

**Absent or Not?:
Classically Conditioning Spike-and-Wave Discharge in Sprague Dawley Rats**

By

**Jeremy A. Taylor
Department of Psychology and Neuroscience
University of Colorado at Boulder**

Defended on November 2, 2016

**Thesis Advisor:
Dr. Daniel S. Barth
Department of Psychology and Neuroscience**

**Honors Council Representative:
Dr. Jerry W. Rudy
Department of Psychology and Neuroscience**

**Thesis Committee:
Dr. Ruth E. Heisler
Department of Integrative Physiology**

Abstract:

Spike-and-wave discharge is an electroencephalographic brainwave found in many rodent species and studied rigorously in inbred laboratory rats (WAG/Rij and GAERS strains) for its similarities to absence epilepsy. The absence epilepsy research field, however, is divided on just how similar SWD in rats is to absence epilepsy in humans. Albino Sprague Dawley rats are an outbred strain used in many fields of research but presently are not used to study absence epilepsy despite being known to exhibit SWD. Five 10 month old male albino Sprague Dawley rats that presented with SWD were rewarded with a 45 mg sugar pellet three seconds after an SWD burst ended. An infrared photo-beam diode was used to track reward tray checking behavior in relation to reward dispensation. It was found that pairing a sugar reward with SWD resulted in a reduced average SWD duration ($<1/2$ of baseline) and that the rats would preemptively check the reward tray for sugar after completing a burst of SWD. Rats were then given ethosuximide, a known anti-epileptic drug, to confirm that SWD was suppressed by ethosuximide as it is in other SWD rodent models of absence epilepsy. SWD was found to be suppressed in the five Sprague Dawley rats when given ethosuximide. Together, these results suggest that Sprague Dawley rats are conscious during and have voluntary control of SWD and therefore are not experiencing absence seizures. Furthermore, the findings imply that SWD rodent models of absence epilepsy may be mistakenly studying a non-absence epilepsy phenomena.

Introduction:

A small portion of the Sprague Dawley albino laboratory rat population has been observed to emit a cortical phenomenon originating from the barrel cortex in the somatosensory region during chronic electroencephalographic (EEG) recordings. This phenomenon has been known and described technically as spike-and-wave discharge (SWD), polyspiking activity (PSA), and high-voltage rhythmic spikes (HVRS) (Vergnes et al., 1987; Kaplan 1984; Shaw 2004). Functionally, it has been proposed to be the rat equivalent to an absence seizure, an alpha-tremor, or an alpha / mu rhythm (Vergnes et al., 1987; Semba & Komisaruk, 1984; Wiest & Nicolelis, 2003). Additionally, it is seen in many other strains of laboratory rat (the WAG/Rij and GAERS rats are two Wistar strains selectively inbred for the SWD trait) and other species of animals (from cats to guinea pigs) (Kaplan 1984; Coenen & Luijcklaar, 2003; Luijcklaar et al., 2011). Though the anatomical circuitry and molecular mechanisms behind the phenomena are well known, the behavioral properties of this phenomena are lacking and strongly debated.

SWD in the somatosensory EEG is very distinct from background activity in that it has a mean frequency of 7 Hz ($\pm$ 2) with high amplitude repeating spikes and waves that overshadow background EEG (Fig. 1) (Rodgers et al. 2015; Marescaux et al., 1992; Coenen & Luijcklaar, 2003). The duration of a spike-wave discharge can vary between a few seconds to a minute with an average duration of 17 seconds (Coenen & Luijcklaar, 2003; Luijcklaar et al., 2011; Marescaux et al., 1992). Anatomically, SWD has been found to originate from the barrel cortex and radiate outward through the rest of the surrounding cortex and down into the thalamus (Polack et al., 2007; Vergnes et al., 1990; Pearce et al., 2014). It is worth noting that, although SWD is primarily a thalamocortical rhythm, it has been observed in many other brain structures such as the cerebellum and limbic nuclei (generally post-cortical occurrence) (Semba & Komisaruk, 1984). Additionally, lesion studies of the cortex have shown SWD to be resilient. In Semba and Komisaruk's 1984 article, "Neural Substrates of Two Different Rhythmical Vibrissal Movements in the Rat," it was found that removal of the frontal half of the cortex (containing the somatosensory cortex and barrel cortex) as well as the entire cortex initially caused SWD to cease but later recovered one to four months later. Nonetheless, in an intact rat brain SWD originates and is most prevalent in the somatosensory barrel cortex.

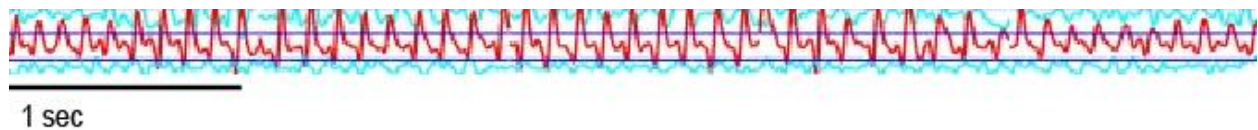


Figure 1. EEG of a highlighted 5 second section of SWD recorded from the somatosensory cortex of a Sprague Dawley 10 month old male albino rat. For this animal, an 8-9 Hz frequency is seen accompanying large amplitude spikes.

Behaviorally, SWD is both simple yet difficult to describe and define. It is the main point of debate and disagreement within the communities that study it. SWD occurs when the rat is in a state of quite wakefulness or immobility (an unaroused state physiologically and psychologically speaking) (Wiest & Nicolelis, 2003; Semba & Komisaruk, 1984; Vergnes et al.,

1987). While immobile, minute vibrissal twitching occurs and in some cases contraction of musculature of the face and neck (Kaplan 1984; Coenen & Luijtelaar, 2003; Luijtelaar et al., 2011). The difficulty of describing and defining SWD begins with the question of what SWD is functionally.



Figure 2. A photograph of a 10 month old male Sprague Dawley rat in the described SWD posture while SWD is electrographically present. The head being lowered toward the ground by approximately 45 degrees was frequently observed along with light twitching of the whiskers and facial and neck muscles.

Two general competing hypotheses exist concerning the function of SWD. The first hypothesis, broadly speaking, is that SWD is a dysfunction of the brain. The pathologies attached to SWD are absence epilepsy, and to a lesser degree, motor diseases such as Parkinson's or Huntington's disease (Marescaux et al., 1992; Semba & Komisurak, 1984). Concerning the motor diseases, SWD is primarily studied as a model of spontaneously occurring motor tremors in which the tremor(s) of the face, neck, and vibrissae are the focus of investigation (Semba & Komisurak, 1984). In contrast, the absence epilepsy hypothesis mainly focuses on the cortical EEG properties of SWD and its response to absence seizure medications (such as ethosuximide and to a lesser degree valproic acid) (Coenen & Luijtelaar, 2003; Marescaux et al., 1992). When given ethosuximide (a T-type calcium channel antagonist), it was found that SWD was eliminated or at the very least drastically reduced in frequency compared to control groups and therefore is the main pillar of support for the absence epilepsy hypothesis (Coenen & Luijtelaar, 2003; Marescaux et al., 1992). The reason behind ethosuximide being the main supportive evidence for SWD being an absence seizure (compared to its' behavioral and electrophysiological properties) is due to a few concerning dissimilarities between SWD and absence seizures.

Absence epilepsy in humans presents with many different types of absence seizures of which two major types are known (typical and atypical). Typical absence seizures (TAS) begin

and end spontaneously during which the patient loses consciousness for a short period of time (2-15 seconds) and has no cognitive or physical impairments following the seizure (Panayiotopoulos, 2008). As such, the patient is generally unresponsive during the seizure and unaware that they had a seizure unless an observer alerts them to its occurrence. Typical absence seizures mainly affect children (≤ 10 of age), tends to improve or subside completely with age, and twin studies have identified a genetic predisposition to develop absence epilepsy (Coenen & Luijckelaar, 2003; Panayiotopoulos, 2008). Electrographically, a bilateral 3 Hz spike-wave event is observed via EEG recordings of absence seizures (Panayiotopoulos, 2008). These spike-wave events consist of a sharp spike component followed by a slow wave and stand out in both amplitude and rhythmicity from background EEG (Sadler et al., 2006). Absence epilepsy is managed with anti-seizure medications, the main and most effective medication being ethosuximide.

In the 1980's, C. Marescaux, M. Vergnes, and A. Depaulis, found that 30% of their Wistar rat population had naturally and spontaneously occurring SWD in their EEG recordings (1992). They observed that when a rat had an SWD event, the rat would be immobile and on occasion have muscular facial twitches (Marescaux et al., 1992). As such, this cohort of rats was inbred over 20 generations; while a control group was outbred for 15 generations (Marescaux et al., 1992). The morphological features of SWD was found to consist of a frequency between 7-10 Hz and last approximately 17.2 seconds on average (Coenen & Luijckelaar, 2003; Luijckelaar et al., 2011; Marescaux et al., 1992). Additionally, rats first displayed SWD as early as 2 months of age, by 6 months were having 16-18 SWD episodes per hour, and at no point during their lifespan recovered or outgrew SWD (Coenen & Luijckelaar, 2003; Marescaux et al., 1992). Marescaux et al. also found that SWD in these rats responded to ethosuximide in the same manner as human patients with absence epilepsy and therefore concluded that SWD must be akin to an absence seizure (1992). Despite differences in EEG morphology, progression, and prognosis, the inbred model has largely been accepted as the best spontaneous animal model of absence epilepsy to date.

A second competing hypothesis does exist and is in opposition to the idea that SWD is pathological. It has been hypothesized that SWD is more likely to be a sensorimotor rhythm, alpha rhythm, or mu rhythm (i.e. an oscillatory idling rhythm) (Kaplan, 1985; Wiest & Nicolelis, 2003; Pearce et al., 2014). Unfortunately, it is difficult to prove that SWD is an idling process rather than an absence seizure (since a rat is unable to communicate with us). Bonnie J. Kaplan, in her 1985 article, "The Epileptic Nature of Rodent Electrographical Polyspiking is Still Unproven," states, in reference to the controversy of SWD being normal or abnormal, that "one factor that would clarify this issue would be evidence showing whether or not rodents were conscious or unconscious while PSA was recorded." Furthermore, if it were also possible to show that rats with SWD were able to control SWD in some manner then SWD would fail to meet the clinical criteria for absence seizures and seizures in general (uncontrollable electrical activity).

One method that could be used to test whether or not an animal with SWD is conscious of and has control of SWD is to use behavioral conditioning techniques such as

classical conditioning or operant conditioning. Due to impaired consciousness and an unawareness of the absence seizure, it should not be possible to get an animal with SWD to associate SWD with a reward because to the animal the presentation of a reward would seem random and unconnected to any specific event or action that it has done. If an animal with SWD is capable of associating SWD with a reward then it must be aware of SWD and its surroundings. Even more, the animal may be able to override SWD and stop doing SWD once it realizes it is doing SWD since SWD would be associated with a reward (assuming the animal is motivated to get the reward) and as such would demonstrate that SWD is not uncontrollable electrical activity (i.e. seizure). It was hypothesized that pairing a sugar reward with the self-termination of SWD would result in a decrease in mean SWD duration and that the animal would preemptively check the pellet tray post SWD cessation and prior to reward dispensation.

Methods and Materials:

Subjects:

Eight ten month old adult male Sprague Dawley rats (Envigo) were double housed in clear plastic cages (48 cm (L) x 26 cm (W) x 20 cm (H)) with approximately one inch of bedding and filter tops. The colony room was kept at a temperature between 23-27° C and had a 12h/12h light/dark cycle (lights on at 7:00 AM) for two weeks with *ad libitum* access to food and water. All procedures were followed according to University of Colorado Institutional Animal Care and Use Committee guidelines.

Surgery:

In sterile conditions, rats were implanted with stainless steel cortical electrode screws (E363-20 Elect W- 3.2 mm screw, 20 mm length; Plastics One, Medical Design and Manufacture, Roanoke, VA). Two electrode screws were placed bilaterally over parietal (AP 2.0 mm, ML $\pm$ 2.0 mm from bregma) and occipital (AP 2.0 mm, ML $\pm$ 4.0 mm from lambda) cortex, one reference electrode screw (AP 4.0 mm, ML -2.0 mm from bregma), one ground electrode screw (AP -3 mm, ML -4 mm from lambda), and two anchor screws (one rostral to bregma at AP 4.0 mm, ML 2.0 mm; one caudal to lambda at AP -3 mm, ML 4 mm) under Isoflurane gas (2.5%) anesthesia. Electrode wires were seated in a round polyimide six socket Electrode Pedestal (Plastics One) and secured with dental cement powder mixed with anti-biotic powder (1.8 g cement : 0.2 g anti-biotic; Uniprim, Macleod Pharmaceuticals, INC.) and cement liquid (2.0 g cement mix : 2 mL cement liquid). Anti-biotic ointment (Triple Antibiotic Ointment + Pain Relief; Bacitracin zinc, Neomycin sulfate, Polymyxin B sulfate, Pramoxin HCl; Signature Care Safeway, Better Living Brands LLC) was applied to the incision site. Rats were given 2 mL saline (0.9 % NaCl) subcutaneously over the right hind limb and an intramuscular injection of Buprenex (0.05 cc / 100g) (once immediately post-surgery and once 24 hours later). Rats had antibiotic added (3.0 mL per bottle; Cherry flavored Sulfamethoxazole and Trimethoprim Oral Suspension, USP; 200 mg / 40 mg per 5 mL; Hi Tech Pharmacal. CO., INC; Amityville, NY) to their water for one week and given a total two week recovery period before behavioral training began. Post-surgery rats were singly housed (housing conditions were kept identical to pre-surgery conditions).

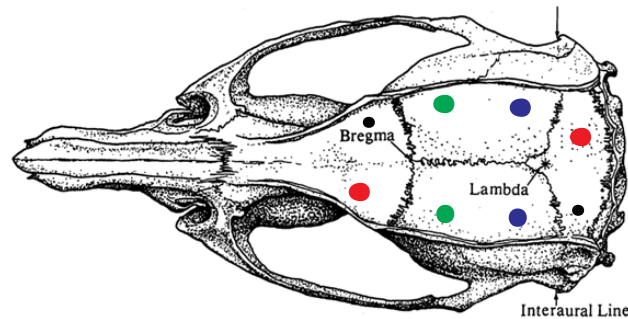


Figure 3: Electrode screw placements on the dorsal surface of a rat skull. Red denotes reference and ground, green parietal cortex screws, and blue occipital cortex screws. Black represents anchor screw locations.

Conditioning Box:

An open-top conditioning box was constructed using three clear Plexiglas panels and one black Plexiglas panel as walls and one clear Plexiglas panel for the floor (30 cm (L) x 30.5 cm (H) x 31.3 cm (W)). Within the box a square pellet tray made of aluminum metal (6.5 cm (L) x 2.5 cm (H) x 6.5 cm (W)) was positioned in the front right corner under the pellet dispenser. The pellet dispenser (45 mg Pellet Dispenser, www.med-associates.com) was bolted to the upper right Plexiglas panel and loaded with 45mg sugar Dustless Precision Pellets (bio-serv.com). The box was raised 5.4 cm high on four Styrofoam pads to allow for an infrared diode photo-beam to be vertically positioned under the pellet dispenser tube. The conditioning box was centrally positioned on a gyroscopically stabilized table on top of which supported a Faraday Cage (85.5 cm (L) x 135 cm (H) x 75 cm (W)). An iron support stand equipped with an iron clamp (57 cm above conditioning box) allowed for the EEG six channel cable (Plastics One, 363-SL/6) and slip-ring commutator (Plastics One, SL6C) to hang centrally over the conditioning box with maximal freedom of movement for the rats. Two speakers were used to play continuous white noise (from the website www.simplynoise.com) during the reward segment of the conditioning procedure. They were aimed at and placed to the left and right with a distance of 13 cm away from the conditioning box. Volume of the speakers was set to a medium volume, tuned based on the animals reaction to avoid a volume that may have been damaging or frightening but still noticeable. Computer and website volumes were set to maximum volumes.

Conditioning Procedure:

Rats were selected for classical conditioning based on the presentation of SWD in the EEG; five of the eight presented with SWD. Daily each rat was individually transferred from the colony home room to the behavioral testing room, placed in the conditioning box, and hooked up to the six channel EEG cable for 2 hours and 30 minutes for conditioning during which it had no access to food or water (except for sugar pellets). Two rats were trained at 9:00 AM, two at 12:00 PM, and one at 3:00 PM. Training lasted for three weeks and test data was collected for six days after training was complete. The conditioning paradigm consisted of an initial 30 minute habituation period followed by two consecutively alternating 30 minute segments (no-reward, reward, no-reward, reward). The first 30 minute segment presented was a no-reward plus no white-noise condition (no-reward). The second 30 minute segment was a reward plus

white-noise condition (reward). The last two segments were repeats of the first and second segment, respectively. During reward segments rats were rewarded three seconds post self-termination of an SWD burst that had a duration of longer than 2 seconds with one 45 mg sugar pellet.

Ethosuximide:

At the end of behavioral testing, rats were ran through the conditioning procedure three additional times. Once while under the effects of ethosuximide, once 24 hours post-ethosuximide administration, and once with saline. Ethosuximide (100 mg / kg; MW 141.17 g / mol, Sigma-Aldrich) was dissolved in saline (0.9% NaCl) and injected intraperitoneally prior to initiating the 2 hour 45 minute conditioning procedure (saline injections followed the same procedure). Saline and ethosuximide injections were counterbalanced.

Automatic SWD Detection:

Two MatLab software programs were used in conjunction with one another to automatically detect SWD (similar to Rodgers et al., 2015) and reward SWD that met the criteria for reward (2 second durations followed by 3 seconds of no SWD). The first MatLab program generated a template pattern of SWD based on its signature frequency and amplitude for each rat. The second MatLab program used this template to recognize SWD in real time and dispense the reward, initiate the reward and no-reward segments, and record the EEG and infrared diode photo-beam data for analysis. For each rat, human observation of day one training was conducted to ensure correct program functioning after which no humans were present in the behavioral testing room during training or testing.

Data Analysis:

SWD EEG data was analyzed visually via an EEG MatLab output program. SWD burst length duration for each rat over six days of data was averaged for each condition (like conditions combined) and compared between reward and no-reward using mean $\pm$ s.e.m. and Two-Sample Assuming Equal Variances t-tests. Infrared diode photo-beam data was analyzed visually in the EEG and graphically generated using a MatLab program to display frequency of checking behavior in relation to SWD.

Results:

Conditioning:

During reward rats had a mean $\pm$ s.e.m. SWD burst length duration of 4.63 ± 0.27 seconds ($n = 60$) and during no-reward a mean $\pm$ s.e.m. SWD burst length duration of 9.14 ± 0.29 seconds ($n = 60$); an average reduction in duration by 4.51 seconds. Two-Sample Assuming Equal Variances t-test was found to be significant between reward and no-reward conditions (Fig. 4A; $t(116) = -11.5305$, $p = 5.83E-21$). The percentage of SWD burst lengths (seconds) for each reward and no-reward condition was examined to determine whether or not rats were shifting towards and favoring shorter burst lengths during reward. It was found that

rats were increasing the percentage of shorter burst lengths and decreasing the percentage of longer burst lengths during reward compared to no-reward (Fig. 4B).

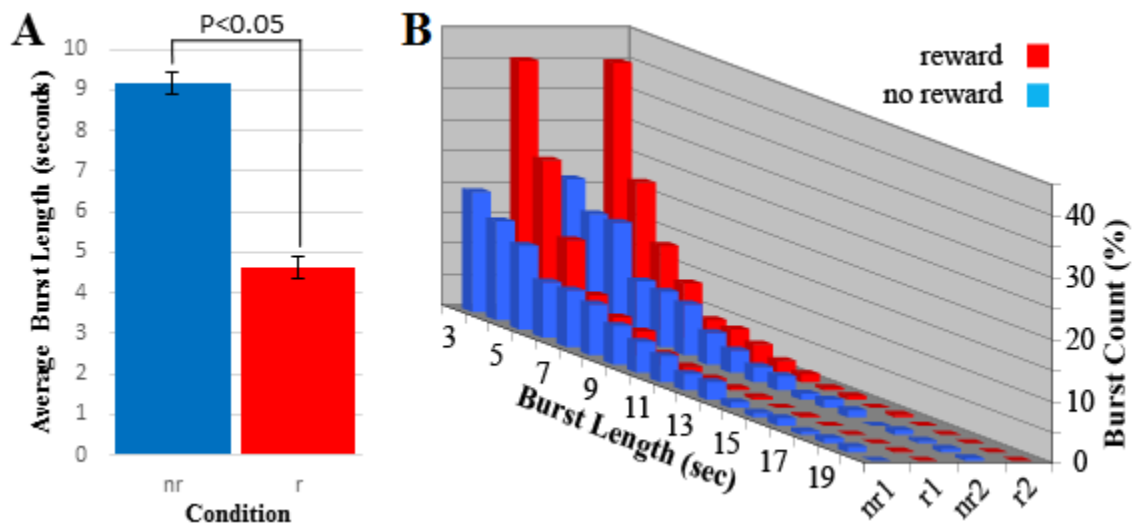


Figure 4. A) The averaged SWD burst length duration for combined reward (red, “r”) and no-reward (blue, “nr”) conditions across five Sprague Dawley rats. During reward, white noise was played and rats were given a 45 mg sugar pellet 3 seconds after completing a burst of SWD. In contrast, during no-reward, no white noise was played and rats were not rewarded with a sugar pellet. No-reward and reward mean $\pm$ s.e.m. SWD burst length durations were found to be 9.14 ± 0.29 seconds and 4.63 ± 0.27 seconds ($n = 60$), respectively ($t(116) = -11.5305$, $p = 5.83E-21$). B) The distribution of SWD burst length percentages for each separate condition. During reward conditions (r1 and r2) an increase in the percentage of shorter burst lengths and a decrease in the percentage of longer burst lengths is seen compared to no-reward conditions (nr1 and nr2).

Analysis of the tray checking behavior (recorded by the infrared diode photo-beam) revealed that 73 % of first checks (the first time the animals snout breaks the beam) occurred within 6 seconds of an SWD burst ending during reward (“pre + post”; Fig. 5B & C). The other 27 % of checks occurred during periods between bursts (inter-burst intervals or “IBI”; Fig. 5B & C). Comparison between reward and no-reward conditions revealed that checking behavior grossly increased during reward (8-fold) and nearly diminished during no-reward (Fig. 5C).

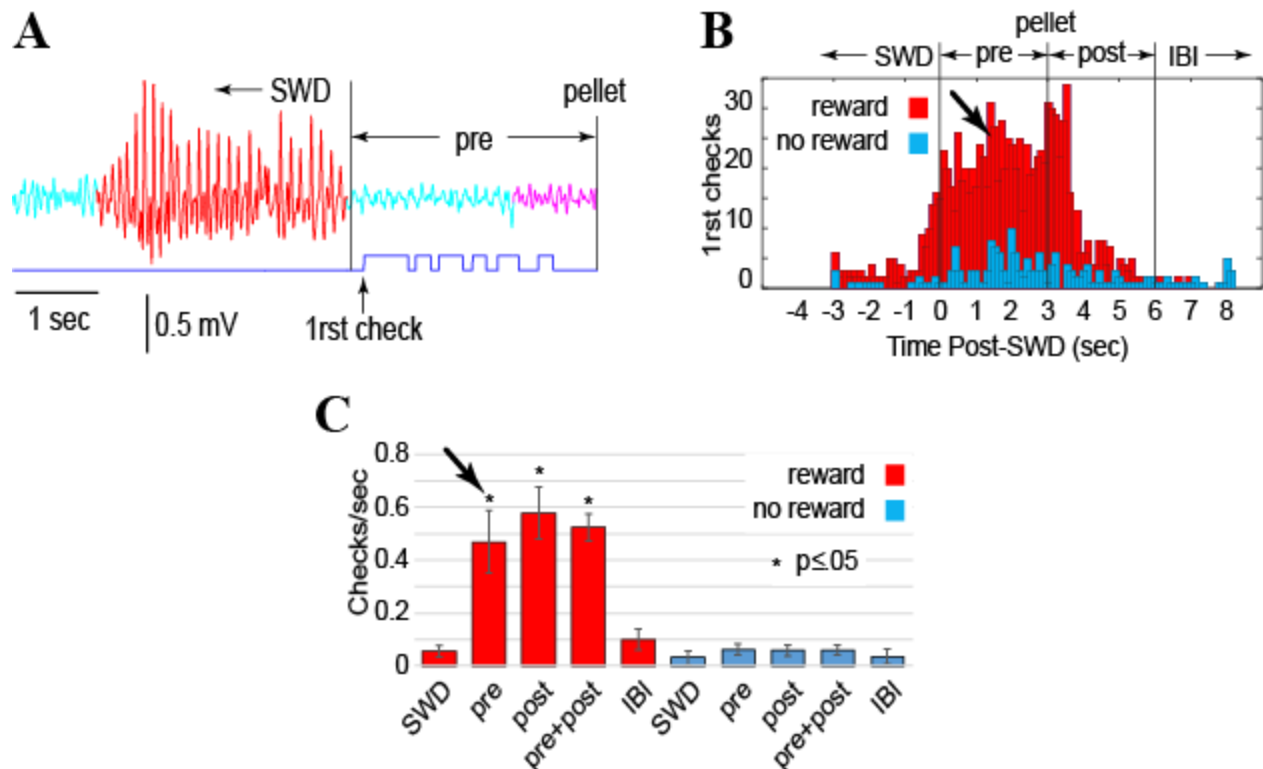


Figure 5. A) The top line is an example of EEG SWD (red) followed by the “pre” period prior to a pellet being dispensed during the reward phase (the purple segment indicates that the Matlab program has detected no SWD for three seconds after an SWD burst and thus dispensed a pellet). The blue line below represents the infrared diode photo-beam and when the beam is broken in relation to the EEG line above. The first time the beam is broken is considered to be the first check conducted by the rat (“1st check”). B) The distribution of first checks for all five rats during reward (red) and no-reward (blue) conditions for each time point (zero seconds is when an SWD burst ends, three seconds is when a pellet is dispensed, and six seconds is considered the end of reward consumption and the beginning of a new inter-burst interval (“IBI”). C) The frequency of checks per second for all five rats are broken down into four time periods; during SWD, after SWD ends but before a pellet is dispensed (“pre”), after a pellet has been dispensed (“post”), and the time between SWD bursts (inter-burst interval; “IBI”). The reward (red) and no-reward (blue) frequency of checks was found to be significant between the two conditions ($p \leq 0.05$). Arrows indicate that a majority of first checks were found to occur during the “pre” pellet dispensation period.

Ethosuximide:

The total amount of SWD (in seconds) per one hour was significantly reduced after the administration of ethosuximide (Fig. 6). Twenty-four hours after administration SWD had recovered but had not completely returned to baseline. The vehicle injections revealed no effect on SWD.

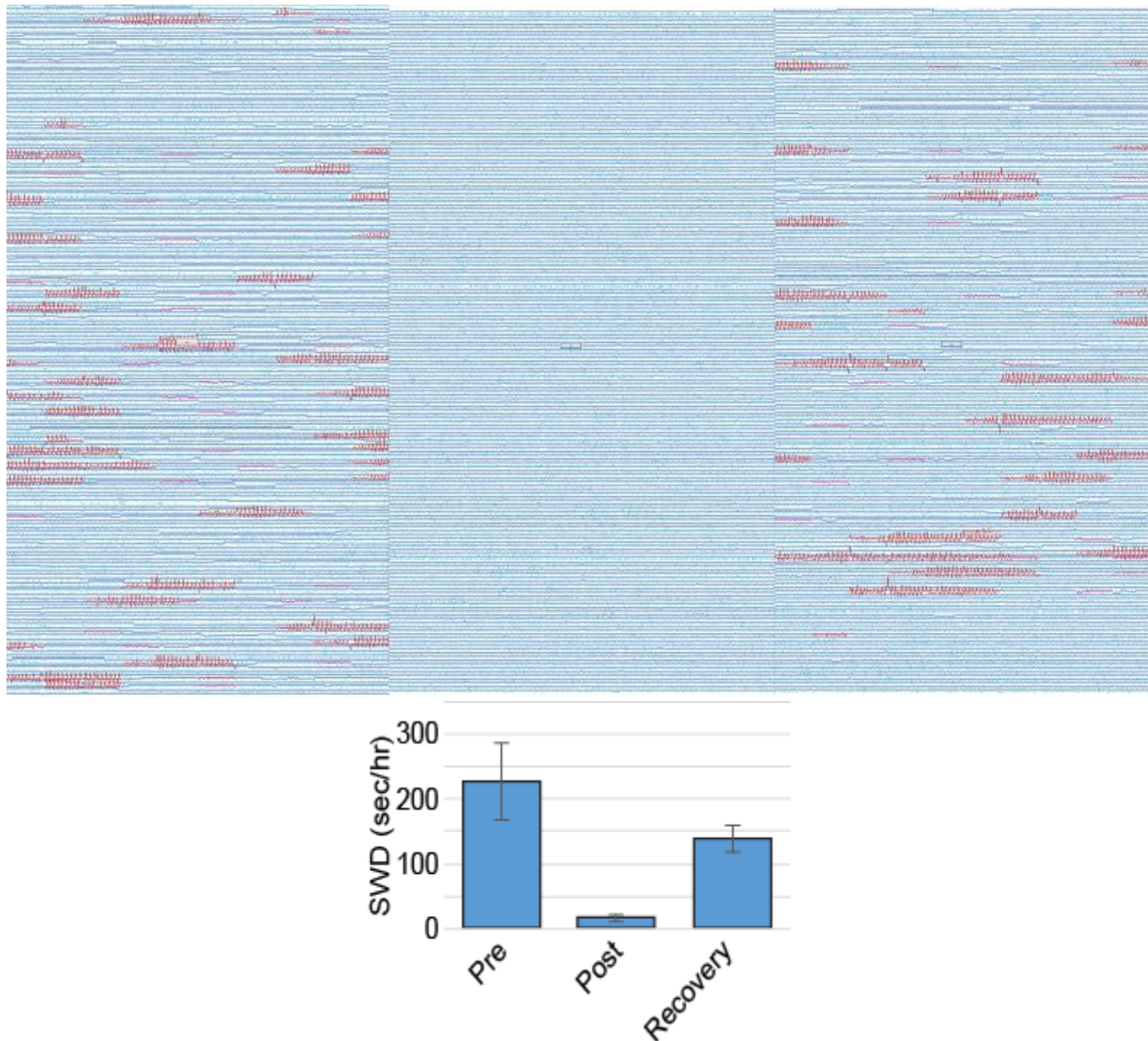


Figure 6. Top: Three 30 minute snapshots of separate EEG recordings taken from one Sprague Dawley rat while in the reward condition. Left is pre-ethosuximide, middle is 30 minutes after being administered ethosuximide, and right is 24 hours later. Bottom: The rate of SWD in seconds per hour for all rats prior to ethosuximide (“Pre”), after being given ethosuximide (“Post”), and 24 hours later (“Recovery”).

Discussion

This study suggests that SWD in adult male Sprague Dawley rats is voluntarily controllable and does not interrupt or impair consciousness. A decrease in the burst length duration of SWD indicates that these rats have some degree of voluntary control over it. The degree of voluntary control, however, is difficult to ascertain from this data. We did not see any significant changes in the number of SWD being produced (or initiated) between conditions, which was surprising to find. One might imagine that pairing a sugar reward with a behavior would result in the animal doing that behavior more often. Perhaps, this wasn't found due to a lack of motivation on the part of rats and therefore a flaw in the design of the experiment. These

rats were not food deprived or brought down to a lower body weight to increase motivational food seeking behaviors (as many behavioral conditioning studies do). It is also possible that the sugar reward was not desirable to the rats. The testing time and training time may have had an impact on the degree of control (the ability to initiate) as well due to the rats being nocturnal. In order to avoid these drawbacks from occurring, it would be best in the future to restrict food intake, test during the night, and use a more desirable reward (such as a grain-based pellet).

Nonetheless, a reduction in burst length is suggestive of the rats' ability to terminate SWD. Unfortunately, how the rat terminates SWD is not known but there must be some type of sensorimotor feedback mechanism that is consciously recognized and when paired with a reward causes SWD to cease upon recognition. The infrared diode photo-beam is a strong piece of evidence that supports the idea of SWD not interrupting consciousness. If consciousness were impaired in some manner, it would be incredibly difficult (if not impossible) to pair SWD with a reward because the rat would have no awareness of SWD having occurred and therefore sugar pellets would seem (to the rat) to appear without rhyme or reason. With the infrared diode photo-beam results showing rats checking the tray prior to a reward being dispensed (with no stimulus heralding its arrival) it can be concluded that these rats are conscious of their own SWD and have learned that SWD (or more correctly, the sensory "feeling" of SWD) results in a sugar pellet being dispensed.

Even though Sprague Dawley rats are not used to study absence epilepsy, SWD is strikingly similar to genetic rat models of absence epilepsy (such as the GAERS and WAG/Rij models). The response to ethosuximide found here, as well as by others, only adds to the similarities found between these outbred and inbred rat strains (Pearce et al., 2014). It is therefore suggestive of the possibility that SWD in Sprague Dawley rats is the same phenomena being studied in the GAERS and WAG/Rij models. Though, finding that Sprague Dawley rats have control of and are aware of doing SWD then it may not be the same phenomena as it no longer meets criteria for an absence seizure (inability to control, lack of awareness of the seizures, and impairment of consciousness). There are two possible implications to these findings: 1) these are two different phenomena or 2) the genetic models are not models of absence epilepsy.

The first and second implication can be tested relatively easily by applying the method used in this study to a cohort of GAERS or WAG/Rij. We have already conducted this experiment with a group of five WAG/Rij rats purchased from Yale University (New Haven, CT) and found similar results (a significant reduction in burst length duration during reward conditions and pre-emptive tray checking after an SWD burst ends). The results were not presented here in order to keep the focus of this paper on the Sprague Dawley cohort. Even so, the WAG/Rij portion of the study suggests that SWD is a trait conserved across rodent breeds and most likely not absence-like epilepsy.

The remaining explanations as to what SWD is in Sprague Dawley rats are the alpha-tremor, alpha or mu rhythm, or sensorimotor rhythm ideas. It is difficult with data from this study to disprove or prove the hypothesis that SWD is an alpha-tremor. It's plausible that SWD is somatosensory activity representing a sensory-motor loop stemming from the tremor of

vibrissae, facial, and/or cervical muscles. If that were the case, the results found here would be of no surprise. Though, a major assumption is being made, which is that SWD is a spontaneously arising idiopathic tremor rather than a non-pathological mechanism of the nervous system or a natural rodent behavioral. Which leaves the remaining idea that SWD could be an alpha or mu rhythm or some form of idling behavior. Perhaps, once the rat has habituated to its environment, and has no pressing internal homeostatic matters to attend to, it shifts its attention towards monitoring the environment for changes. It is in this state of quiet-wakefulness (or attentiveness) that SWD is generated. Of course, this is pure speculation as well and further experiments are needed to determine the role of SWD in the rodent nervous system.

In ending, the classical conditioning procedure used here solves the question posed as to whether or not these animals are conscious during and have voluntary control over SWD. It has high applicability to animal models, especially those involving diseases or disorders that affect consciousness. The degree of impairment to consciousness could potentially be assessed using this procedure. Lastly, these results stress the importance of examining and considering all aspects of a phenomena by using multi-disciplinary techniques and methods to study it.

References

- Coenen, A. M. L., & Van Luijtelaar, E. L. J. M. (2003). Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behavior genetics*, 33(6), 635-655.
- Kaplan, B. J. (1985). The epileptic nature of rodent electrocortical polyspiking is still unproven. *Experimental neurology*, 88(2), 425-436.
- Marescaux, C., Vergnes, M., & Depaulis, A. (1992). Genetic absence epilepsy in rats from Strasbourg—a review. In *Generalized Non-Convulsive Epilepsy: Focus on GABA-B Receptors* (pp. 37-69). Springer Vienna.
- Marten, F., Rodrigues, S., Benjamin, O., Richardson, M. P., & Terry, J. R. (2009). Onset of polyspike complexes in a mean-field model of human electroencephalography and its application to absence epilepsy. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, 367(1891), 1145-1161.
- Panayiotopoulos, C. P. (2008). Typical absence seizures and related epileptic syndromes: assessment of current state and directions for future research. *Epilepsia*, 49(12), 2131-2139.
- Pearce, P. S., Friedman, D., LaFrancois, J. J., Iyengar, S. S., Fenton, A. A., MacLusky, N. J., & Scharfman, H. E. (2014). Spike-wave discharges in adult Sprague-Dawley rats and their implications for animal models of temporal lobe epilepsy. *Epilepsy & Behavior*, 32, 121-131.
- Polack, P. O., Guillemain, I., Hu, E., Deransart, C., Depaulis, A., & Charpier, S. (2007). Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *The Journal of neuroscience*, 27(24), 6590-6599.
- Rodgers, K. M., Dudek, F. E., & Barth, D. S. (2015). Progressive, seizure-like, spike-wave discharges are common in both injured and uninjured sprague-dawley rats: implications for the fluid percussion injury model of post-traumatic epilepsy. *The Journal of Neuroscience*, 35(24), 9194-9204.
- Sadleir, L. G., Farrell, K., Smith, S., Connolly, M. B., & Scheffer, I. E. (2006). Electroclinical features of absence seizures in childhood absence epilepsy. *Neurology*, 67(3), 413-418.
- Semba, K., & Komisaruk, B. R. (1984). Neural substrates of two different rhythmical vibrissal movements in the rat. *Neuroscience*, 12(3), 761-774.
- Shaw, F. Z. (2004). Is spontaneous high-voltage rhythmic spike discharge in Long Evans rats an absence-like seizure activity?. *Journal of neurophysiology*, 91(1), 63-77.
- Van Luijtelaar, G., Sitnikova, E., & Luttjohann, A. (2011). On the origin and suddenness of absences in genetic absence models. *Clinical EEG and Neuroscience*, 42(2), 83-97.

- Vergnes, M., Marescaux, C., & Depaulis, A. (1990). Mapping of spontaneous spike and wave discharges in Wistar rats with genetic generalized non-convulsive epilepsy. *Brain research*, 523(1), 87-91.
- Vergnes, M., Marescaux, C., Depaulis, A., Micheletti, G., & Warter, J. M. (1987). Spontaneous spike and wave discharges in thalamus and cortex in a rat model of genetic petit mal-like seizures. *Experimental neurology*, 96(1), 127-136.
- Wiest, M. C., & Nicolelis, M. A. (2003). Behavioral detection of tactile stimuli during 7–12 Hz cortical oscillations in awake rats. *Nature neuroscience*, 6(9), 913-914.
- Wyrwicka, W., & Sterman, M. B. (1968). Instrumental conditioning of sensorimotor cortex EEG spindles in the waking cat. *Physiology & Behavior*, 3(5), 703-707.