, and then post treatment EEG until brain wave patterns returned to baseline or death intervened. The raw EEG signal was reviewed for polyspike activity or isoelectric phases that other researchers have interpreted as indicating a humane stun. Also, power spectra analyses of the EEG prior to and post stimulation in conjunction with behavioral events were used to determine the effectiveness of the stuns. The high current/low frequency stunning caused a substantial difference in EEG's baseline and post treatment EEG. The low current/high frequency stunning caused a moderate, but less dramatic change between baseline EEG's and post treatment EEGS. The EEG's of the shocked birds prior and post treatment were not different.

Key Words: Electroencephalogram, stun, unconsciousness

INTRODUCTION

Electrical stunning is the most commonly used method for immobilizing or killing poultry prior to processing. It provides two major benefits: it is economical and has generally believed to be humane. Gregory (1998, p.84) states when sufficient current flows through the brain, stunning renders the bird unconscious, and the electric shock is not perceived. He describes the introduction of electrical stunning "…in the 1930's [researchers] thought that it produced unconsciousness by restricting blood flow to the brain. Since then it has been learned that blood flow to the brain actually increases…" Cook *et al.* (1992) demonstrated in sheep that unconsciousness is brought on by a physiological response similar to a *grand mal* epileptic seizure produced by the release of excitatory amino acids into extracellular space followed by a depolarization shift of the brain. Cook *et al.* (1995) showed that it takes approximately 0.2 seconds from the application of current to the onset of epileptiform discharges.

However, poultry when stunned do not show the same type of waveform as red meat species such as sheep. Instead poultry exhibit a *petit mal* seizure, which is not always indicative of unconsciousness (Gregory, 1998, p. 237). Therefore, EEG analysis alone is not sufficient to make conclusions on the mental state of an animal and must be paired with some other form of physiological indicator, such as muscle tension, heart rate, respiration rate or behavior (for example, an EEG and corresponding behavioral activity associated with unconsciousness). On the other hand, the effectiveness of stunning is maximized by ensuring that the equipment is accurately delivering the recommended current for the duration the bird is in the stunner and that the stunner is in the appropriate position in reference to the birds coming through.

Esplin and Freston's (1960) work on spinal cord convulsions noticed that when the stunning current only passed through the spinal column then no epileptiform waveforms were produced, also changing corresponding behavior. "Instead of showing a phase of tonic activity, followed by clonic activity, it would only show a short period of tonic activity." Gregory (1998, p.86) uses this as a model for explaining what might happen to birds if electrodes were misplaced on the neck for stunning rather than on the head. Moreover, he stated when stunning "at low currents [the bird] might vocalize and show escape behavior." The placement of the pins in the previous experiment, caused concern that the brain might bypassed and the electrical stimulation was following the spinal cord.

This experiment was designed to determine the feasibility of the mode of electrical application and to compare the baseline EEG to post treatment EEG for signal divergences that may indicate the conscious state of the bird. Recording of behavioral responses, such as eye closure, breathing, and kicking, synchronous with the recording of the EEG may give clarity on the mental state of the bird to compare with the previous behavioral study.

MATERIALS AND METHODS

Laying Hen Experiment

Twenty-five Single Comb White Leghorn hens were used in this experiment. A total of 15 birds (pinned birds) were fitted with subcutaneous electrodes immediately behind the comb (head electrode) and on the underside of the tail above the cloaca (cloacal electrode). The electrodes were standard safety pins. Wires to direct electrical impulses to the birds were connected to the electrodes using alligator clips. Ten additional hens were fitted with lightweight clips (clipped birds) secured to their combs and to their cloacas. The wiring harness for both pinned and clipped groups was aligned along the neck, back and under the wing to avoid the wires distracting the bird's vision. The wires were attached to an electrical source to allow a head to tail application of stunning current similar to a commercial stunner. When the wiring harness was attached, both head and cloacal electrodes were dampened with salt water solution to enhance electrical contact. The hens were divided into five treatment groups. The five treatments were 1) A 120V sinusoidal AC electrical impulse at 60 Hz applied subcutaneously through safety pins. 2) A 120V sinusoidal AC at 60 Hz applied through external clips. 3) A 16V pulsed DC at 500 Hz applied subcutaneously through safety pins. 4) A 16V pulsed DC at 500 Hz applied through clips. 5) A 3.1V pulsed DC at 500 Hz (shock) applied subcutaneously through safety pins.

On the day of testing all twenty-five birds had the feathers behind the comb plucked for attachment of the EEG electrodes. The plucked area was wiped clean with a saline solution. Three external electrodes[2] were placed on the bird to allow for the recording of the EEGs. The positive and reference electrode were placed on the plucked area behind the comb (cleaned with a saline solution to help adherence) with the positive electrode directly behind the comb. The third electrode, the ground electrode, was placed at the base of the neck in an area that was naturally devoid of feathers. The bird was placed on a rubber mat and left to undergo EEG recording.

The results of EEG signals after being registered as voltage differences between an active electrode and a reference electrode were measured, amplified and displayed on a computer monitor. The computer operator also recorded and saved each trial. Recording allowed enough time for at least thirty seconds of "normal" or baseline EEG excluding any artifacts caused by movement. The appropriate electrical stimulus was then applied for 10 seconds. The electrodes were not able to record a signal for 5 seconds after the application. From this point on, the EEG was recorded until brain wave patterns returned to baseline or death intervened (recording lasted

[2] BioRadio model 110, Cleveland Medical Devices Inc., Cleveland, OH 44103

at least two minutes). A split screen video recorder was used to record bird behavior, the EEG display and the ammeter reading throughout the process.

Broilers

A total of twenty-five commercial broilers obtained from the receiving area of a local processing plant were tested over the course of five days. Five birds a day were tested, one from each treatment group. The treatments were identical to the treatments used for the laying hens. Procedures and materials were identical to that of the laying hens except the broilers were held off the mat to reduce artifacts during the pre-stimulus EEG (i.e. head shaking, walking) and only placed back onto the mat after the conclusion of stimulation. Also time frame of at least two minutes was placed on the post stimulus EEG to ensure recording of a return to baseline or death. Commercial broilers were tested for their EEG readings during electrical stimulation for comparison to the laying hen EEGs recorded earlier.

EEG Analysis

In its raw signal form the EEG can be paired with behavioral conditions (i.e. wing flapping, kicking, eye closure, posture, breathing) and artifacts associated with these behaviors can be eliminated from the EEG recordings. Likewise, the raw EEG readings can provide a rough estimate of changes in behavioral state (end of stimulation, return of rhythmic breathing, or death).

The subsequent broiler EEGs were divided into two time periods, before stimulus and post stimulus. Rather than taking a segment of the EEG at a specific time, a representative sample was taken from each time period. A representative sample consisted of 15 seconds which was not effected by artifacts. This was necessary due to two factors. First, the ability to use multiple electrodes to normalize the artifacts was not possible due to the small scalp size of a chicken. Second, some birds displayed clonic-type movements, such as kicking or tremors, after the termination of the electrical stimulus, which appeared as large artifacts on the EEG signal. However, the post sample were take as close to the termination of stimulation as artifacts allowed. The two samples were compared against one another for analysis. A power spectrum analysis shows the distribution of the raw EEG signals by particular frequency bands. By plotting the prior and post-treatment representative samples on the same power spectra, differences in the brain waves can be compared. Because the spectrum is in decibels (which are a function of the logarithmic difference in powers) even a slight difference may be significant. Our transformation was done by exporting data from the Bioradio program into MATLAB[3].

RESULTS AND DISCUSSION

Laying Hens

Figure 3 depicts two EEG's of electrically stunned laying hens. The top EEG depicts brain activity of birds stunned with a high voltage and low frequency (AC current). The bottom EEG depicts birds stunned with a low voltage and high frequency (pulsed DC current). Neither stunning method produced any polyspike activity indicative of a seizure (Gregory, 1987). Directly following the stun, the beginning of an isoelectric phase was not as smooth or quick to appear as earlier research indicates (Gregory, 1987). However, this varied highly from bird to bird.

The sequence of events for a high voltage/low frequency stun was as follows. The nictitating membrane covered the eye, the muscles contracted and the legs extended becoming rather tense for the duration of the stimulus, similar to the low voltage high frequency stun. Some hens experienced tremors for several seconds. Upon the end of stimulation for the high voltage/low frequency group, the muscles would relax, breathing would stop and defecation

[3] MATLAB version 7, The MathWorks Inc., Natick, MA 01760-2098

might occur. No tremors or other involuntary movement were noted. The EEGs for the birds showed the time until death (when the EEG “flatlines”) between 27 and 46 seconds.

The sequence of events for a low voltage/ high frequency stun was as follows. The nictitating membrane covered the eye, the muscles contracted and the legs extended becoming rather tense for the duration of the electrical stimulus. Once the stimulus ended, the muscles relaxed and the hens appeared to have little to no control over bodily movements. These hens sometimes experienced tremors for less than ten seconds. The hens’ lack of coordination included no neck muscle tension, very little movement, no rhythmic breathing and salivation if head was unsupported.   The characteristics the hens displayed after being stunned were similar to the characteristics seen in birds stunned in a commercial plant. The EEGs for the these birds showed an immediate or gradual return, between 5 and 30 seconds, an EEG resembling the baseline pattern. During this time period, signs of epileptic waveforms were apparent in the raw EEG although to the heartbeat caused some interference in the signal n some birds.

Broilers

Figure 4 depicts the EEGs of three electrically stimulated commercial broilers. The top is a shocked bird, the middle is a high voltage/low frequency stunned bird and the bottom is a low voltage/high frequency stunned bird. The elicited behavior from the birds in the mild shock treatment was as expected; the birds reacted adversely during or post stimulation by vocalization, wing flapping, jumping or some combination of all three. The low voltage/high frequency stunned broilers reacted similarly to the laying hens. During the stun, the nictitating membrane covered the eye and the skeletal muscles went rigid. After the stun, the muscles relaxed, sometimes tremors occurred for less than ten seconds and the broilers had little to no control over bodily movements (no neck muscle tension, no leg retraction, clonic kicking, loss of rhythmic breathing and salivation). The high voltage/low frequency stunned birds reacted the same as the low voltage/high frequency stunned birds during the stimulus. However, after the stimulus the high voltage/low frequency birds the muscles would relax, but no tremors or clonic activity were noted, respiration stopped and in some birds, defecation occurred.

The raw EEG signal for all broilers showed no signs of epileptic wave forms after the stun. However, the time taken for the EEG to return to “normal” by visual examination was 5 seconds or less in all but one (245 seconds) of the high voltage/low frequency stuns and between 5 and 15 seconds for the low voltage/high frequency stuns. In some cases for the high voltage/low frequency stuns the isoelectric phase never smoothed out, but showed a “buzzing” pattern, where swells of rhythmic activity would occur and fade out again in varying time lengths, beginning from 6 to 48 seconds after the end of stimulation.

Figure 5, is a theoretical example generated to show the significant difference of outcomes (prior versus post stimulus signals) one might observe when an EEG signal is transformed into the power spectra. In these graphs the signal has been plotted in bands where the width is equal to the 95% confidence interval at each frequency. Therefore the band is the region where it is most likely (95% likelihood) the spectrum lies. These graphs are fitted with best fit lines, in black, which were made with the assumption that the spectrum is smooth over a medium set of frequencies, i.e. the signal is consistent or representative. Additionally many points were used to determine the line of best fit, so single large spikes caused by artifacts do not affect the overall pattern. The consistency and the multitude of data points used give a very small error on the best fit line, approximately the width of the black line. Consequently, it is possible to state that over the sampled set of frequencies the two spectra are distinguishable from each other, even though they appear to overlap. Specifically, Figure 5 showed a significantly reduced post stimulus signal in comparison to the prior stimulus signal, i.e. the "after" line, post stimulus signal, is below the "before" line, prior stimulus signal. This bird's EEG signal would have greatly increased post stimulus as compared to before treatment application in order to reduce the power spectra. This representation is the basis for comparison for determining significant difference in our EEG power spectra.

Figures 6, 7 and 8 were mild shock, low voltage/ high frequency stun (clipped) and high current/low frequency stun (clipped) treatments respectively. There was no difference between power spectrums with the mild shock treatment, i.e. the power spectra for prior and post stimulus were superimposed. On the other hand, nine out of ten high voltage/low frequency stunned birds showed a definite difference between prior and post power spectrums. In the low voltage/ high frequency treatment the EEG responses differed. Six out of ten birds in the low voltage/ high frequency stun treatments showed reduced post responses for the majority of their spectra, but not as reduced as the high voltage/low frequency treatments. Three birds showed increased post responses for the majority of their spectra. One bird was not significant either way. No differences would suggest some birds may go through the stunner without the electrical current disrupting brain activity. It is imperative to note that this is the first time external electrodes have been used to record brain activity in response to stunning because some error may have been due to the operator rather than the procedure.

The waveforms (amplitude and frequency) and patterns for broilers were similar to those found in laying hens. The similarities between the laying hens and the broilers were evident in their behavior responses to the stimulus and EEG readouts, dispelling any concern of the physiological difference between growing and mature birds on the effectiveness of the stun when stunned with the same current.

ACKNOWLEDGEMENTS

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Esplin , D.W. & Freston, J.W. (1960). Physiological and pharmacological analysis of spinal cord convulsions. Journal of Pharmacological and Experimental Therapeutics 130, 68-80.

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Gregory, N.G., Wotton, S.B. (1989). Effect of electrical stunning on the somatosensory evoked potential in chickens. British Veterinary Journal 143, 175-183.

Gregory, N.G. (1998). Animal welfare and meat science. New York: CABI Publishing. p 243

Figure 3. Representative EEGs of two treatments, prior and post stimulus.

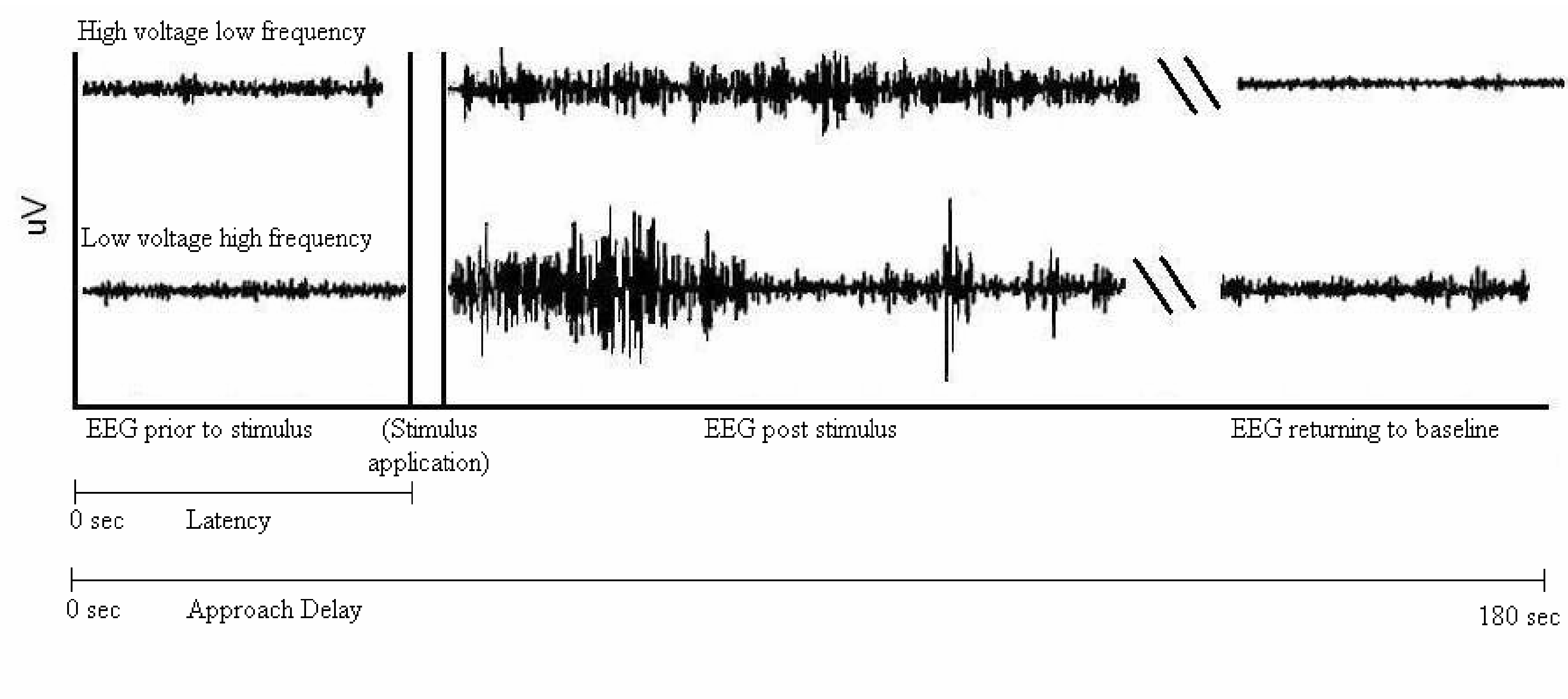


Figure 4. Representative EEGs of three treatments, prior and post stimulus.

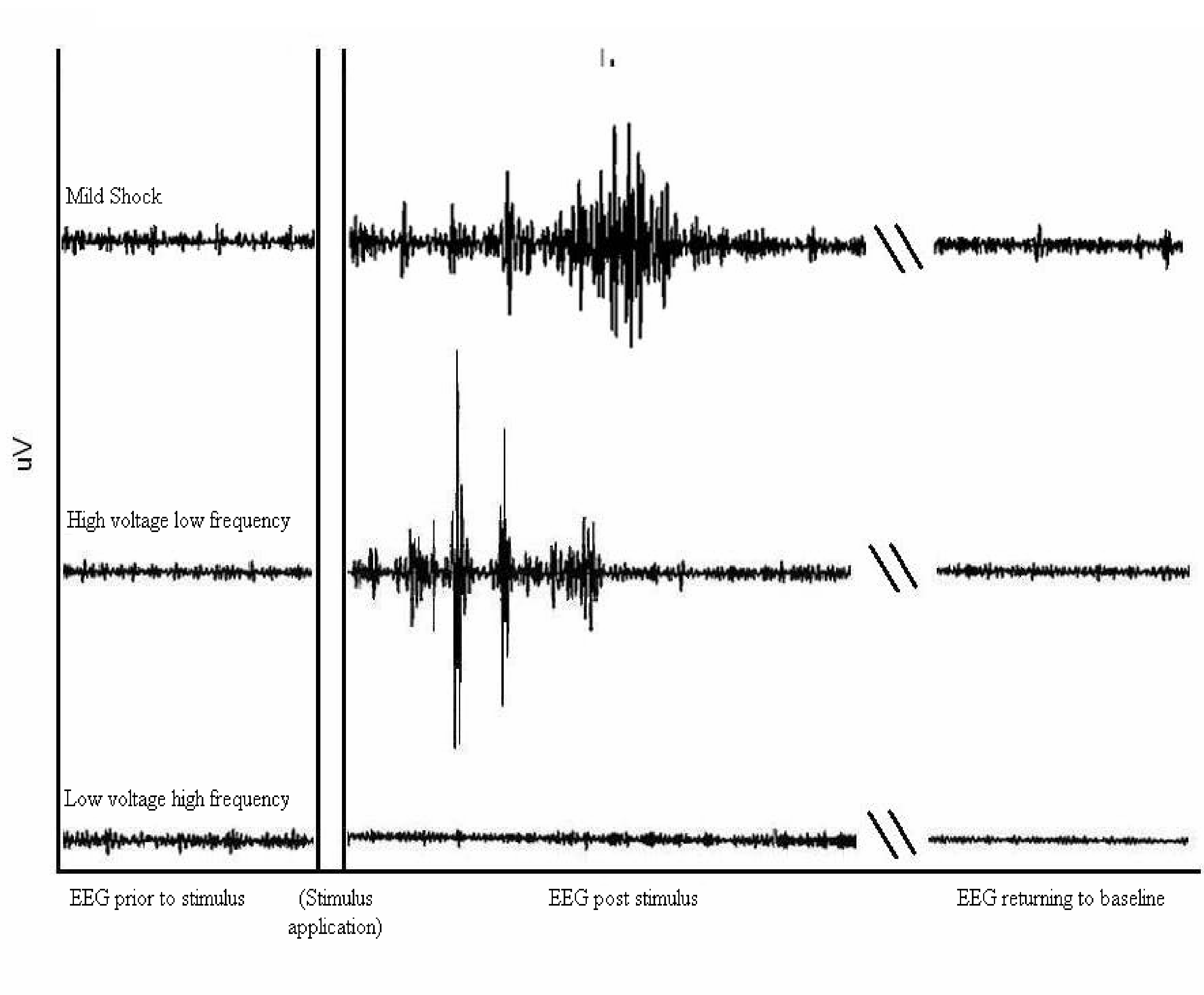


Figure 5. The spectrum analysis of a theoretical EEG showing a significantly reduced post stimulus signal in comparison to the prior stimulus signal. The band width is equal to the 95% confidence interval at each frequency.

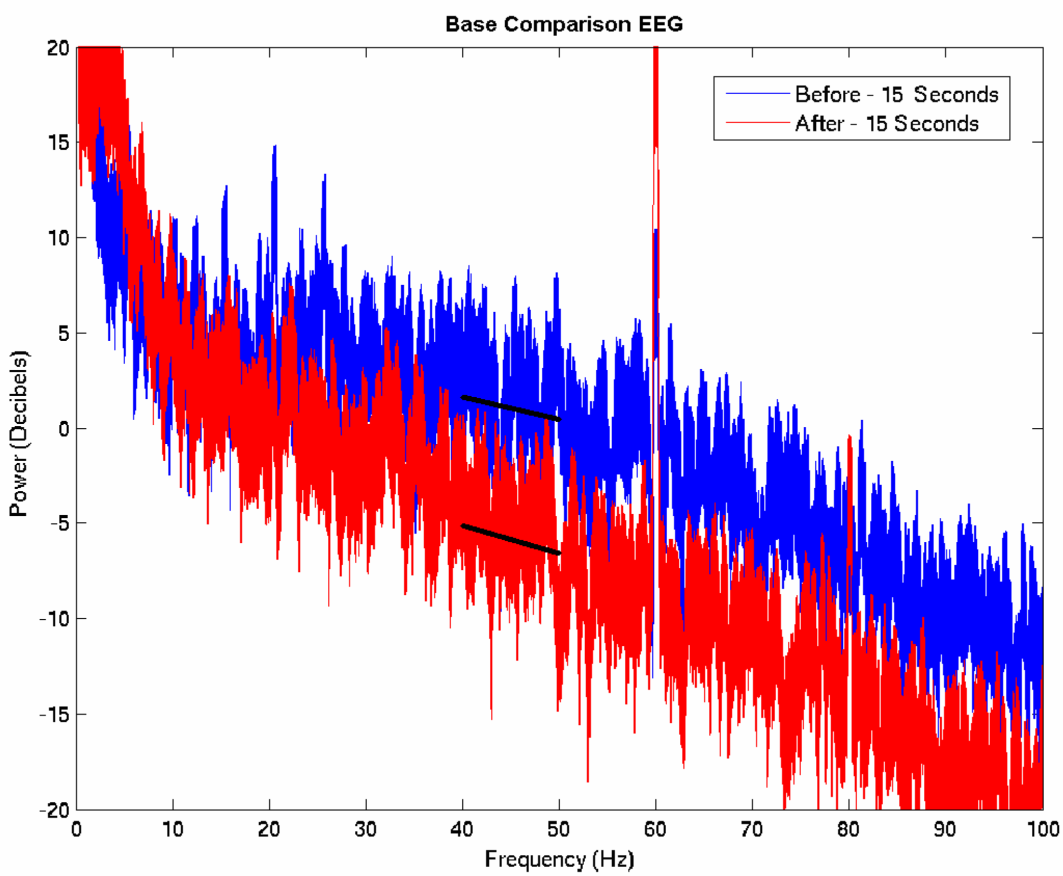


Figure 6. The spectrum analysis of an EEG of a bird receiving a mild shock. No difference between prior and post stimulus.

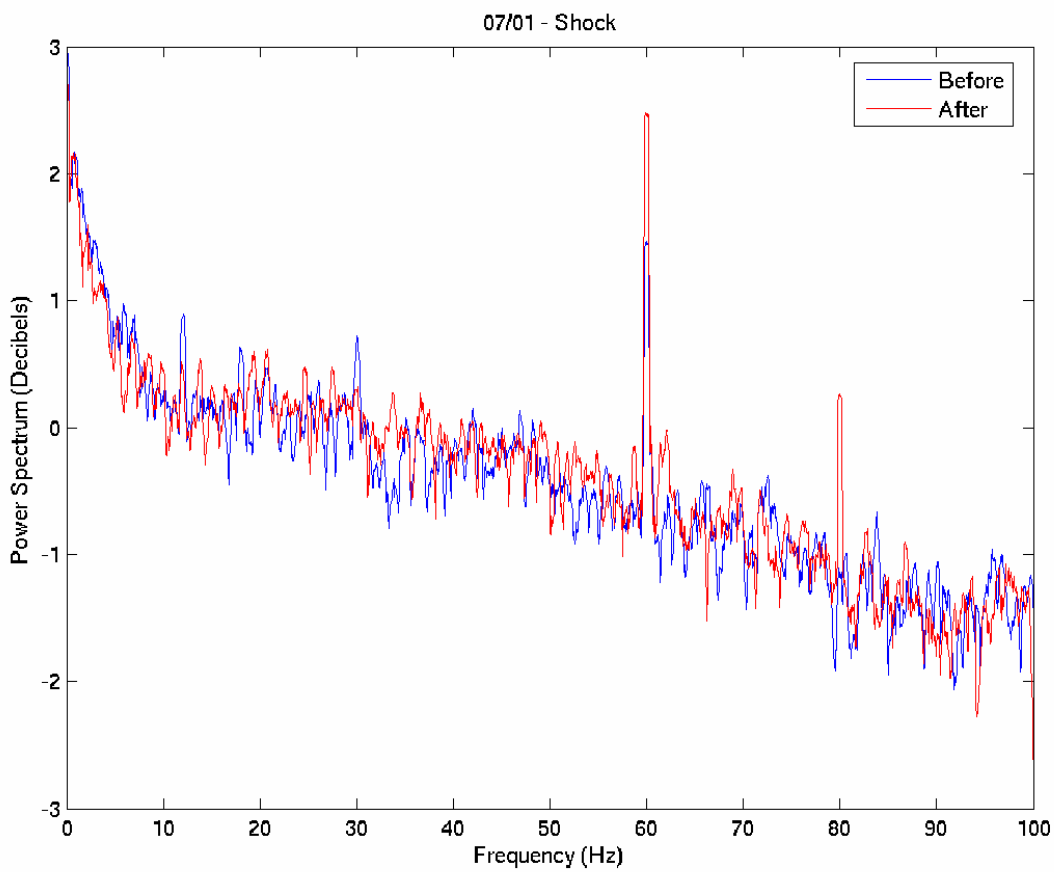


Figure 7. The spectrum analysis of an EEG of a bird receiving a low voltage/high frequency stun. Moderate, though significant, difference between prior and post stimulus.

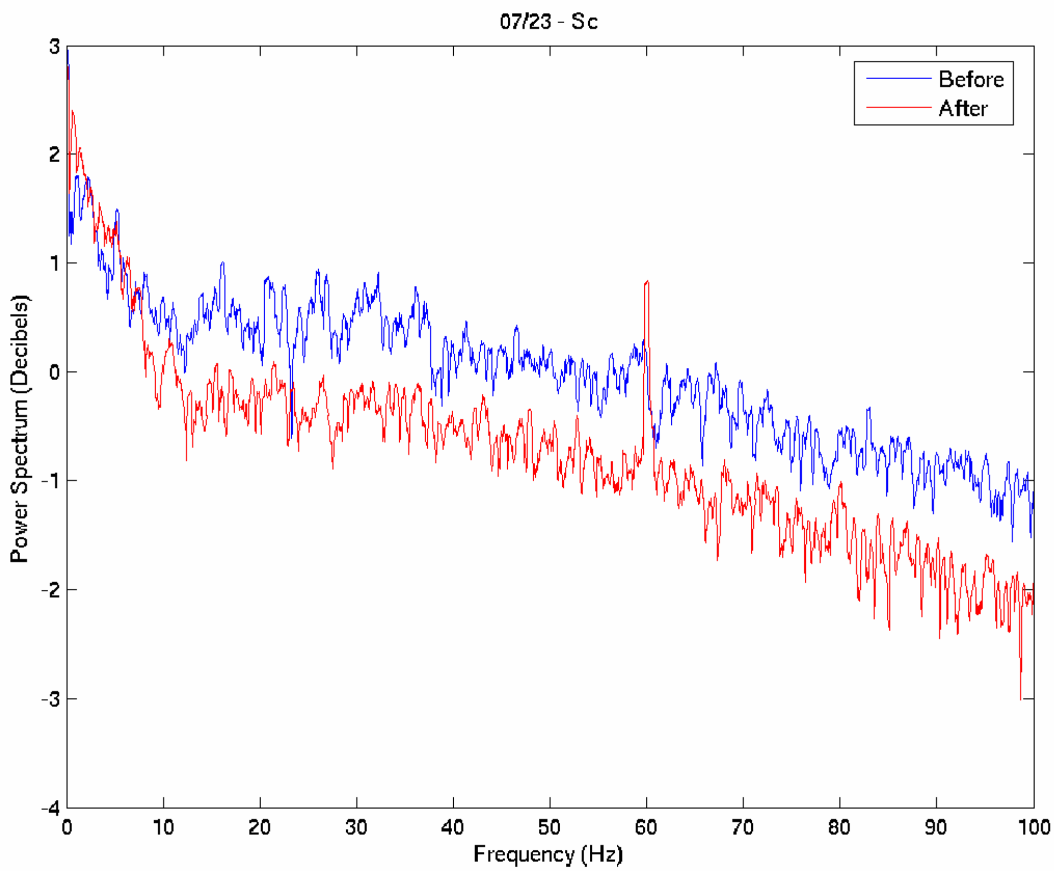
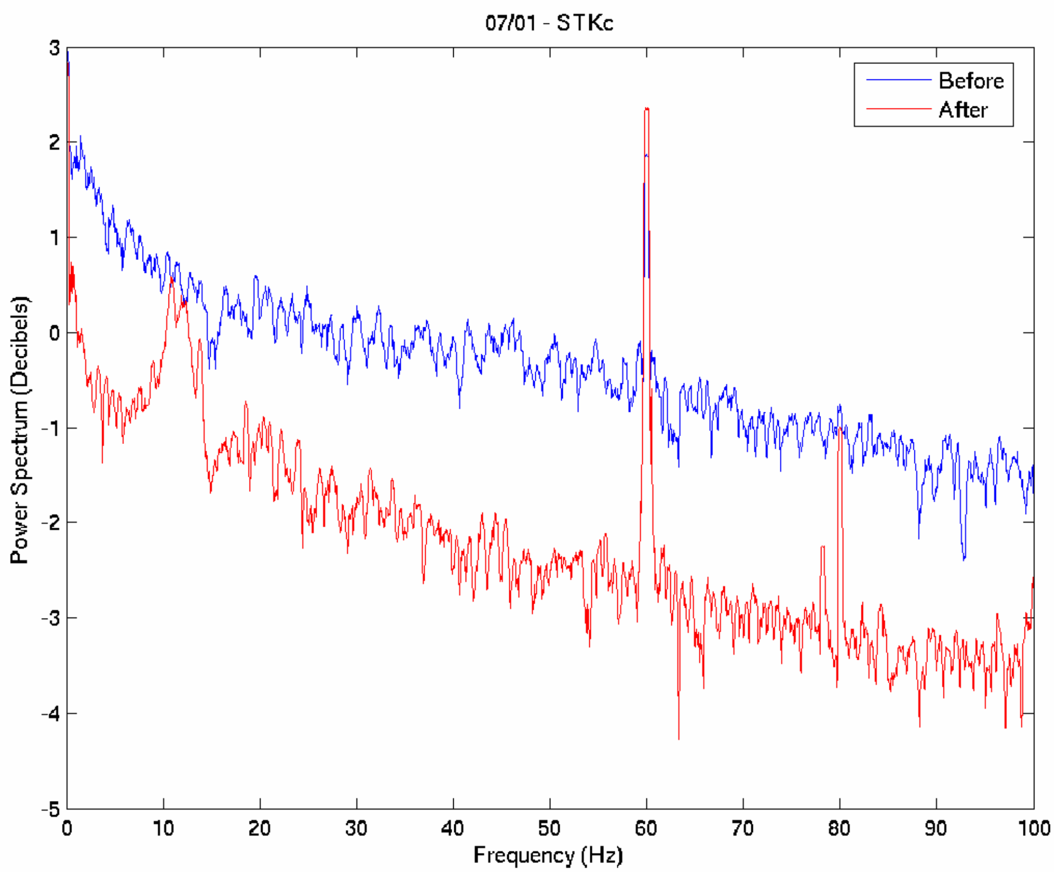


Figure 8. The spectrum analysis of an EEG of a bird receiving a high voltage/low frequency stun. A large difference between prior and post stimulus.



CHAPTER 5

SUMMARY AND CONCLUSIONS

The results of the behavioral experiment showed all of the electrically stimulated treatments were perceived as aversive by the subjects. Similarities between treatment groups' behavior and the marked dissimilarity of the control group's behavior suggest that all treatment birds perceived the event as an aversive. Admittedly, one cannot directly conclude birds stunned at 16V recall the actual unpleasant electrical experience associated with a discriminative cue, because of the possibility that the bird did not perceive pain during the stun, but rather the negative after effects, such as soreness or fatigue. If the soreness or fatigue caused by repeated trials is not overcome by the reward, then it is perceivable that the aversion would continue. Hunger or reward size may also affect the behavior. If the reward or the time at the feeder is not perceived as satisfying by the bird, it is conceivable that the bird's aversion behavior would persist. These factors: soreness, fatigue, hunger, and adequate rewards, may factor into the aversion experience. These results do not conclude that the bird experienced pain or suffering during the stun, only that there is a memorable event taking place accompanying the stun. The hen behaviors' can only be evaluated in conjunction with a reliable physiological response, such as an EEG.

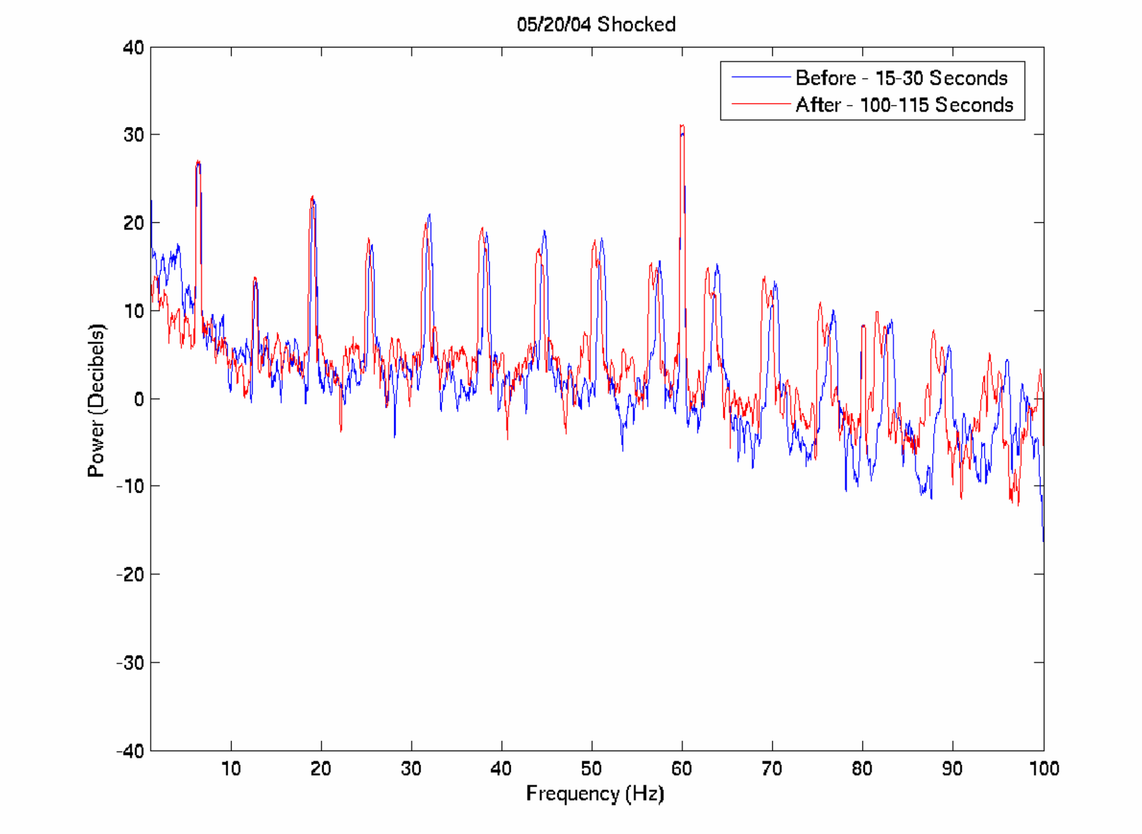
To expand on this, hens' and broilers' brain waves were recorded and compared (prior and post stimulus); the EEGs showed that there is a difference between the types of electrical stimuli. The power spectra show a difference in brainwaves prior and post stimulus, in order of magnitude: high voltage/low frequency, low voltage/high frequency and shock. The high voltage/low frequency birds demonstrated a significant difference. The low voltage/ high frequency birds demonstrated a moderate difference, whereas, the shock treatment shows no differences in prior and post stimulus. These results are important because they show the stun had some effect on the brainwave activity of the bird. However, because of the delay in the sampling method we can not conclude anything about the bird immediately after stunning. The implications this has on slaughter are related to an accurate neck cut. The results of the significant differences of the prior and post power spectra on some low voltage/high frequency stunned birds and the delayed time frame during which the samples were taken suggest that brain activity is disrupted during neck cutting in some birds. If neck cutting is accurate and bleed out is sufficient then the bird likely die of ischema before the resuming consciousness is possible.

Appendix Key

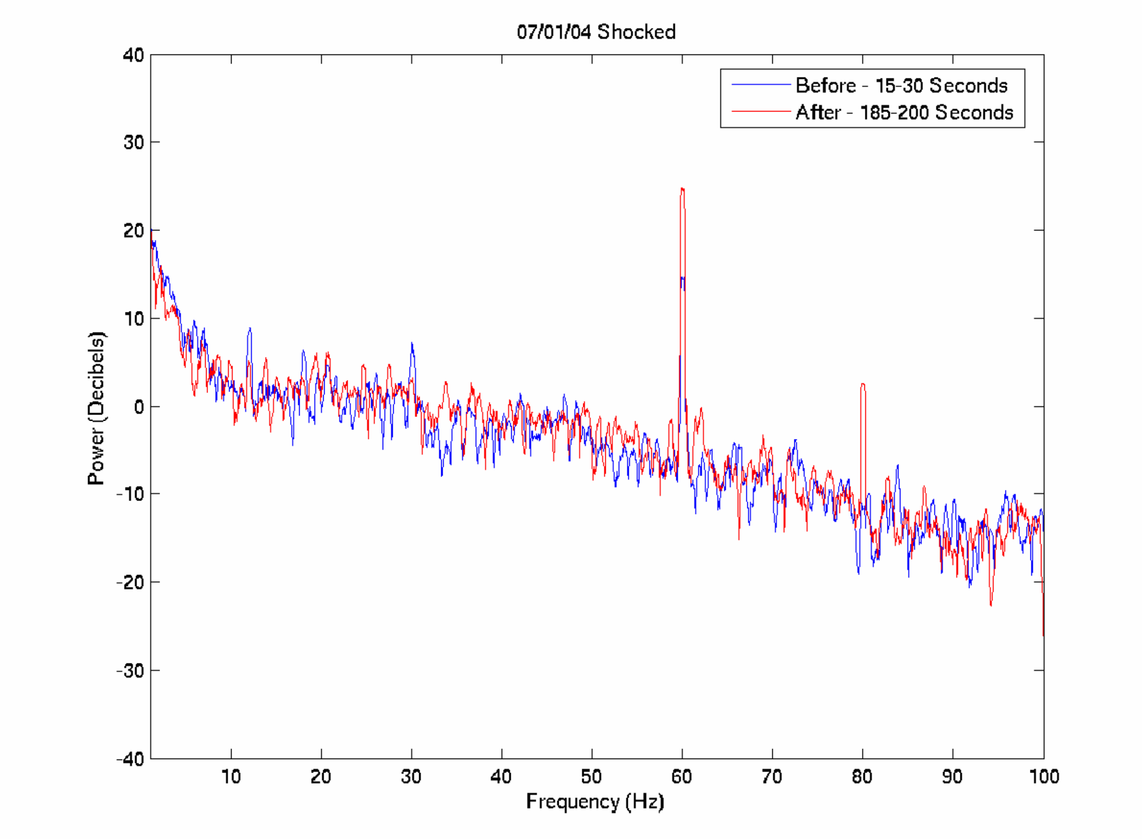
BIRD ID	TREATMENT	BEFORE	STIMULUS	AFTER	AFTER 2
Appendix 1	Mild Shock	15-30 sec	69-79 sec	100-115 sec	
Appendix 2	Mild Shock	15-30 sec	159-169 sec	185-200 sec	
Appendix 3	Mild Shock	15-30 sec	69-79 sec	85-100 sec	
Appendix 4	Mild Shock	15-30 sec	78-88 sec	100-115 sec	
Appendix 5	High Voltage Stun	15-30 sec	75-86 sec	100-115 sec	
Appendix 6	High Voltage Stun	15-30 sec	65-75 sec	85-100 sec	100-115 sec
Appendix 7	High Voltage Stun	15-30 sec	151-161 sec	170-185 sec	
Appendix 8	High Voltage Stun	15-30 sec	66-78 sec	85-100 sec	
Appendix 9	High Voltage Stun	15-30 sec	79-91 sec	100-115 sec	
Appendix 10	High Voltage Stun	15-30 sec	74-85 sec	100-115 sec	
Appendix 11	High Voltage Stun	15-30 sec	225-235 sec	245-260 sec	
Appendix 12	High Voltage Stun	15-30 sec	118-128 sec	140-155 sec	
Appendix 13	High Voltage Stun	15-30 sec	66-81 sec	90-105 sec	
Appendix 14	High Voltage Stun	15-30 sec	86-100 sec	105-120 sec	
Appendix 15	Low Voltage Stun	15-30 sec	105-115 sec	130-145 sec	
Appendix 16	Low Voltage Stun	15-30 sec	76-86 sec	100-115 sec	
Appendix 17	Low Voltage Stun	15-30 sec	201-211 sec	225-240 sec	
Appendix 18	Low Voltage Stun	15-30 sec	73-83 sec	90-105 sec	
Appendix 19	Low Voltage Stun	15-30 sec	132-143 sec	155-170 sec	
Appendix 20	Low Voltage Stun	60-75 sec	120-130 sec	145-160 sec	
Appendix 21	Low Voltage Stun	15-30 sec	106-116 sec	125-140 sec	
Appendix 22	Low Voltage Stun	15-30 sec	51-61 sec	70-85 sec	115-130 sec
Appendix 23	Low Voltage Stun	15-30 sec	954-105 sec	125-140 sec	
Appendix 24	Low Voltage Stun	15-30 sec	97-107 sec	120-135 sec	

- The time given in the individual appendix legends are from the initial start point of 0 seconds. Consequently, the “post” period is not the seconds after the stimulus was applied, but the duration of to the entire EEG at which the sample was taken.

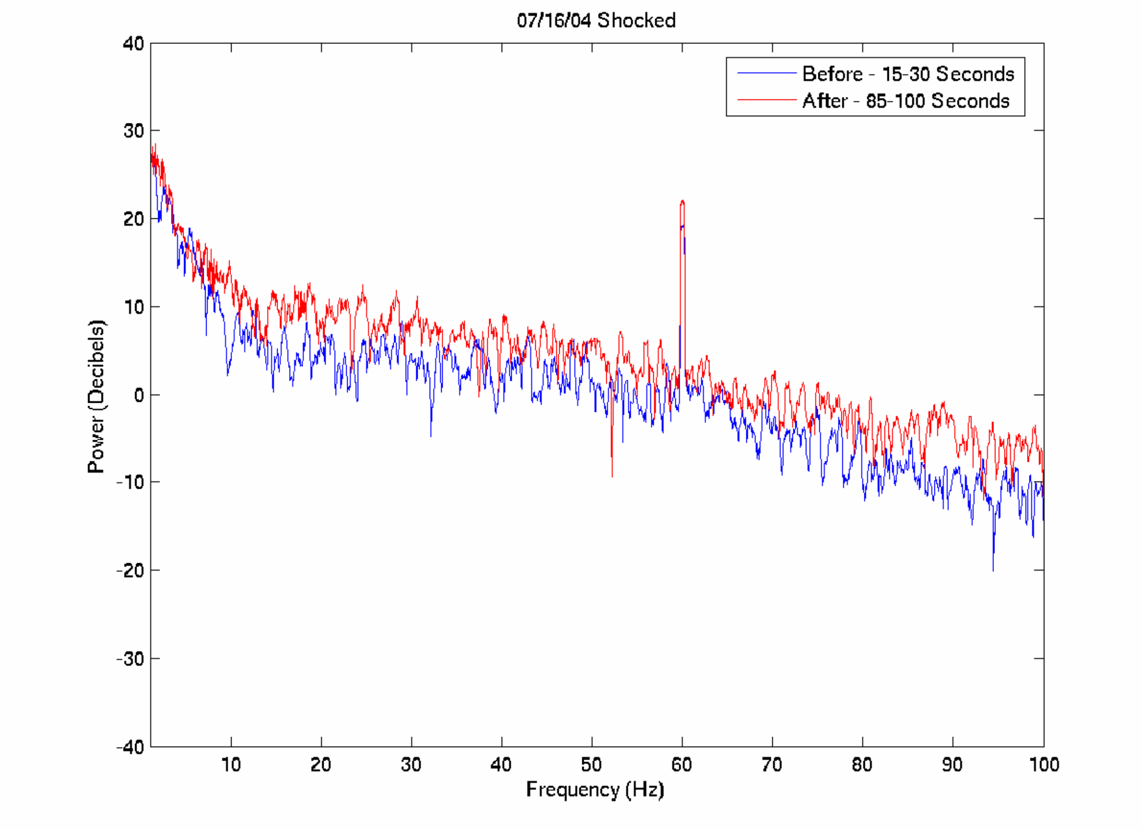
Appendix 1 Mild shock treatment



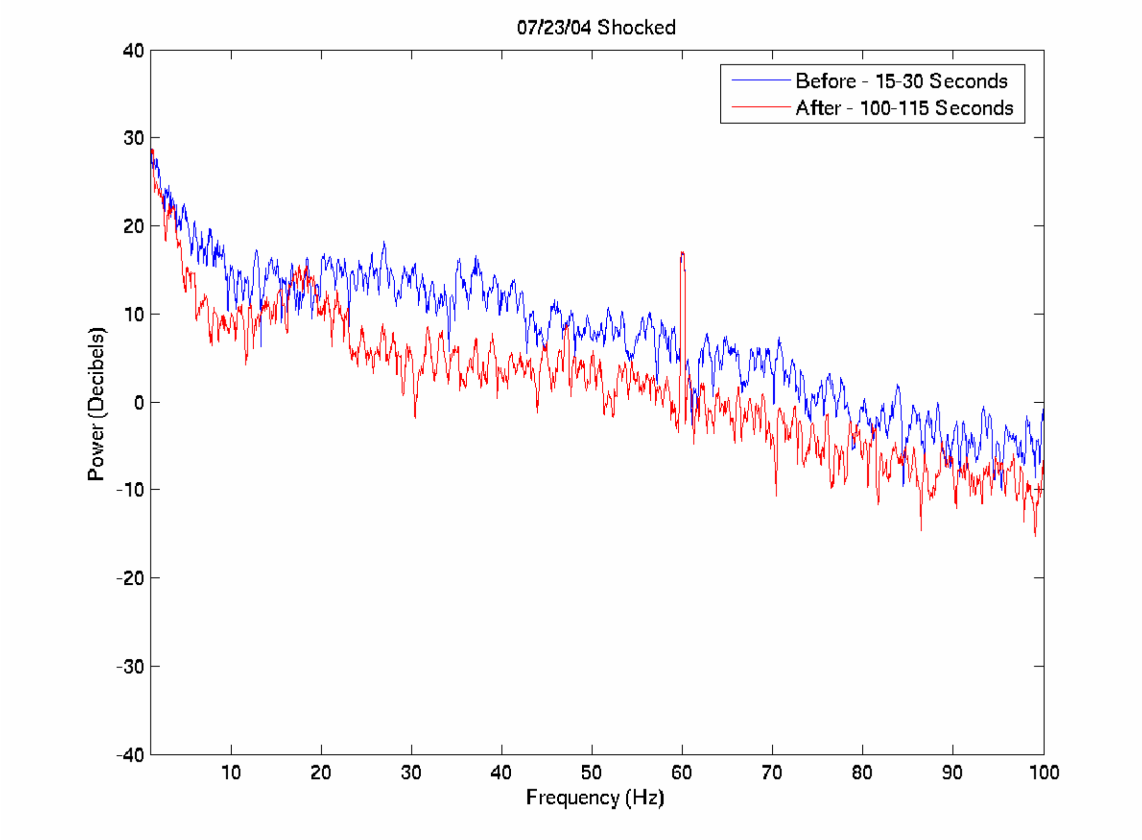
Appendix 2 Mild shock treatment



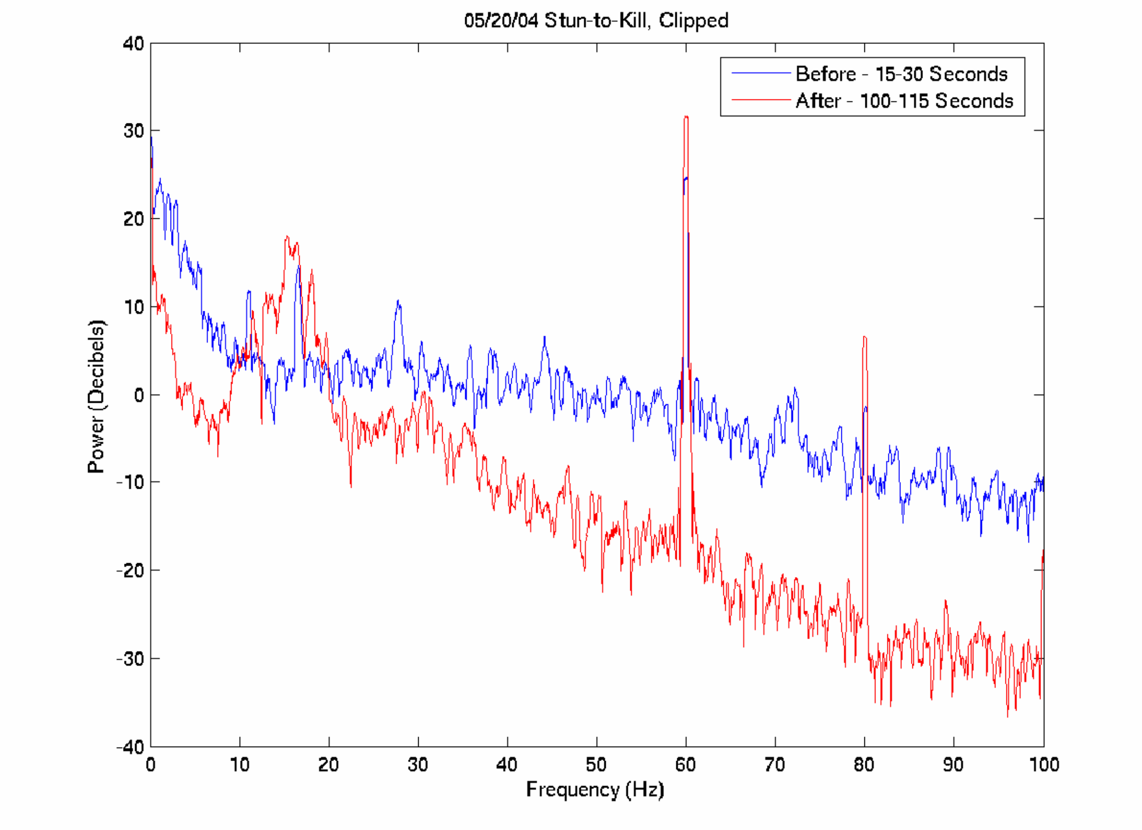
Appendix 3 Mild shock treatment



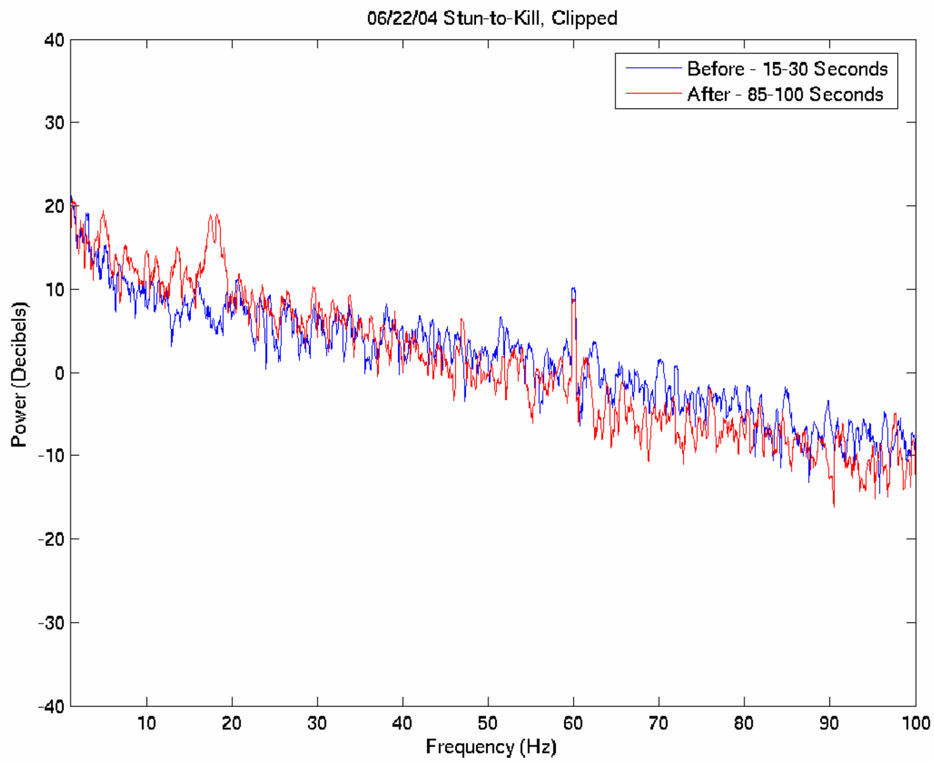
Appendix 4 Mild shock treatment

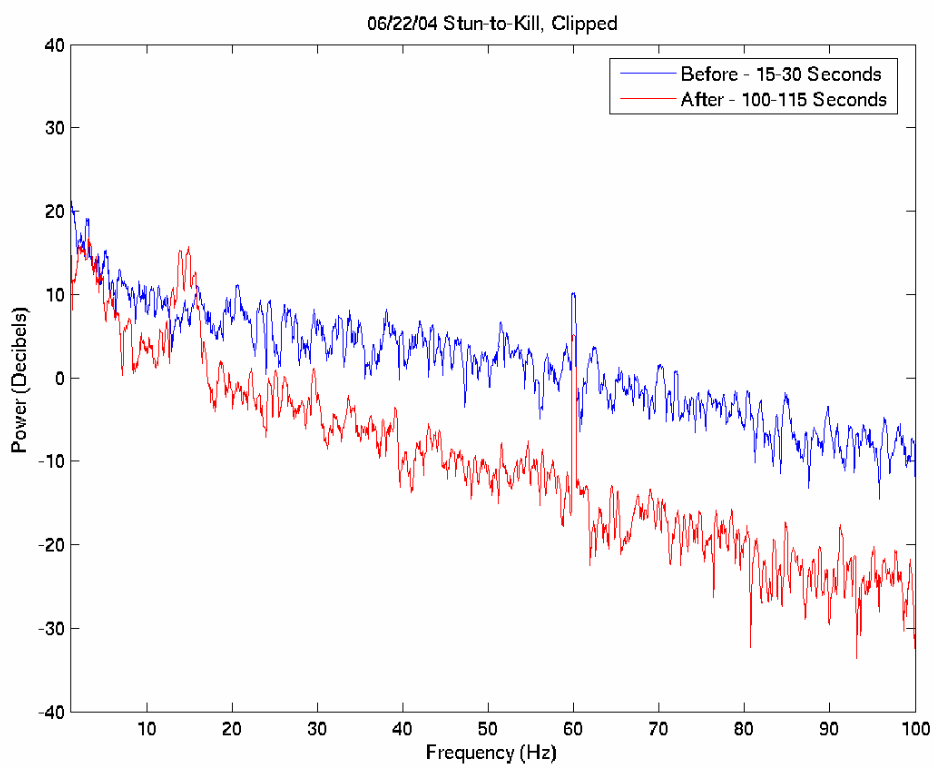


Appendix 5 High Voltage/Low Frequency treatment



Appendix 6 High Voltage/Low Frequency treatment





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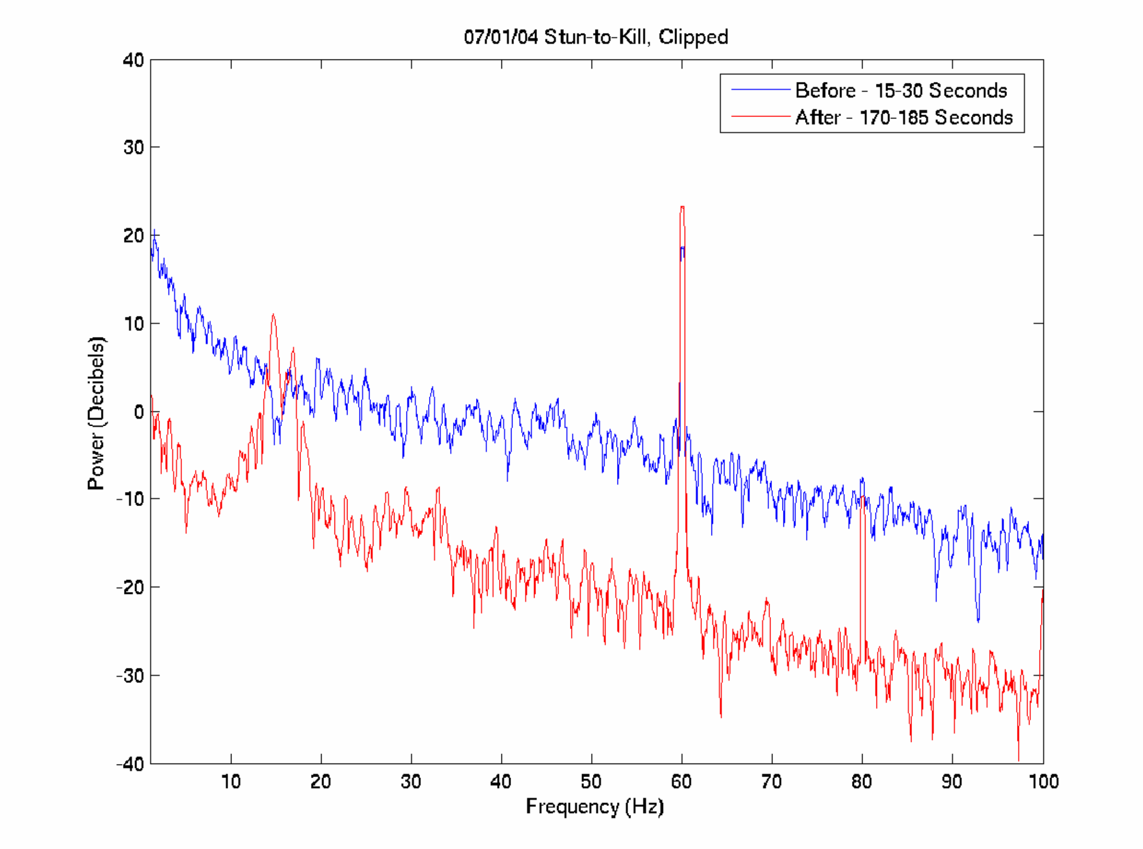
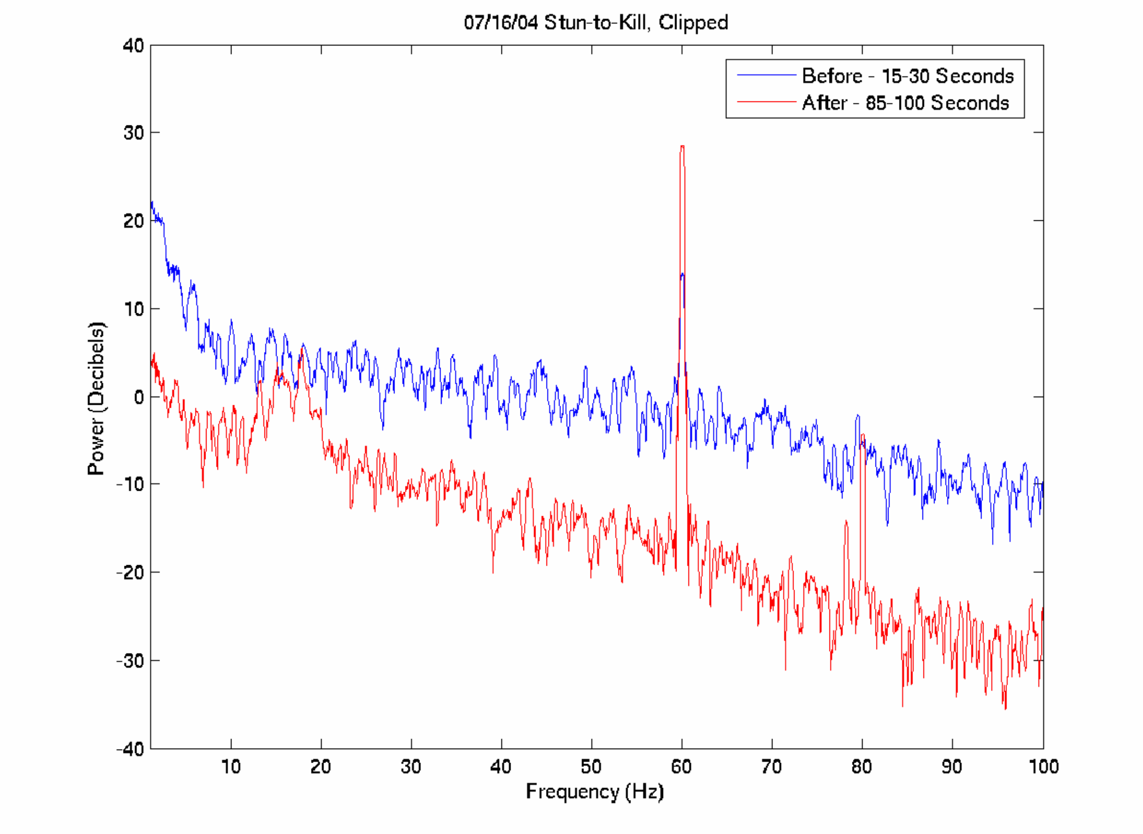
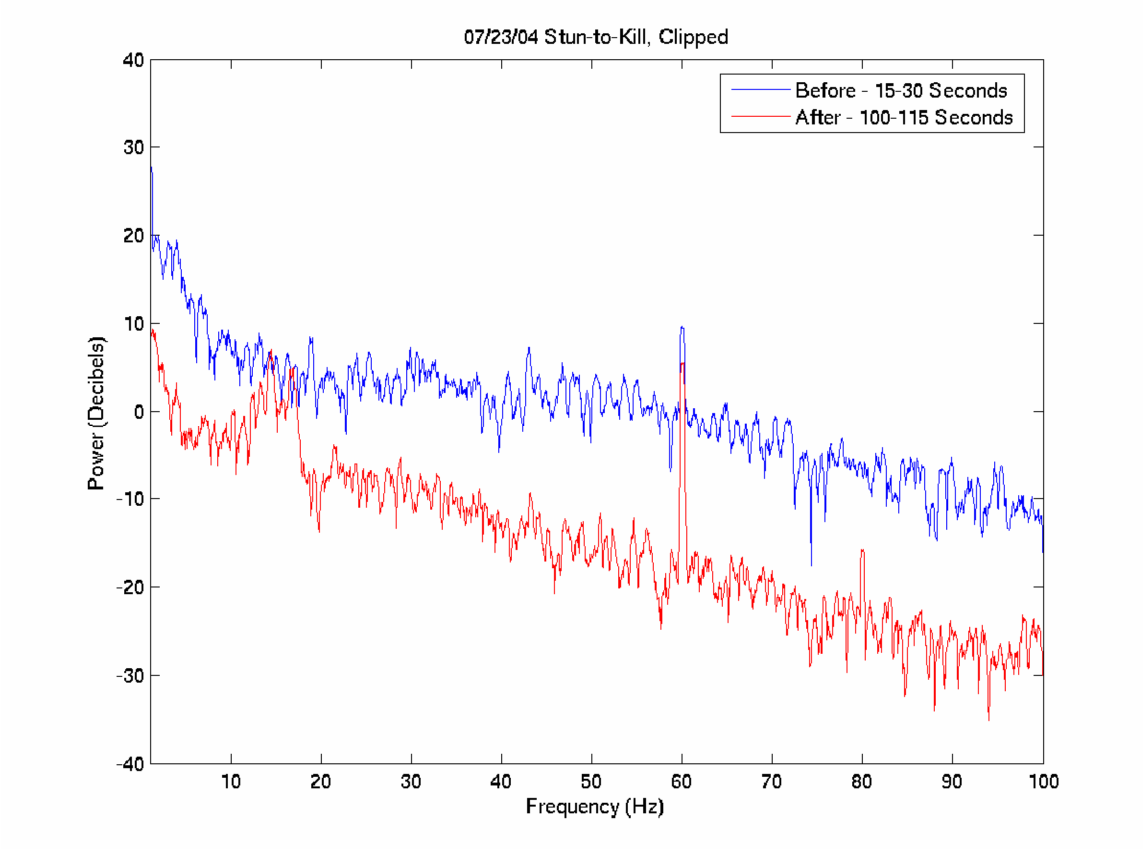


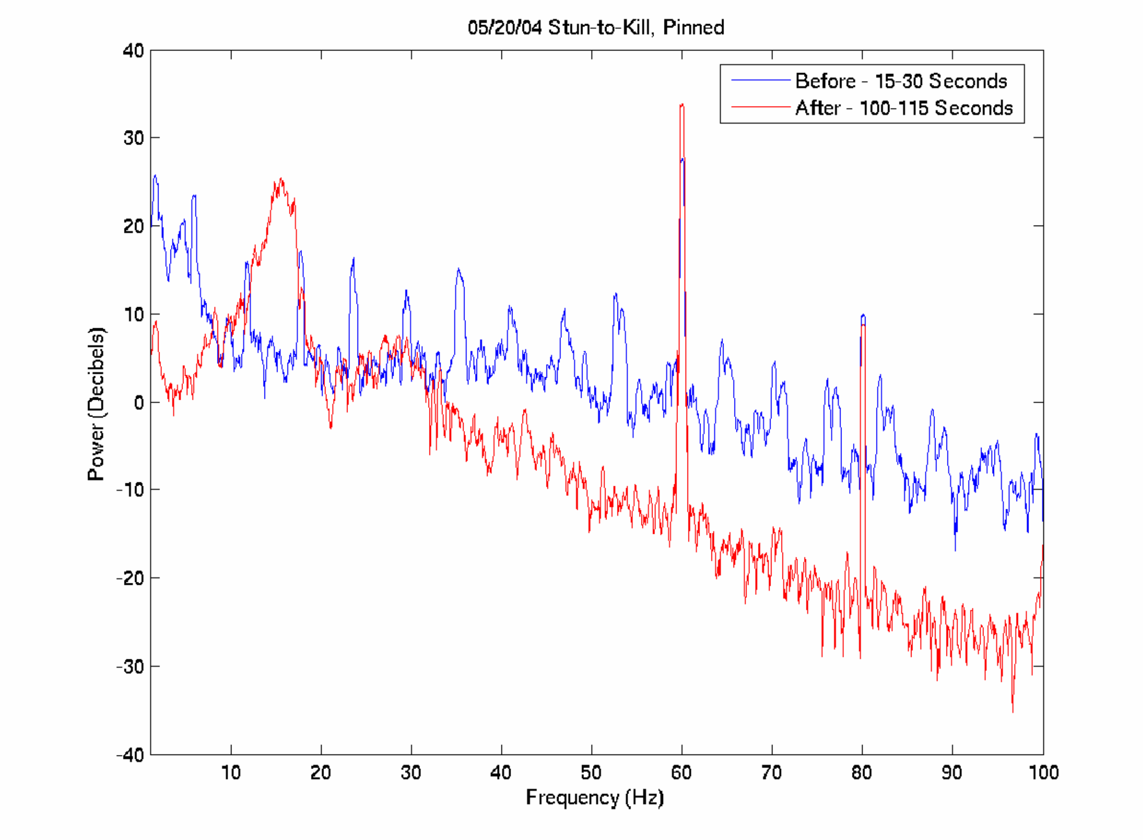
Appendix 8 High Voltage/Low Frequency treatment



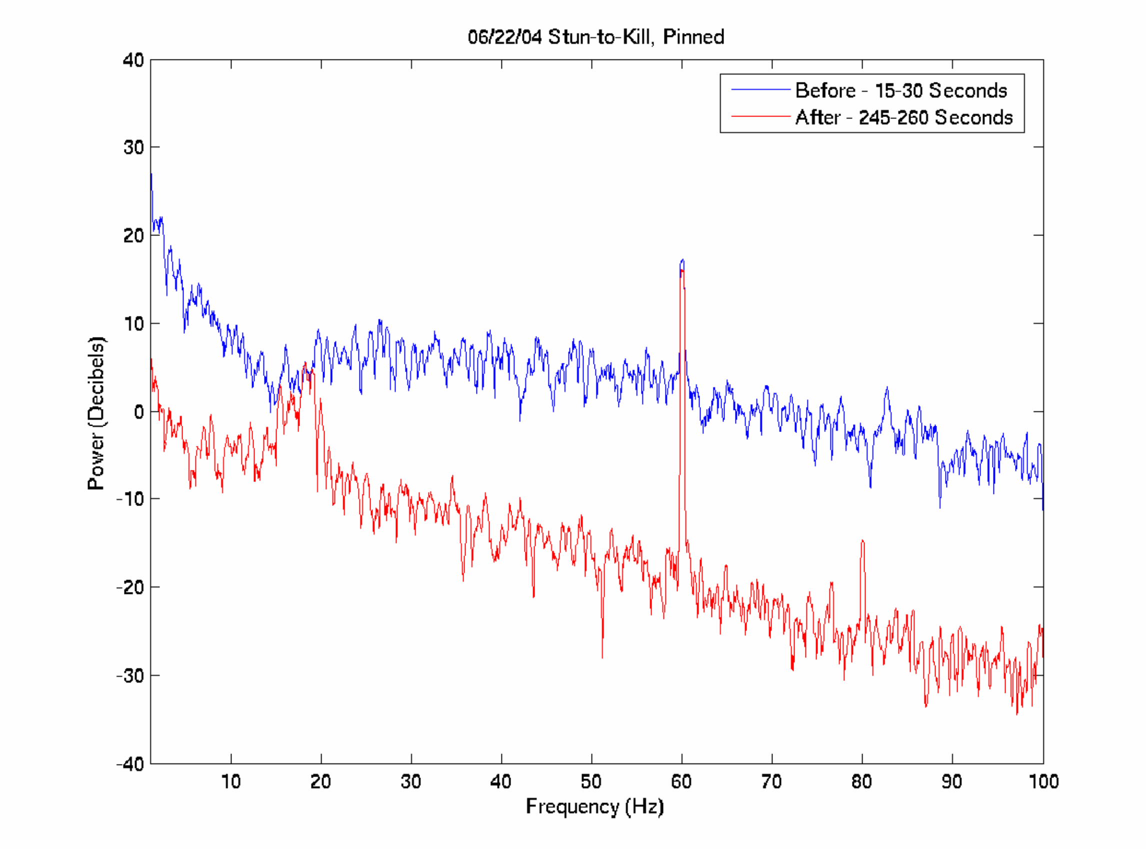
Appendix 9 High Voltage/Low Frequency treatment



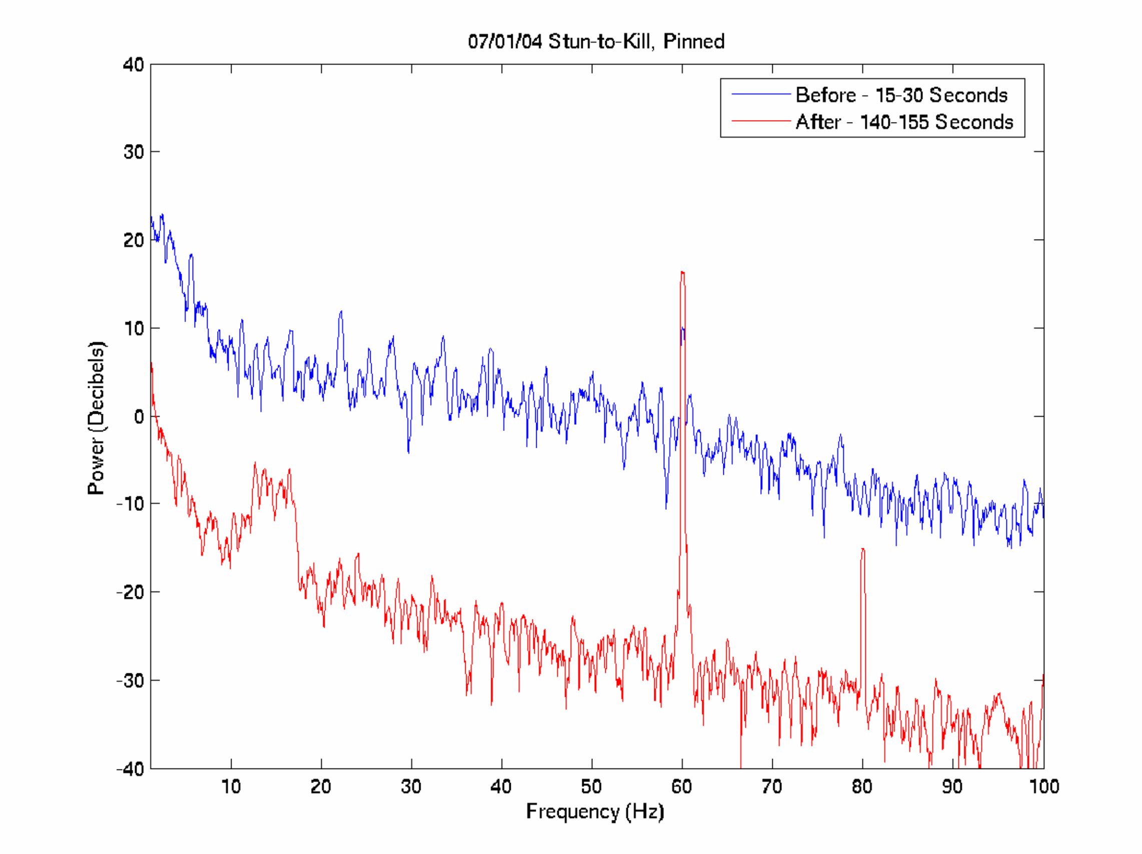
Appendix 10 High Voltage/Low Frequency treatment



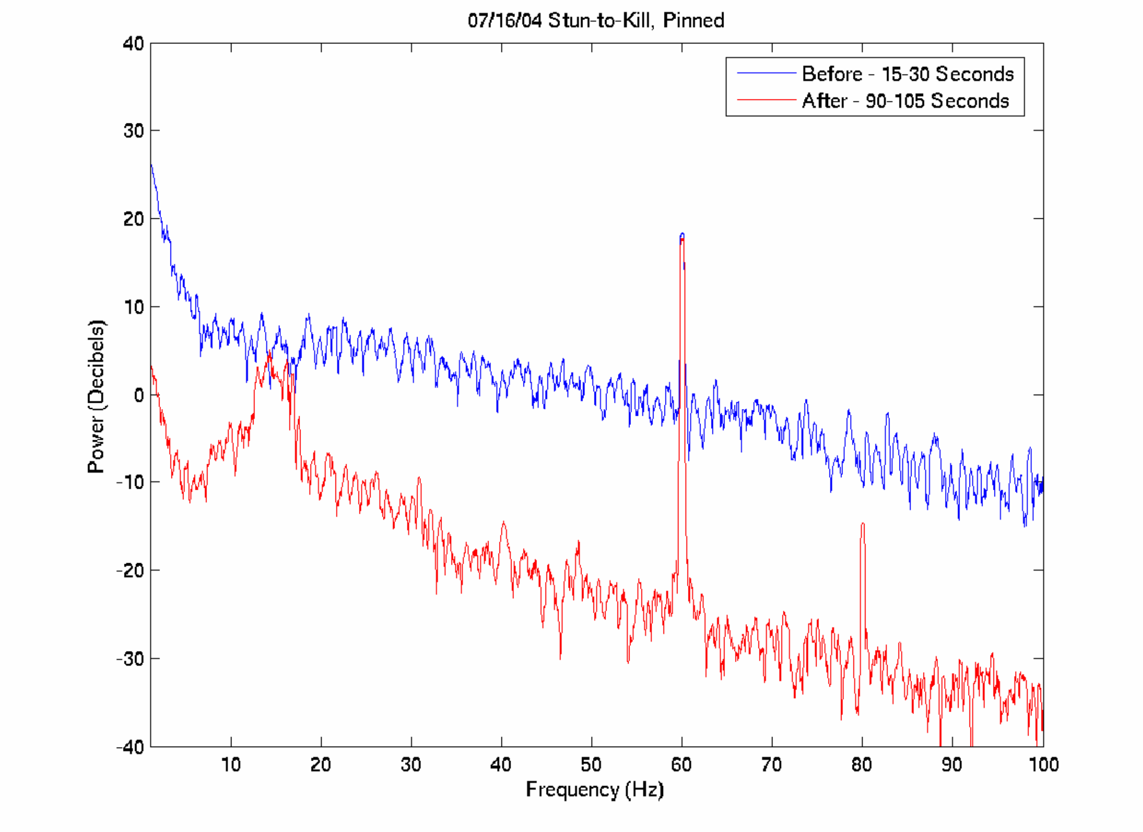
Appendix 11 High Voltage/Low Frequency treatment



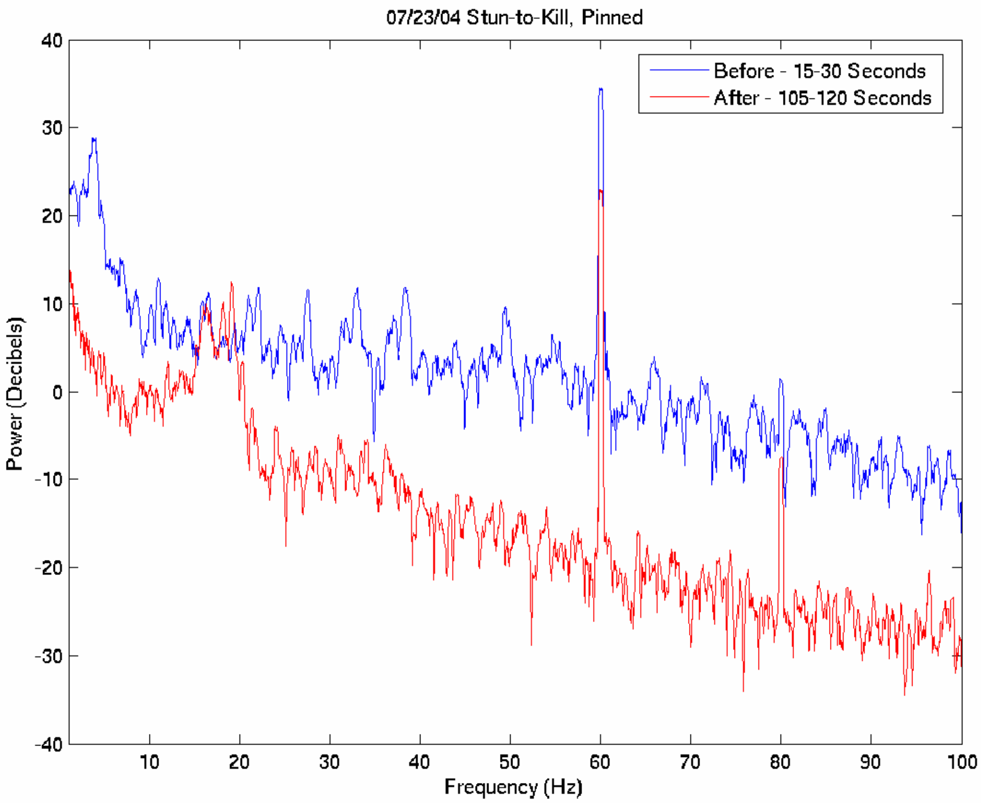
Appendix 12 High Voltage/Low Frequency treatment



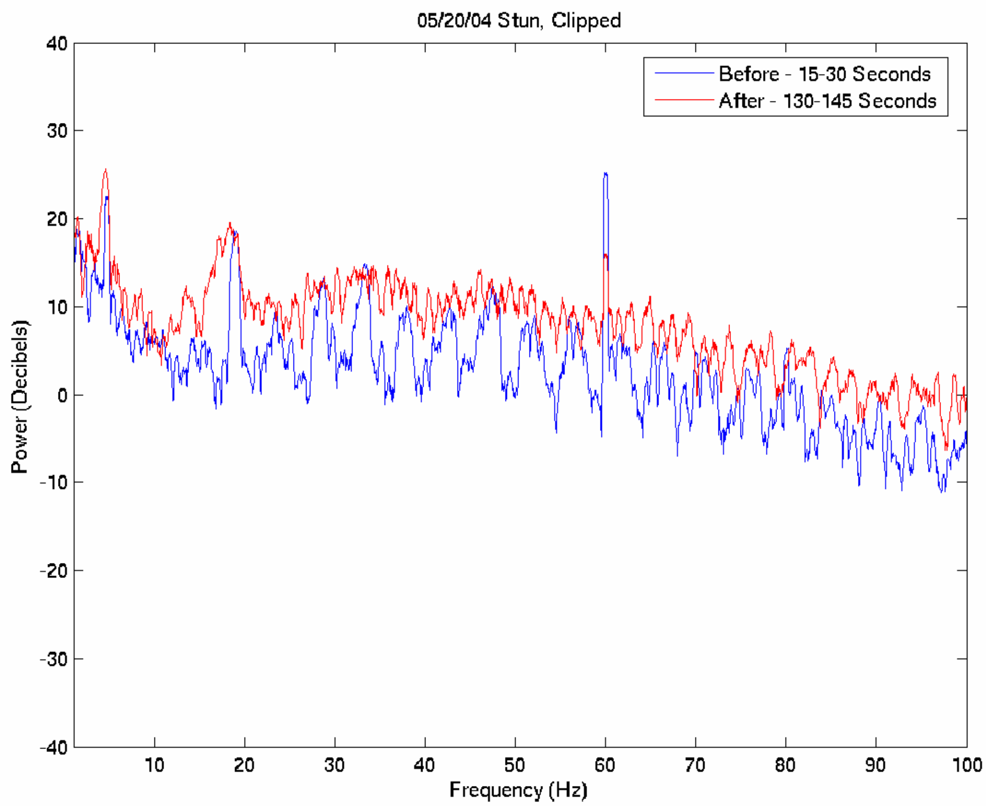
Appendix 13 High Voltage/Low Frequency treatment



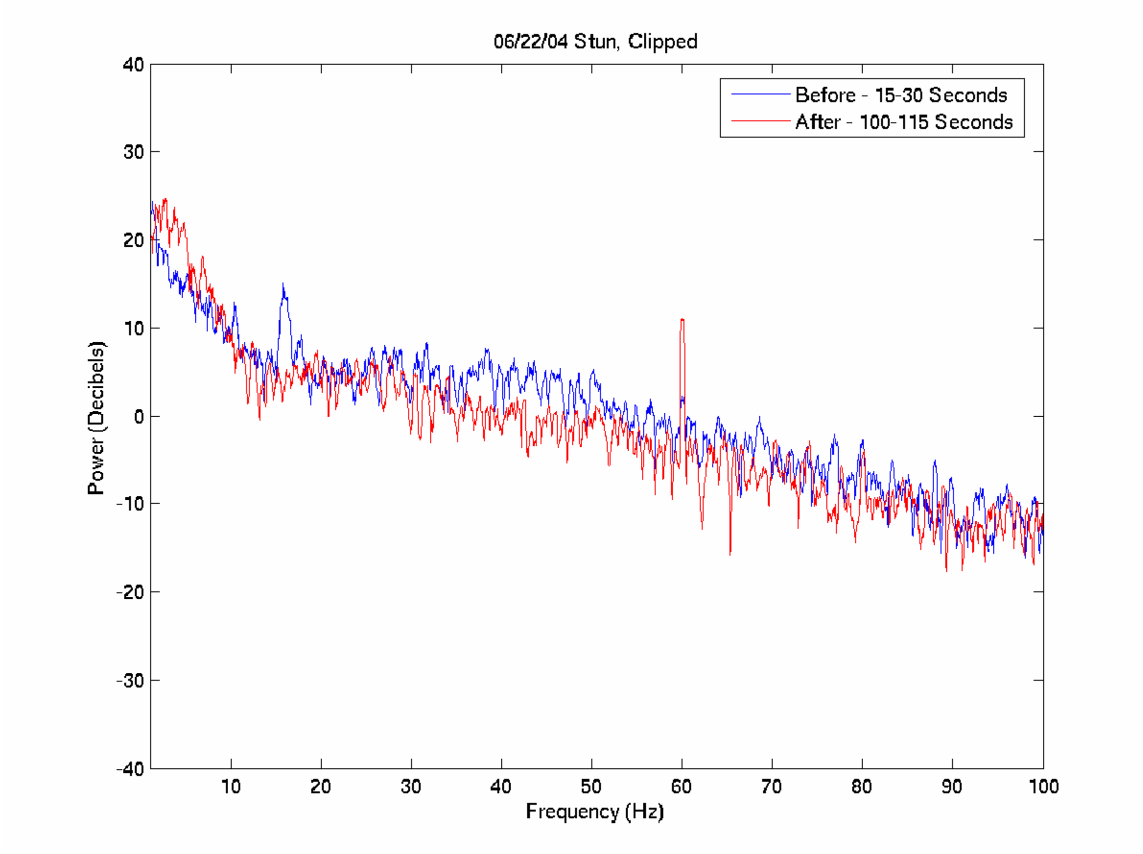
Appendix 14 High Voltage/Low Frequency treatment



Appendix 15 Low Voltage/ High Frequency treatment



Appendix 16 Low Voltage/ High Frequency treatment



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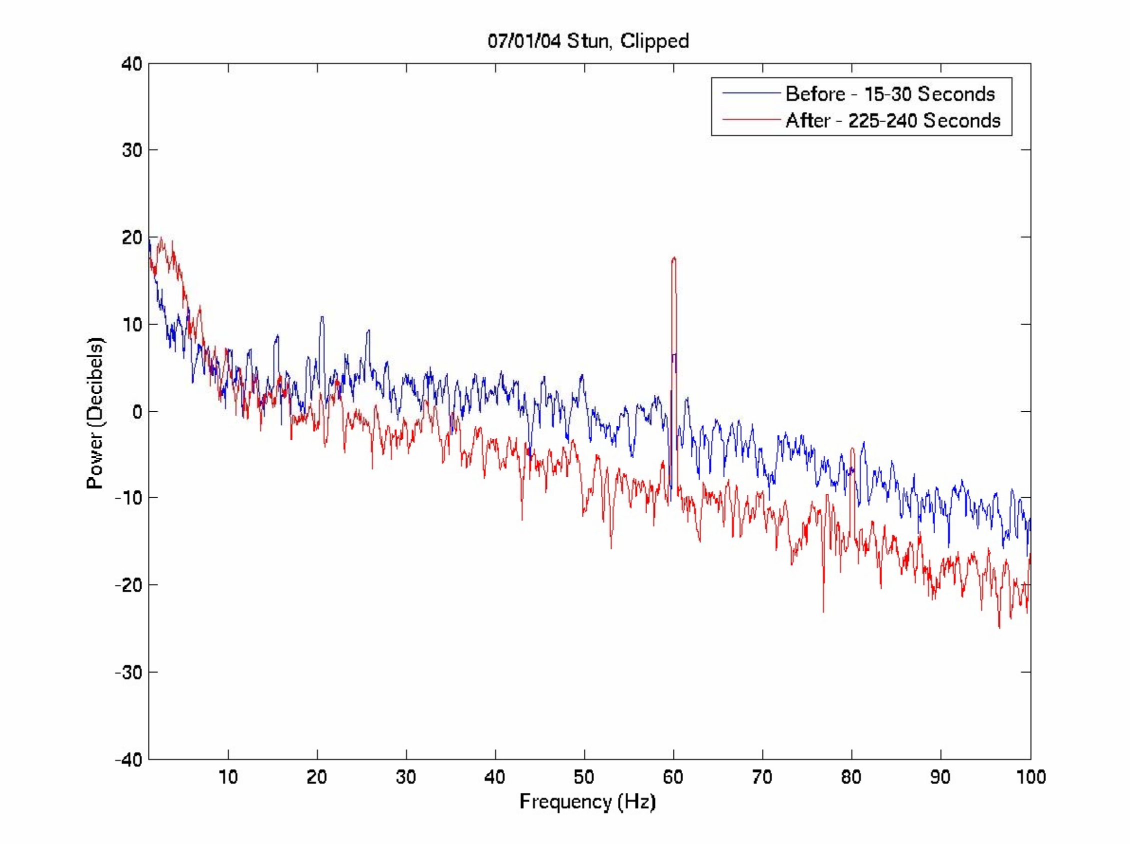
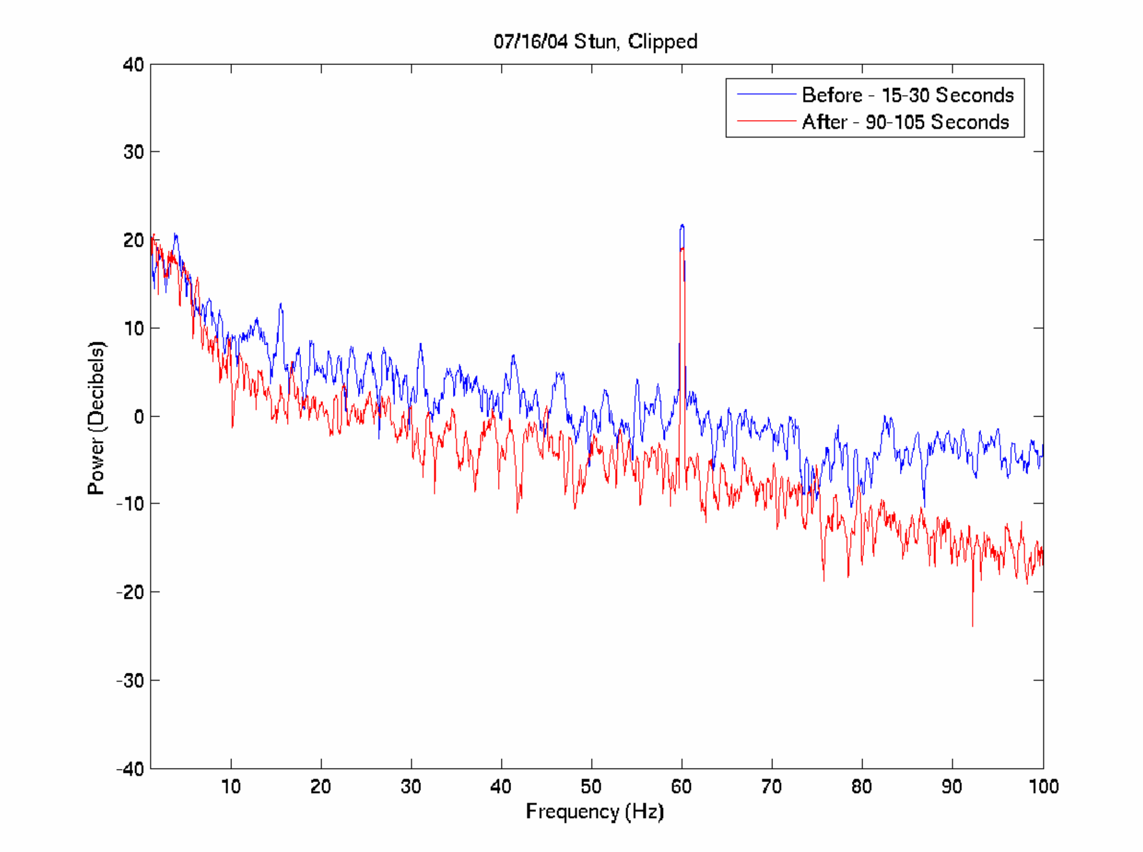
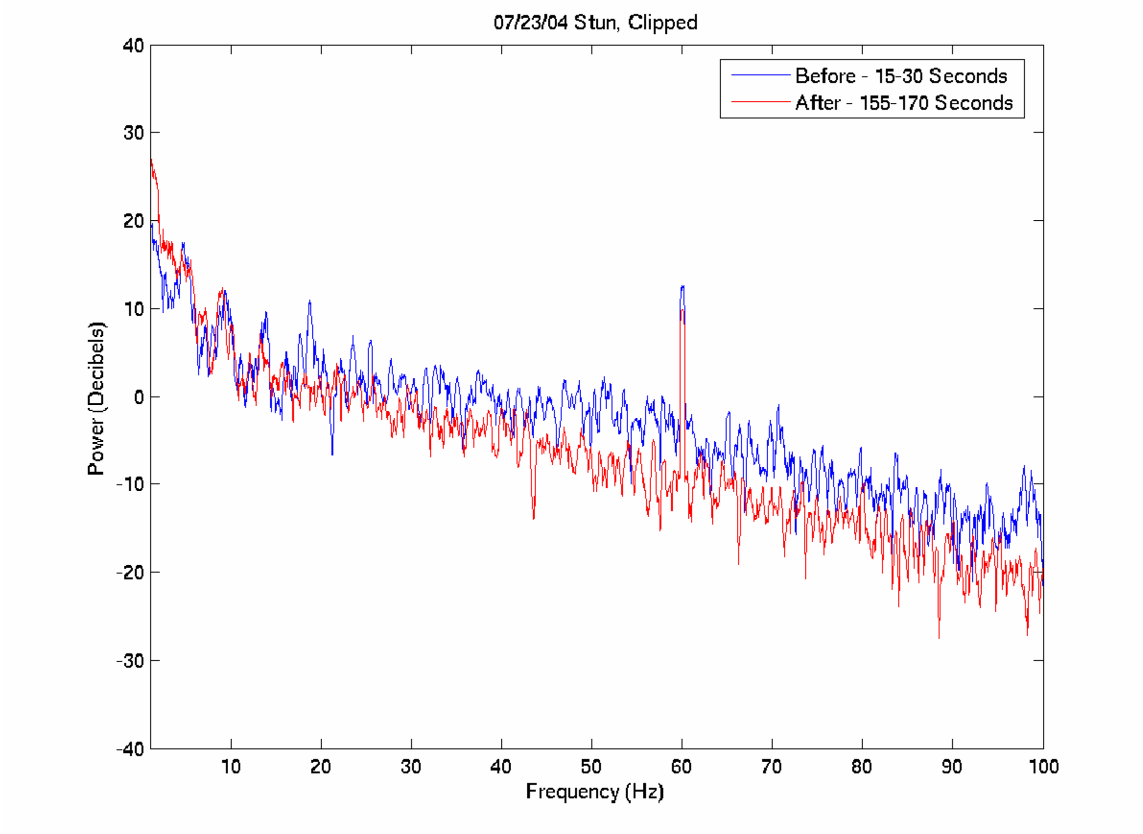


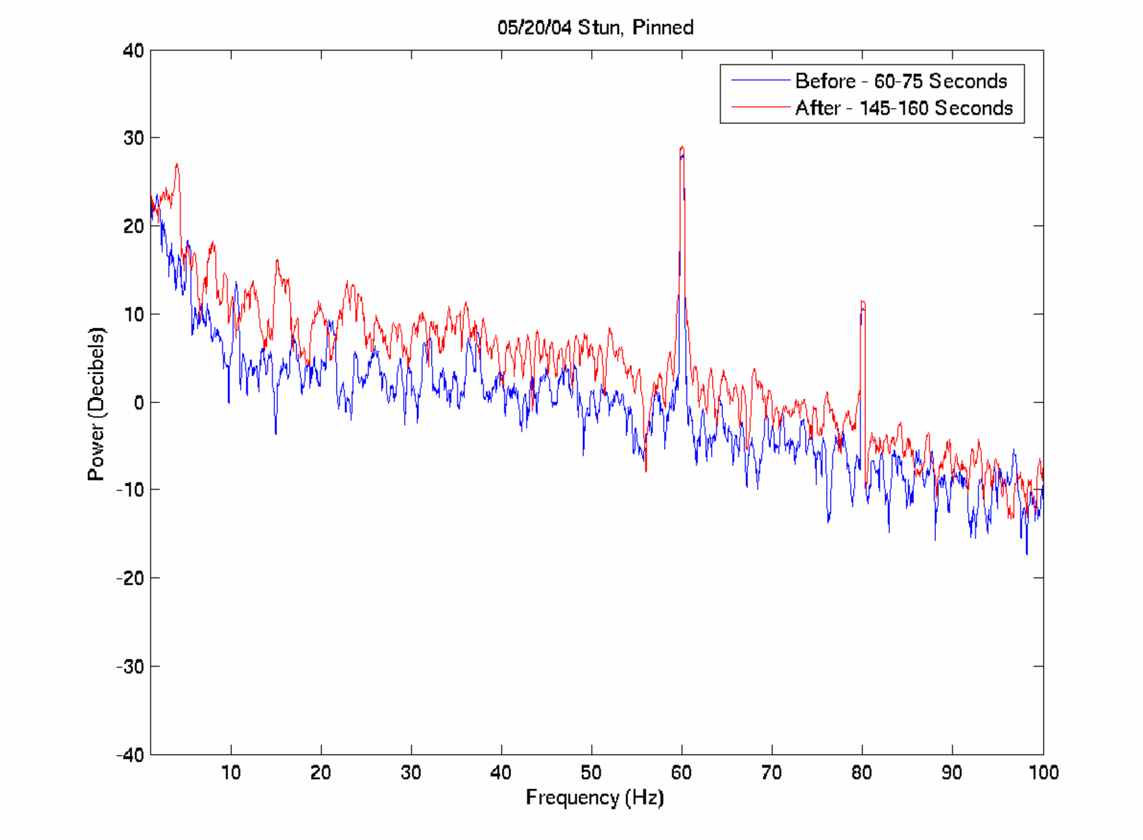
Appendix 18 Low Voltage/ High Frequency treatment



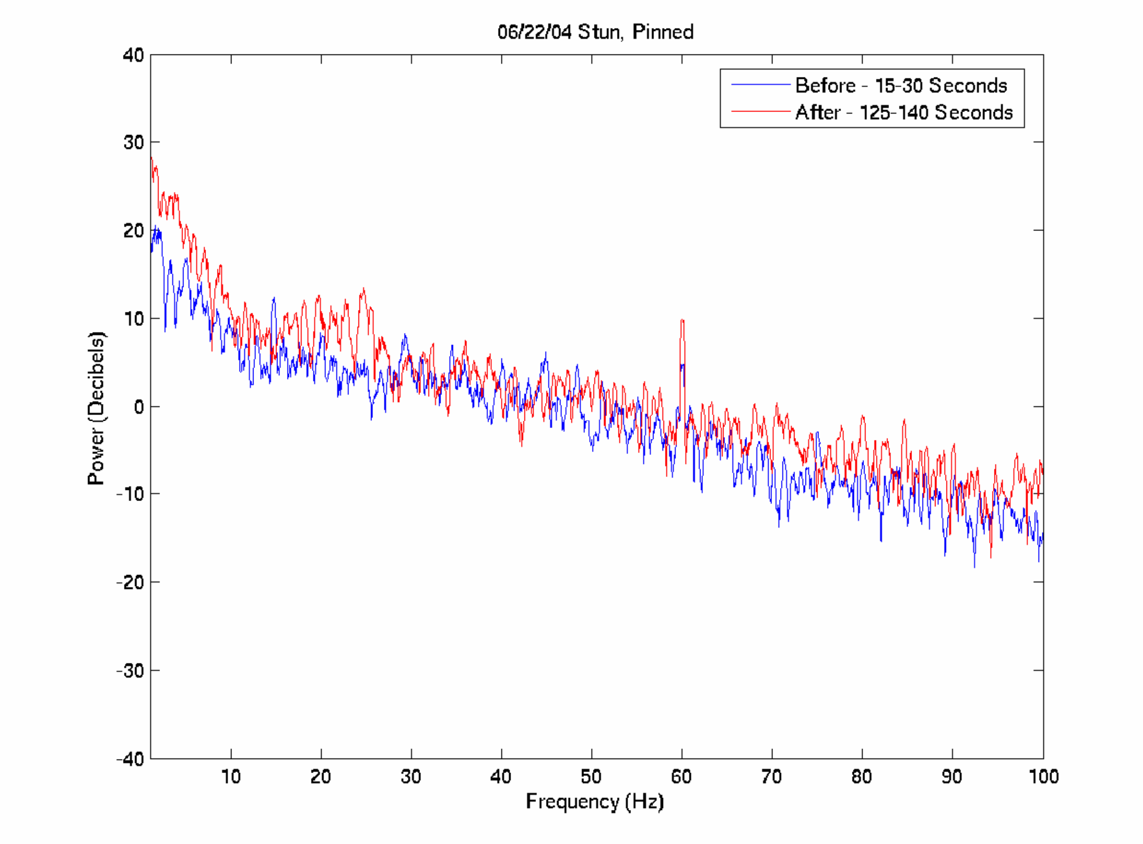
Appendix 19 Low Voltage/ High Frequency treatment



Appendix 20 Low Voltage/ High Frequency treatment



Appendix 21 Low Voltage/ High Frequency treatment



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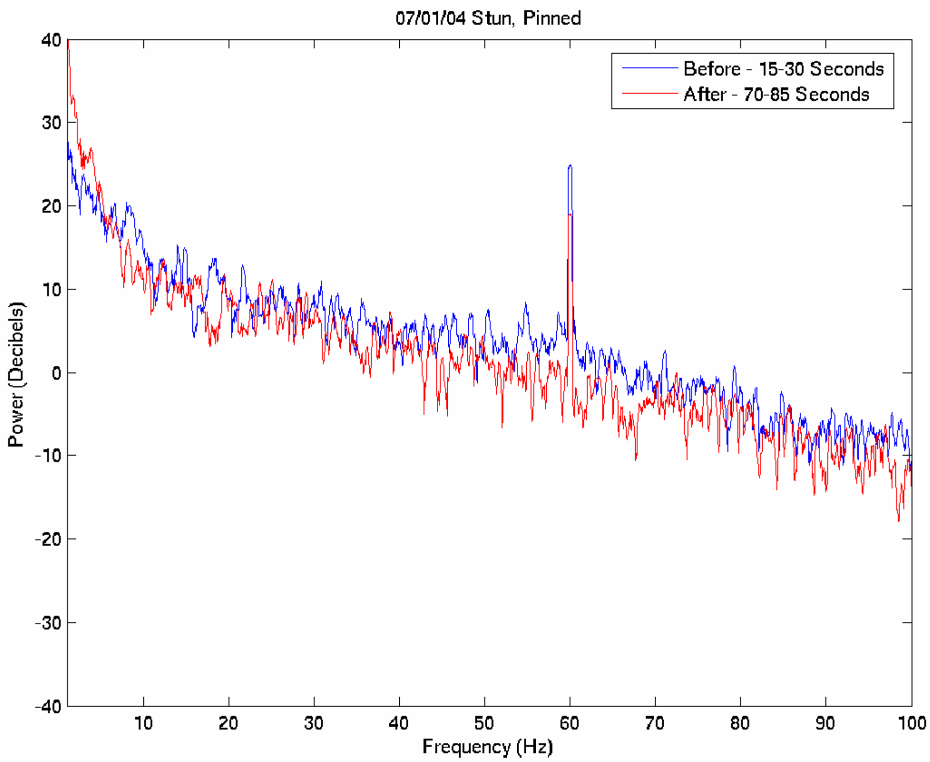
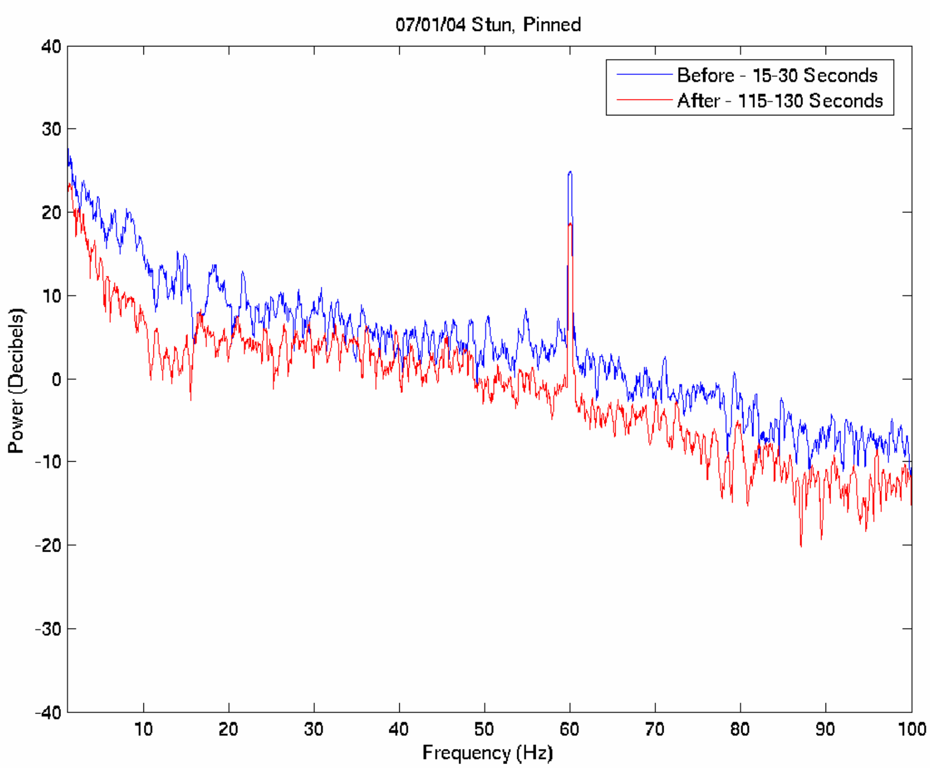
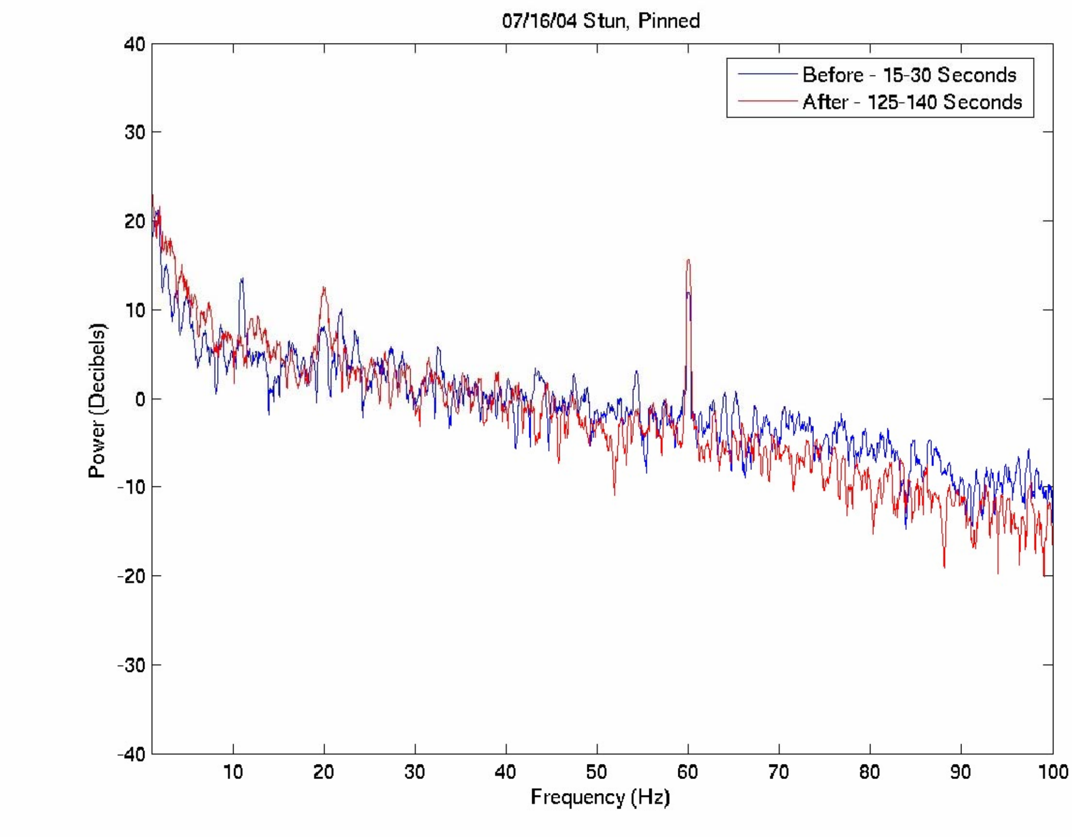




Appendix 23 Low Voltage/ High Frequency treatment



Appendix 24 Low Voltage/ High Frequency treatment