

2012

# Influence of Daily Electrical Stimulation of Periaqueductal Grey on Vocalization and Depressive-Like Behavior during Separation in Guinea Pigs

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INFLUENCE OF DAILY ELECTRICAL STIMULATION OF PERIAQUEDUCTAL  
GREY ON VOCALIZATION AND DEPRESSIVE-LIKE BEHAVIOR DURING  
SEPARATION IN GUINEA PIGS

A thesis submitted in partial fulfillment  
of the requirements for degree of  
Master of Science

By

Jennifer Elaine Dazey

B.S., Wright State University, 2008

2012  
Wright State University

WRIGHT STATE UNIVERSITY  
SCHOOL OF GRADUATE STUDIES

August 24 2012

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY  
SUPERVISION BY Jennifer Elaine Caudill ENTITLED  
Influence of Daily Electrical Stimulation of Periaqueductal Grey on Vocalization and  
Depressive-like Behavior During Separation in Guinea Pigs  
BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
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## Abstract

Jennifer Dazey, M.S., Anatomy Program, Department of Neuroscience, Cell Biology, and Physiology, Wright State University, 2012. **Influence of daily electrical stimulation of Periaqueductal Grey on vocalization and depressive-like behavior during separation in guinea pigs.**

Maternal separation has been shown to promote the onset of depression. This early life stressor produces a biphasic response marked by an active “protest” phase followed by a passive “despair” phase in humans as well as several other species. In infant guinea pigs, active phase behaviors include increased locomotion and species-typical distress vocalizations, whereas the passive phase is marked by depressive-like behaviors including a crouched stance, eye-closure and extensive piloerection. The mechanism underlying the transition from one phase to the next is still unknown. The purpose of this study was to determine if daily stimulation of the neural pathway initiating the active behaviors would lead to enhanced expression of the passive behaviors. Guinea pigs were separated into experimental and control groups. The control group received daily stimulation of a brain region not anticipated to produce vocalizations (cortex) while the experimental group received daily stimulation of the periaqueductal grey (PAG). Although stimulation of the PAG produced vocalizations that decreased across the 10 days of testing, the PAG stimulated animals did not show more passive depressive-like behaviors than pups receiving control-region stimulation.

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## Acknowledgements

The authors would like to thank Jackie Floyd and Christopher Fitch for technical assistance. The work was supported by grant from the Hope for Research Fund (HDRF).



## *I. Introduction*

### *Depression and maternal separation*

Major depression affects millions of people every year. About 10% of adult Americans suffer from this debilitating illness, and the instances of depression are on the rise for teen and young adults (Kroes et al., 2007). At the present, antidepressants in the form of pharmaceuticals are the main treatment option for patients, but antidepressants are not always effective; therefore, it is vital to gain an understanding of the mechanisms that drive the onset of this illness. It has been hypothesized that early life stressors are among one of the contributing factors that increase vulnerability in adulthood to depression (Kendler et al., 1992). Maternal separation is one such early life stressor that has been shown to affect the developing brain in such a way that leads to depression in adulthood (Gillespie and Nemeroff, 2007).

### *Biphasic response to maternal separation*

The link between early attachment-figure separation and depression was first recognized by Spitz in the 1940's (Spitz, 1946). Children who had spent prolonged time separated from their parents due to hospitalization or quarantine exhibited a syndrome he called "anaclitic depression" marked by profound sadness, social withdrawal, and behavioral changes including lying down or stereotypic rocking. Later, investigators searching for an animal model for anaclitic depression noted that when nonhuman

primates were separated from their mothers, they exhibited a biphasic response. Kaufman and Rosenblum (1967) called the first stage “protest” and noted it was marked by species-typical distress vocalizations and increased locomotor activity. This protest phase is believed to be the infant actively attempting to reunite itself with its mother. The second phase “despair” was exhibited after an extended period of separation (days to weeks) and was marked by a decrease in vocalizations and motor activity; infants may lie down or assume a hunched posture while engaging in self-clasping or other stereotypic soothing behaviors (Bowlby et al., 1952; Kaufman and Rosenblum, 1967). Furthermore, the despair phase was characterized by what appeared to be emotional despondency or even the appearance of physical sickness in the infant. Interestingly, infant guinea pigs exhibit behavioral changes following maternal separation that are similar in form to those observed in primates, but over a shorter period of time. Immediately following separation, infant guinea pigs display an increase in behavioral activation and emit species-typical “whistle” vocalizations, characteristic of distress (Berryman, 1976), and increased locomotor behavior, both of which subside within 60-90 minutes of maternal separation (Hennessy et al., 1995) and give way to behaviors that are similar to those observed during despair in primates. During this “passive” stage in guinea pigs, infants exhibit a crouched posture in which the back is hunched and all four feet are drawn under the body, extensive piloerection and near or complete eye closure (Fig. 1).

#### *PAG and vocal control*

Concurrent with the onset of active, distress-related behavior in separated guinea pig pups, maternal separation evokes activation of the hypothalamic-pituitary-adrenal (HPA) axis, increased expression of corticotropin-releasing factor in the paraventricular

nucleus (Maken et al., 2010), and circulating levels of adrenocorticotrophic hormone (Hennessy et al., 1989) and cortisol (Hennessy and Ritchey, 1987).

Neural pathways supporting the production of distress vocalizations have been identified, and it appears likely that these regions are activated at the same time as the HPA system. The periaqueductal grey (PAG) is critical for the production of distress calls in infant guinea pigs; activation of this region triggers the onset of vocalizations and lesioning this region causes animals to become mute without akinesia (Jurgens and Pratt, 1979; Jurgens and Richter, 1986; Zhang, et al., 1994 ). These brainstem regions appear to be suppressed by opioids, as indicated by a decrease in distress vocalizations following opiate administration (Panksepp et al., 1988). It was discovered that this effect is related to the endorphin-mediated system. During stimulation of the PAG, an analgesic effect occurs; the concentration of endorphins increases in the cerebrospinal fluid, which causes reduction in distress vocalizations by acting on the brain stem (Herman and Panksepp, 1981). In contrast, administration of opioids antagonists, like naloxone, triggers an increase in vocalizations (Panksepp et al., 1988).

In 1915, T.G. Brown first suggested the role of the PAG in vocal control during his work with the chimpanzee. When a stimulating electrode was placed near the aqueduct, a vocalization was produced. Similar studies have shown that stimulation of a variety of sites in the PAG of cats and rhesus monkeys also elicited vocalizations. The types of vocalizations induced through stimulation of the PAG are typically natural agonistic calls: hissing, growling, and howling in cats, and shrieking, yelling, and cackling in the squirrel monkey (Jurgens, 1994; Mangoun, et al., 1937). Within each species of animal, non-agonistic calls also occurred: meowing in cats and purring in

squirrel monkeys. But do the vocal reactions represent a direct stimulation of motor neurons or are they a secondary reaction due to emotional changes based on stimulation location? Studies on rat and squirrel monkey showed that the specific site of stimulation within the PAG determined the emotional effect whether positive or aversive. In contrast, studies conducted on squirrel monkey revealed that the emotional reaction elicited had no correlation to the type of vocalization observed. Different sites within the PAG that produced a specific vocalization elicited very different emotional responses, thus leading researchers to conclude that vocalizations are not secondary reactions due to upstream emotional changes (Jurgens, 1994).

Sugiyama et al. (2010) used electrical stimulation along the brainstem from the PAG to the nucleus retroambiguus (NRA) to determine sites that produced vocalizations. The circuitry proposed for the pathway of the PAG-induced vocalizations begins in the PAG and extends to motor neurons activating the laryngeal muscles in order to produce vocalizations (Larson, 1991; Larson and Kistler, 1984, 1986; Holstege, 1989). Using a cat model, Sakamoto et al. (1996) discovered that the neurons for the PAG occur laterally and then run dorsally to the pontine reticular formation. The PAG receives input from many sensory areas via glutamatergic neurons and is directly connected to the periaqueductal reticular formation, an area of the brainstem which has a direct connection with all the phonatory motor nuclei. Herman and Panksepp (1994) discovered while mapping the brain for distress vocalizations that locations including the ventral septum-preoptic area and doromedial thalamus provided the most favorable areas for obtaining vocalizations. The research also revealed that distress vocalizations resulting from stimulation of the forebrain area appeared similar to separation distress vocalizations

observed in infant guinea pigs. Not only do we need to have a thorough understanding of the neural circuitry responsible for triggering the active vocal behaviors, but the immune system has also been demonstrated to have an impact on the cessation of active behaviors via pro inflammatory cytokines.

In the guinea pig, passive behavior appears to be strongly influenced by pro-inflammatory cytokines. Levels of tumor-necrosis factor- $\alpha$  increase with prolonged separation and administration of several different anti-inflammatory compounds reduces the occurrence of crouch, eye-close, and piloerection (Hennessy et al., 2007; Hennessy et al., 2009; Perkeybile et al., 2009; Schiml-Webb et al., 2006). Thus, it appears that one of the mechanisms triggering the shift from active to passive behaviors is the rise in pro-inflammatory factors that occurs following maternal separation. What is unknown, however, is whether the expression of active behaviors is a necessary behavioral precedent for the onset of passive behaviors.

The PAG, which is situated within the limbic system, is thought to enhance the generation of the emotions that mediate pro-social behaviors as well as separation distress calls (Panksepp, 1988). The PAG also receives input from the forebrain limbic system, which impacts the motivational aspect of vocal behavior (Kittelberger et al. 2006). The PAG and other brain sites including medial diencephalon, amygdala, bed nucleus of the stria terminalis (BNST) and nucleus accumbens all play a role in the circuitry called the “PANIC” system (Panksepp, 1988). Electrical stimulation of the PAG in human subjects has led to feelings of fear, anxiety and agitation (Wright & Panksepp, 2011). Panksepp proposes that other emotional systems including FEAR, RAGE and SEEKING all converge onto the PAG the location of central emotional processing (Panksepp, 2011).

### *Hypothesis and Study*

The present study identifies the PAG as one of the brainstem regions involved in infant guinea pig distress vocalizations (DVs). The study describes the effects of PAG electrical stimulation on the rate of DVs, and also looks at the effect of the elicitation of DVs for several days on the occurrence of passive behaviors during placement in a novel environment. Additionally, the likelihood of producing an aversive state by electrical stimulation is evaluated by measuring place preferences of animals exposed to distinctive environments during PAG stimulation. We predicted driving DVs via electrical stimulation would accelerate the onset and amplify the amount of passive behavior expressed in later days of testing. We also predicted that artificial stimulation of DVs would be somewhat aversive as measured by place preference.

## II. Methods

### *Subjects*

Female albino guinea pigs (*Cavia parcellus*) arrived at the laboratory ranging in age from 30-32 days old. Animals were housed in pairs in polycarbonate cages measuring 73 x54 x 24 cm with saw dust bedding. Water and food were continuously available. The colony room where the animals were housed was maintained on a 12:12 light/dark cycle, with lights turned on at 0700. The testing and colony rooms were maintained between 22 and 25°C. Guinea pigs were assigned to one of two separate groups: the PAG group (n=10), the cortical control group (n=9). All procedures were approved by the Wright State University Laboratory Animal Care and Use Committee.

### *Surgery*

Between days 34 and 40 (birth considered day 0), all pups underwent surgery for placement of an indwelling, left unilateral electrode aimed at the periaqueductal grey (PAG) located around the cerebral aqueduct within the tegmentum of the midbrain. Pups were pretreated with atropine (0.05 mg/kg) and anesthetized throughout surgery with isoflurane (1-5%). A local anesthetic (0.25 mg/0.1 ml 0.25% bupivacaine) was administered subcutaneously at the surgical site. Electrodes were constructed with two poles, one anterior and the other posterior; stereotaxic coordinates were determined using

the posterior pole. For the PAG group, the electrode was placed relative to bregma with coordinates of -5.5 mm anterior-posterior, +0.5 mm medial-lateral and -6.5 mm dorsal-ventral; stainless steel screws were placed adjacent to the electrode to help secure the cranioplastic cement. The control group electrode was placed relative to bregma with coordinates of +2.0 mm anterior-posterior, +5.5 mm medial-lateral and -1.0 mm dorsal-ventral. All supplies were sterile at the time of surgery and electrodes were purchased from Plastics One (Roanoke, VA). All pups were treated with buprenorphine (0.015 mg/0.05 ml) upon recovery from surgery and again 24 hours later to control pain. Each day, animals were weighed, surgical site checked, and overall health assessed. Animals were allowed to recover from surgery for at least 3 days prior to threshold testing.

#### *Threshold Testing and Testing Protocol*

Guinea pigs were placed in a clean plastic cage in the testing room under full room lighting for a series of electrical stimulations. The electrode was connected to via an insulated cable to the commutator positioned directly above the testing area to allow the animal to move freely about the testing cage. The commutator was connected to a stimulus isolation unit, which was connected to the output of a stimulator. A 10-sec train of 0.1-ms electrical pulses were delivered at increasing intensities to help identify the optimal current level for further testing. The levels of stimulation were applied in increasing intensity beginning with 100  $\mu$ A up to a maximum of 800  $\mu$ A (in increments of 100  $\mu$ A) and each current was applied once. After a 10-sec of stimulation each animal was allowed 2-mins to recover before the next stimulus was applied. During the 2-min recovery, the number of vocalizations was recorded.



For the purposes of this study, threshold is defined as the current level in milliamps (mA) at which clear vocalizations were observed during stimulation and without abnormal behaviors (e.g., aberrant motor responses, pain screams or atypical involuntary movements). If these responses were observed, the stimulation for threshold testing was terminated immediately. Level of threshold stimulation and the number of vocalizations helped to determine the current level chosen for behavioral testing. If at threshold the animal emitted fewer than 150 clear vocalizations, then the testing intensity was set to 100 $\mu$ A above threshold for behavioral testing. However, if the animal emitted 150 vocalizations or more then this was the intensity used for behavioral testing. The stimulus intensities for the cortical control group were chosen to match those of the PAG group. Both the anterior and posterior poles on the electrode were tested, and the pole that yielded the clearest vocalizations was chosen.

Beginning 1 to 2 days following threshold testing, between 36-44 days of age, animals began the testing sessions which lasted 10 consecutive days followed by an 11<sup>th</sup> day of place preference testing. On Day 1, the animal was removed from its home cage, quietly carried in a small transport cage from the colony room to the testing room, and placed in a clean Plexiglas chamber (11 x 9 x 11 in). The electrode was attached to the stimulator. The chamber walls had a distinct pattern made by black electrical tape on the outside of 3 of the walls; the 4<sup>th</sup> wall remained clear for observations. The patterns were either vertical or horizontal lines to provide a distinct context for each animal; the pattern choice was determined quasi-randomly for each animal. The cage with the horizontal lines was lined with a plain piece of white typing paper on the cage bottom, and the floor of the vertical chamber was lined with a piece of 60 grit sand paper. The test began with a

5-min acclimation period followed by a 5-min pretest period. No data were collected during the acclimation period. During the 5-min pretest period, the number of low and high whistle vocalizations spontaneously emitted were counted via a hand counter, and passive behaviors were scored in a one-zero fashion per minute using paper and pencil. Following this period, the testing phase began. During the testing phase, both the cortical control and PAG groups received a series of stimulations for 30-min (current was applied for 10 sec at 2-min intervals). The numbers of high and low vocalizations were recorded in 15-min intervals using a hand counter, and the passive behaviors were recorded as above. At the end of the 30-min testing phase, animals were moved to a “home cage” testing area for the home cage or post-stimulation test. The home cage from the colony room had been moved to the testing area prior to the pretesting; bedding remained but all other animals were removed. The animal was placed in this area for 15- min undisturbed. During this time, vocalizations and passive behaviors were recorded in order to determine if electrical stimulation of the PAG caused a change in behavior in the home cage.

The testing procedure was repeated on Days 1-4 and Days 6-9. On Day 5 and Day 10 of testing, a probe session was performed. Handling was the same except that animals did not receive electrical stimulation; vocalizations and passive behaviors were still recorded as during previous days. These probe tests were performed in order to determine if the physical environment had an impact on the behavior of the animals in the absence of electrical stimulation.

The final day of testing, Day 11, the animal was placed in a Plexiglas testing cage (9 x 33 x 11 in) that was divided into three compartments. One end of the testing cage, the compartment walls were marked with electrical tape in a horizontal pattern and the

cage floor was lined with white typing paper. At the other end of the cage, compartment walls were marked with electrical tape in a vertical pattern and the cage floor was lined with a piece of 60 grit sand paper. These two enclosures, thus, mimicked the testing cages, with one being similar to that in which the animal received stimulation and the other novel. The center compartment of the testing cage was left clear/free of tape and no paper was placed on the floor. The three testing areas were separated by Plexiglas walls that had entrances placed so the animal could move between the three compartments. The animal was placed in each of the lined areas for 30 sec, and then placed in the middle “neutral” section last; the cage lid was placed on the top, and the animal was left alone for 1-hr. This 1-hr test was recorded with a video camera placed in front of the testing cage so that behavior, duration of time spent in each of the three compartments, could be scored later.

### *Scoring Behavior*

During the testing procedure, a trained observer recorded the total number of high and low vocalizations (Berryman, 1976). Vocalizations were scored with a hand-held counter. The observer scored the characteristic crouched posture in which the feet are tucked beneath the body, complete or near-complete closure of one or both eyes, lying down (chest is supported completely by cage), and extensive piloerection (over 50% of the body). The measure employed was the number of intervals in which the guinea pig simultaneously exhibited all three passive behaviors (designated “full passive” response). Because single instances of crouch/lying down, eye-closure, and piloerection typically occur over an extended period of time, these behaviors were scored with one-zero sampling as in previous studies (Schiml-Webb, et al., 2006). The passive behaviors were

scored with pencil on prepared scoring sheets. Behaviors were scored during the 5-min pre-stimulation period, the 30-min stimulation period, and the 15-min post-stimulation period. The testing chamber was cleaned with detergent prior to each test.

### *Histology*

At the end of Day 11, the animals in the cortical control and PAG group were administered a lethal dose of Euthasol (0.25-0.5 ml; 390 mg/ml pentobarbital/phenytoin solution) and carbon dioxide inhalation. A 10-sec train pulse of 1200 – 1300  $\mu$ A was used to mark the electrode placement. Guinea pigs were then perfused intra-cardially with saline followed by 10% formalin solution. The brains were extracted and placed in a 10% formalin solution to fix for 3-7 days. After the fixing period, the brain was sliced using a freezing microtome or a vibratome and then mounted onto glass slides. Once mounted, the slides were left to air dry for between 24-48 hr. The slides were stained with cresyl violet and Prussian blue to determine the location of the probe and stimulation site.

### *III. Data Analysis*

The data were analyzed using non-parametric tests due to the number of scores of zero. The vocalizations and full passive behaviors of the experimental and control groups were compared using the Mann-Whitney U-test. Friedman 2-way analysis of variance of ranks examined the changes over days for the vocalizations and full passive behaviors of the experimental and control groups during each of the three test sessions: pre-stimulation, stimulation and post-stimulation. Selected follow-up tests were performed using the Wilcoxon signed-rank test which compared the first day of testing to each of the other test days. The Wilcoxon signed-rank test was also utilized to compare the amount of time that the experimental and control groups spent in either the familiar or unfamiliar area of the place preference testing cage during the 1- hr place preference test. The familiar area of the cage was defined as containing the same type of cage lining and electrical tape markings on the three walls that the animals experienced on each of the 10 days of testing.

#### IV. Results

##### *Vocalizations during the Pre-Stimulation Period*

As Figure 2 illustrates, the only apparent differences seen between the experimental and control groups were on Day 1 and Day 3 of testing. However, the Mann-Whitney analysis showed that the number of vocalizations emitted by animals did not differ between groups on any day. Nonetheless, for the experimental group, there was a significant difference in vocalizations across days ( $p < 0.05$ ).

##### *Vocalizations during the Stimulation Period*

Figure 3 illustrates that the median value of vocalizations occurring during the stimulation period of testing for the experimental group were initially high and then declined over the days of testing. The same decline over days was not seen with the control group.

Mann-Whitney tests revealed a significant difference between the Experimental and Control Groups on Day 1 ( $p < 0.05$ ), Day 3 ( $p < 0.05$ ), Day 6 ( $p < 0.01$ ), Day 7 ( $p < 0.01$ ), Day 8 ( $p < 0.01$ ), and Day 9 ( $p < 0.01$ ). The Friedman analysis yielded no significant difference across days for the control group, but the experimental group did show a significant difference across days ( $p < 0.01$ ). The follow-up test demonstrated that when compared to Day 1 there was a significant reduction on Day 2 ( $p < 0.01$ ), Day 3 ( $p < 0.01$ ),

Day 4 ( $p<0.01$ ), Day 5 ( $p<0.05$ ), Day 6 ( $p<0.01$ ), Day 7 ( $p<0.05$ ), Day 8 ( $p<0.01$ ), Day 9 ( $p<0.01$ ), and Day 10 ( $p<0.05$ ).

#### *Vocalizations during the Post-Stimulation Period*

Overall, the median number of vocalizations for the experimental and control groups began high, and over the 10 days of testing, vocalizations declined (Figure 4). The Mann-Whitney U test analysis showed that there was no significant difference between the experimental and control groups on any days. Analysis of the experimental and control groups showed a significant difference across days for both groups ( $p<0.01$ ), indicating a general decline over the days of testing.

#### *Full Passive Responses during the Pre-Stimulation Period*

As Figure 5 illustrates, the median number of full passive responses during the pre-stimulation period for the experimental and control groups was zero. The Mann-Whitney analysis revealed no significant difference between the experimental and control groups for any day. Further, neither group showed a significant difference across days.

#### *Full Passive Responses during the Stimulation Period*

The median values of full passive responses for the control group were larger than those for the experimental group on all days except Day 1, Day 6 and Day 10 (Figure 6). In contrast to Day 1 and Day 6, when the median value of full passive responses was zero for both groups, on Day 10, the median value for the experimental group was larger than the control group. Although the control group exhibited more full passive response compared to the experimental group, the largest difference between the two groups was

only 4. The Mann-Whitney test revealed that when comparing the experimental and control groups, significant differences were found on Day 2 ( $p < 0.01$ ), Day 3 ( $p < 0.05$ ), Day 7 ( $p < 0.05$ ), and Day 8 ( $p < 0.05$ ). Friedman analysis of variance yielded no significant changes in the number of full-passive responses for the control group across days; however the experimental group did show significant variation across days ( $p < 0.01$ ).

#### *Full Passive Response during the Post-Stimulation Period*

Figure 7 illustrates that the median full passive response score for the experimental and control groups during the post-stimulation period was zero. There were no differences between the groups on any day, nor were there differences across days within either group.

#### *Place Preference Test*

As Figure 8 illustrates, not all of the animals observed during the place preference test chose to spend time in the familiar compartment (the area of the cage that was the same environment they were exposed to during the testing phase) or unfamiliar compartment (area of the cage containing a novel cage lining and different striping pattern on the cage walls). However, of the 7 experimental animals that made a choice, 6 of these animals chose to spend the majority of the 30-min test in the unfamiliar area, while only 1 animal spent more time in the familiar area of the test cage. In contrast, of the 6 control animals that made a choice during the test, it was evenly split; 3 spent the majority of the test time in the unfamiliar area, while 3 spent either most of the time in the familiar area, or spent some time in the familiar and the rest of the time in the neutral area, the untaped/unlined part of the cage linking the familiar and unfamiliar area.



There were no differences between the experimental and control groups in the preference for the familiar or unfamiliar compartments. However, a marginal positive significant difference was seen when the familiar and unfamiliar areas were compared and the experimental and control groups were combined ( $p < 0.087$ ).

## V. *Discussion*

The data presented here replicate previous work that stimulation of the PAG increases vocalization rates compared to control stimulations. Sugiyama, et al. (2010) found that in guinea pigs the area of the brain stem that produced vocalizations extended from the PAG to the pyramidal tract in the medulla. Cats also produce vocalizations when a similar area of the PAG is stimulated electrically or chemically (de Lanerolle, 1990).

As mentioned above, human and non-human primates and guinea pigs demonstrate a biphasic response to maternal separation. The first (protest) and second (despair) stage of this biphasic response are marked by specific behavioral responses; active vocalizations and depressive-like behaviors, respectively. The mechanism for transition from active phase to passive is not understood completely but may involve elevations in pro-inflammatory cytokine during maternal separation resulting in behavioral changes (Wright and Panksepp, 2011). Alternatively, the theory of Panksepp whereby overstimulation of the PANIC system leads to generation of DV and future depressive behavior is an additional possibility.

The current study was designed to determine if electrical stimulation of the PAG, a part of the PANIC system, which causes the active behavioral response of vocalization, would promote an increase in the number of full passive behavioral responses seen over the 10 days of testing. Although the median values of vocalizations for the experimental

group was higher than in the cortical control group, there was no clear effect that increasing the number of vocalizations during testing prompted an increase in passive behavior later on. The procedure achieved the first goal of producing vocalizations by stimulating the PAG. Throughout testing, both groups showed a decline in the number of vocalizations but the experimental group showed a much greater decline than the control group. The decline in number of vocalizations could not be attributed to fatigue of the animal because there was 24-hour recovery between tests, but instead it is possible that the repeated EBS may have caused damage to the neurons. Since the procedure was designed to avoid such damage by setting the stimulating level at minimal levels' a more likely explanation is that repeated stimulation of the PAG impacted the input on the vocal neurons and perhaps reduced their sensitivity.

Animals receiving PAG stimulation not only exhibited increased vocalizations while receiving EBS, but also a decrease in passive behavior during those trials; however, the effect on passive behavior did not extend beyond this time. When PAG-stimulated animals were moved into the home cage for post-stimulation observation, no increase in passive behavior was observed over control animals. Likewise, there was no increase in pre-stimulation levels of passive behavior over days within the experimental group. Similarly, in studies conducted on squirrel monkeys, it was found that electrical stimulation of the PAG had no effect on the emotional response of the subjects at a later time (Jurgens, 1994). Though stimulation of the PAG did not drive an overall increase in either active or passive behaviors beyond the period of stimulation, it may have produced an adverse reaction resulting in avoidance behavior. This possibility was tested during the place preference testing. There was a slight tendency for PAG-stimulated animals to

avoid the compartments associated with the stimulation environment during the place preference testing. This tendency may have been due to the aversiveness of overstimulation of the PAG.

The present experiment was conducted after another laboratory member had tested an alternate location in the PANIC system, the BNST. She tested guinea pigs using the same experimental procedure but with a different brain location and found similar results, in that driving active behaviors did not increase animals' likelihood of displaying passive behaviors (Kardegar, unpublished). Because it was predicted that there would be an increase in full passive behavior beyond the testing phase due to a repeated stimulation of the PANIC system, it must be concluded that repeated stimulation of the PANIC system does not lead to a transition from the active (protest phase) to the passive (despair) phase.

## VI. References

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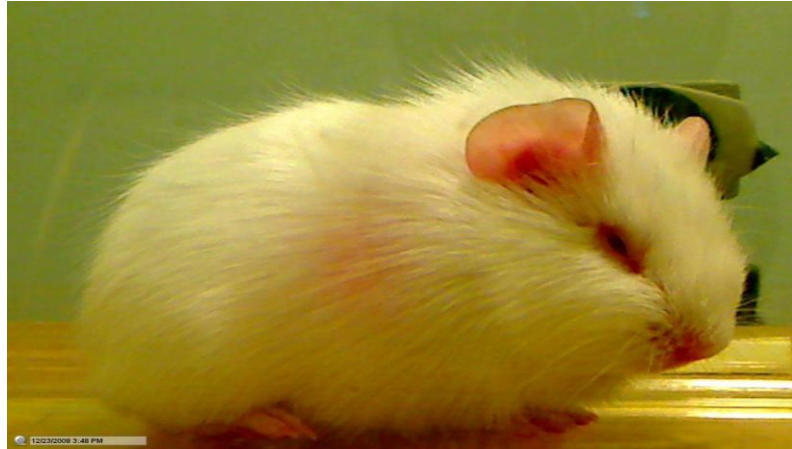


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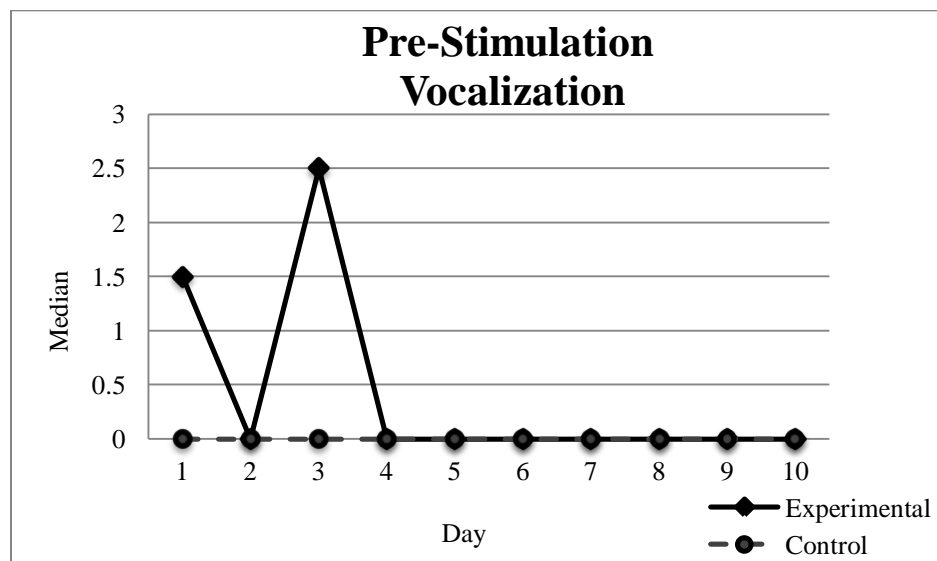
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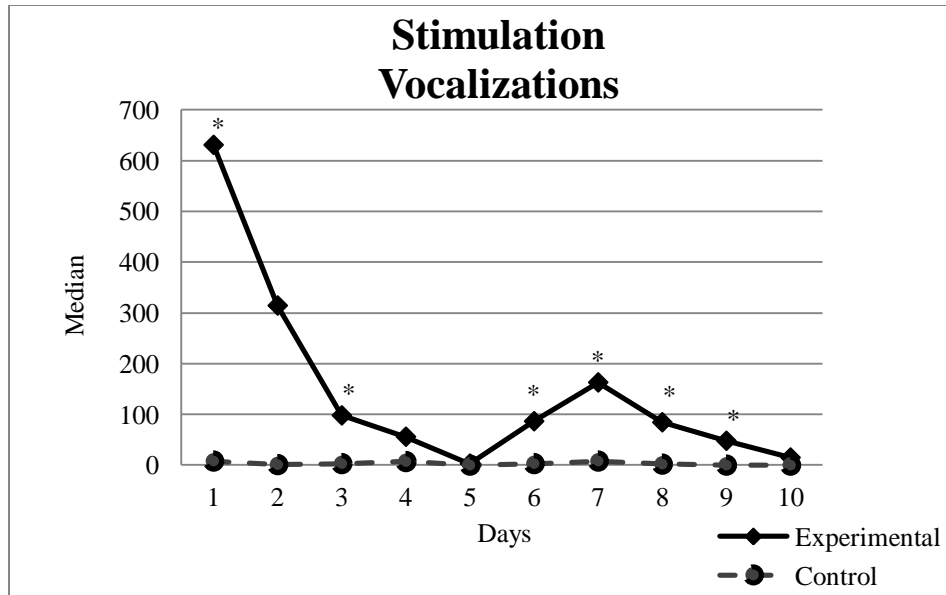
## VII. Appendix



**Figure 1** The guinea pig displaying passive behaviors: crouched, piloerection, and partial eye closure.

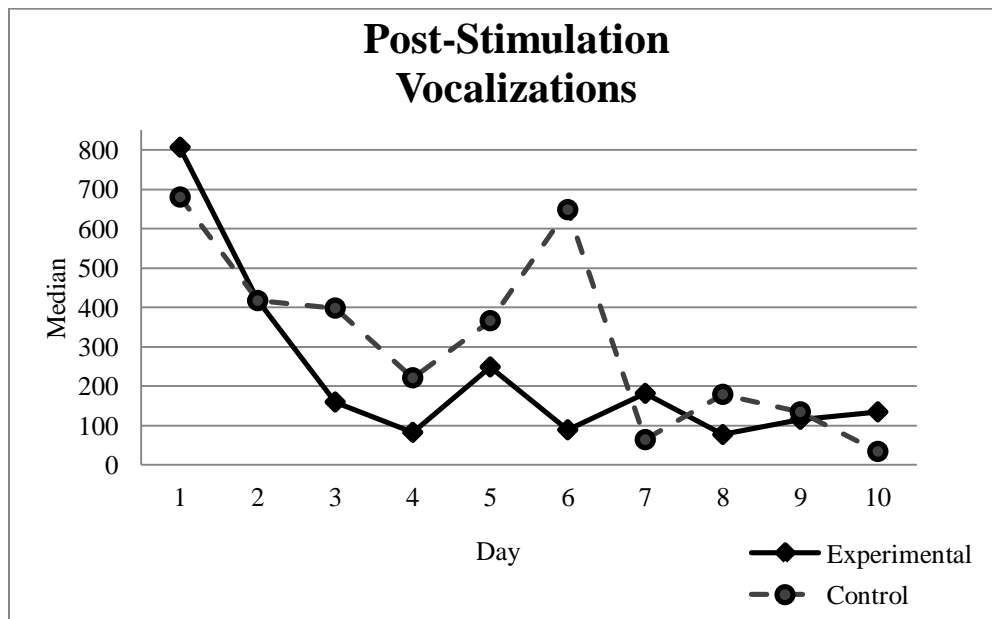


**Figure 2** Median number of vocalizations during the pre-stimulation period of testing for the experimental and control groups for the 10 days of testing

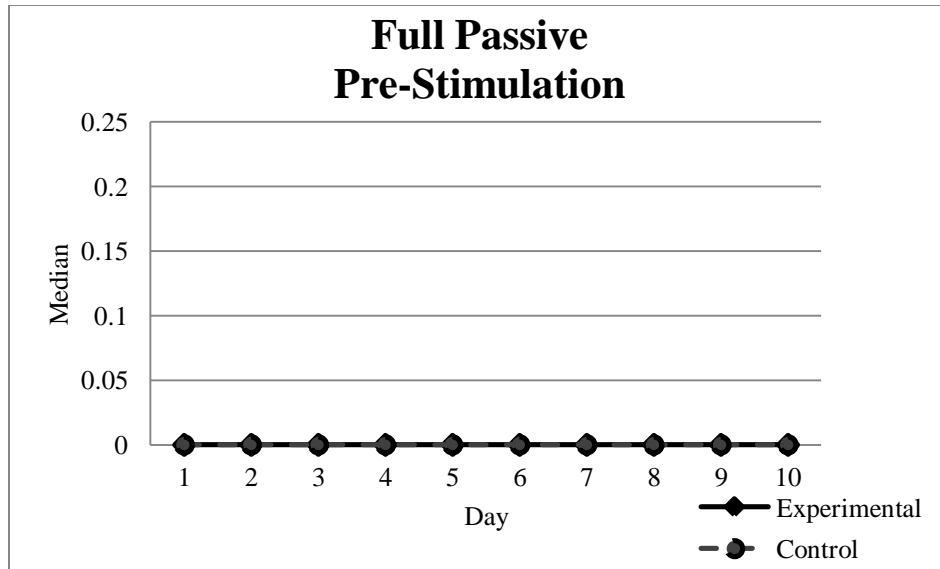


**Figure 3** Median number of vocalizations during the stimulation period of testing for the experimental and control groups for the 10 days of testing

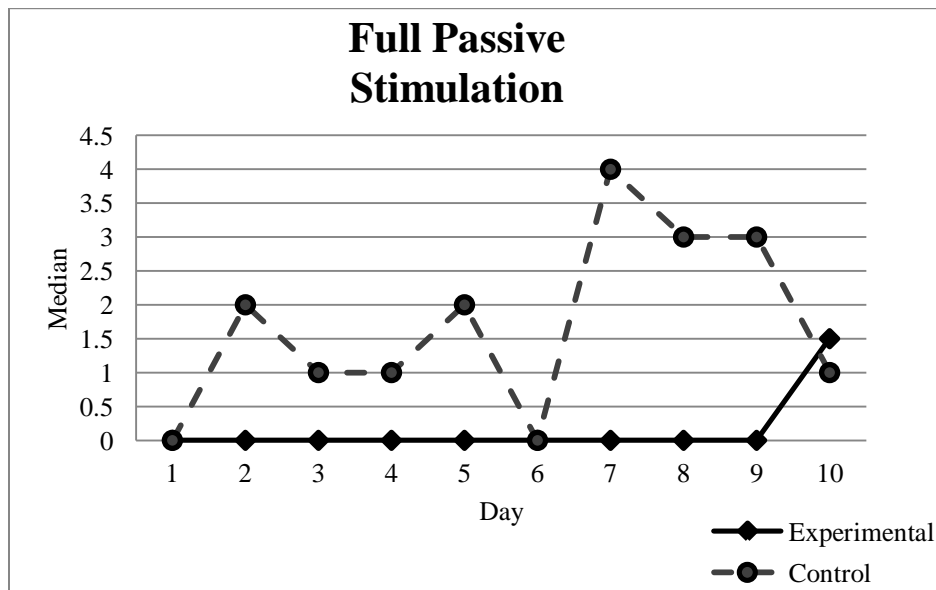
(\*) refers to days where there is a difference between groups



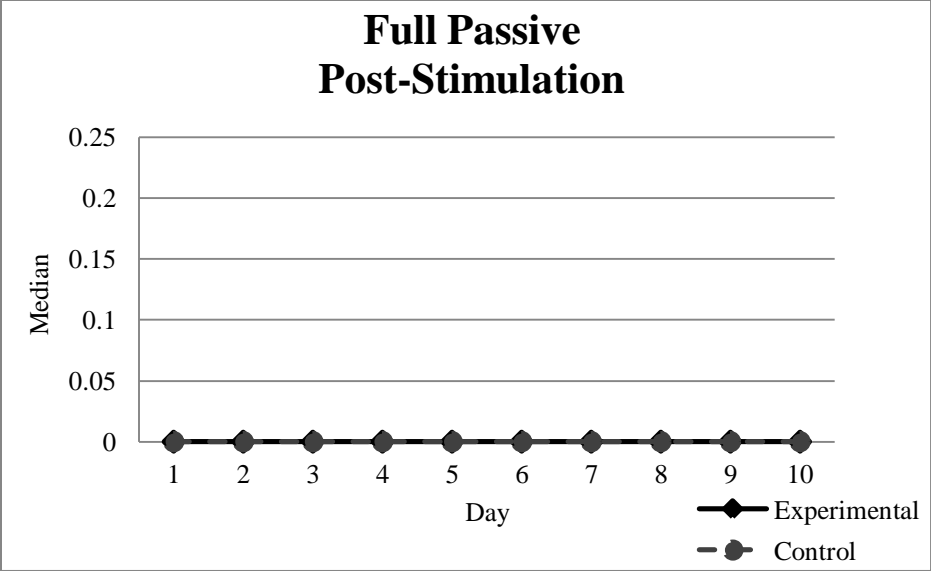
**Figure 4:** Median number of vocalizations during the post-stimulation period of testing for the experimental and control groups for the 10 days of testing



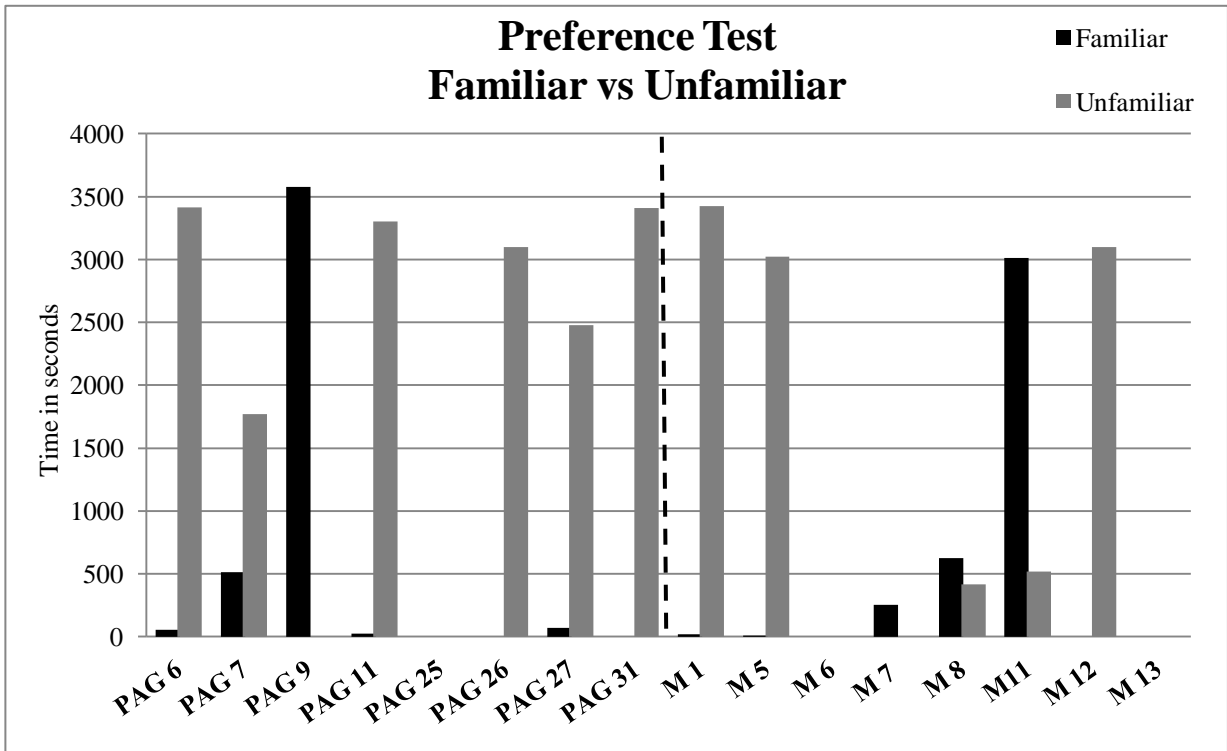
**Figure 5:** Median number of full passive responses during the pre- stimulation period of testing for the experimental and control groups for the 10 days of testing.



**Figure 6:** Median number of full passive responses during the stimulation period of testing for the experimental and control groups for the 10 days of testing



**Figure 7:** Median number of full passive responses during the post-stimulation period of testing for the experimental and control groups for the 10 days of testing



**Figure 8:** Time (in sec) Experimental and Control animals spent in the familiar and unfamiliar areas of cage during place preference test.