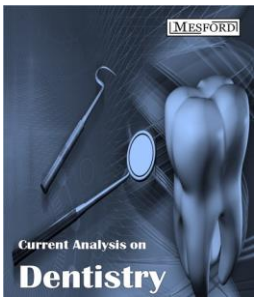

Characterization of Masticatory and Brux-like Motor Patterns in the Laboratory Rat: Electromyography Amplitude Analysis

Branden Bennett[1], Jaclyn Taylor[1], John Devin Wall[1] and Dan Welch[1, 2,*]

[1]*Department of Biological Sciences, Southern Illinois University Edwardsville, 1 Hairpin Dr, Edwardsville, IL 62025, USA.*
[2]*School of Dental Medicine, Southern Illinois University Edwardsville, 2800 College Avenue, Alton, IL 62002, USA*

Abstract:

Background: Bruxism is a repetitive jaw- muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible. Left untreated bruxism can cause orofacial pathology. Central pattern generators are neurological circuits that are involved in the production of complex rhythmic movements. Examining the amplitudes of rhythmic muscle contractions will help us characterize the underlying neurological circuitry of the brainstem that controls the movements of the jaw.

Objective: Our objective was to characterize EMG amplitude activity during masticatory and brux-like movements. Method: We compared the electromyogram burst amplitudes produced for masticatory versus brux-like motor patterns with the masseter, temporalis, and anterior digastric muscles. We used sixteen male Sprague Dawley rats, Rattus norvegicus. We calculated normalized root mean square values to represent the electromyogram burst amplitudes.

Results: Brux-like electromyogram burst amplitudes were significantly smaller when compared to masticatory amplitudes for the masseter, temporalis, and anterior digastric muscles.

Conclusion: Our new findings can help produce an animal model used to examine brainstem circuits, and trigeminal pathways that underlie activation and switching of masticatory and brux-like motor patterns of the jaw.

Publication History: Received: 04 October 2018| Revised: 16 January 2019|Accepted: 05 May 2019

Keywords:
Bruxism, Central Pattern Generator (CPG), Electromyogram (EMG), Mastication, Maxillofacial Pathology, and Root Mean Square (RMS).

INTRODUCTION

Bruxism is a repetitive jaw muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible [1]. Bruxism can lead to worn teeth, lost fillings, fractures, headaches, and temporomandibular disorders [2]. Our aim is to develop an animal model in which to study brux-like motor patterns produced by the underlying nervous system. Most current treatments seek to reduce the physical symptoms of bruxism with occlusal splints or mouth guards. However, some forms of bruxism are induced from medications that might impact components of the nervous system [3]. This suggests that some forms of bruxism may have a neurological etiology. As such, a comprehensive characterization of the underlying nervous system circuitry may allow us to develop new treatment options. In the current study, brux-like motor patterns occurred more frequently when the rats were initially placed into the testing cage. Previous studies suggest that brux-like behaviors were more frequent when rats were anxious [4, 5]. As an animal model, rats have been used for multiple experiments addressing the functioning and adaptive capacities of the mandible [6]. The connection between occlusal dysfunction and stress is consistent with results that suggest emotional stress may be involved in periodontal reactions associated with acute trauma from occlusion [7].

Neurological circuits that produce rhythmic movements are known as central pattern generators (CPGs) [8]. Considerable knowledge of CPGs in vertebrates comes from studying similar circuits in the spinal cord used for locomotion [9]. The CPG that produces the complex and rhythmic movements for the muscles of mastication has not received equal attention. So far, we know that the masticatory CPG exists in the pons and medulla [10], and can be modified as such by the motor cortex [11].

Electromyography (EMG) is a technique that allows for precise measurement of electrical activity produced by muscle fibers. The muscles of mastication are innervated by fibers from the trigeminal motor nucleus, which are driven by fibers from the parvocellular reticular formation, and receive their rhythmic firing patterns from the CPG inside the brainstem [8]. As such, the EMGs from muscles of mastication provides an indirect measurement of the firing patterns of the underlying nervous

*Address correspondence to this author at Department of Biological Sciences, Southern Illinois University Edwardsville, 1 Hairpin Dr, Edwardsville, IL 62025, USA; Tel: (618) 474-7248; Fax: (618) 474-7089 ; E-mail: dwelch@siue.edu

Mesford Publisher Inc
Office Address: Suite 2205, 350 Webb Drive, Mississauga, ON L5B3W4, Canada; T: +1 (647) 7109849 |E: cad@mesford.ca, contact@mesford.ca, https://mesford.ca/journals/cad/

system. Previous investigators have employed EMGs to study brux-like behavior in rats. Brux-like EMGs were found to be shorter and more rapid when compared to mastication EMGs for the anterior temporalis muscle [5]. In a previous study, we implanted EMGs into muscles of mastication and conducted a dual-referent phase analysis. During mastication, we observed an alternation of anterior digastric (jaw opening) and masseter (jaw closing) muscles. However, during brux-like behavior, the digastric and masseter muscles exhibited co-contraction. In addition, brux-like motor patterns exhibited shorter rapid bursts, and occurred at a cycle frequency greater than masticatory patterns [12]. Human subjects with bruxism exhibit a shorter interval between cycles, irregularly shaped envelopes of motion, sudden changes in direction, and a loss of the "teardrop" kinematic pattern seen in normal human masticatory cycles [13].

Coordination and amplitude are perhaps the two most important variables for characterizing motor patterns. In the current study, we expanded on our previous results by examining the EMG amplitudes with the same data set we used initially to analyze cycle period, burst duration, and coordination [12]. In the current study, our goal was to analyze the amplitude of the EMG bursts for the masseter, temporalis, and anterior digastric muscles. However, simple averaging of the raw EMG signal is uninformative. Normalized root mean square (RMS) measurement of EMG activity provides a more accurate characterization of the underlying physiological events than simple measures of mean amplitude [14]. The RMS represents the square root of the average power of the EMG signal for a given period. Examining the RMS of EMG bursts allowed us to characterize the underlying motor control circuitry of the brainstem (trigeminal motor nuclei) that activates the muscles of the jaw.

MATERIALS AND METHODS

1.1. Animals

Sixteen male Sprague-Dawley rats, Rattus norvegicus (200-224 g) were purchased (Harlan Laboratories, Indianapolis, IN) and housed under controlled 12/12 hour light/dark cycles. The rats had ad libitum access to standard chow blocks (Mazuri, Arden Hills, MN) and water (tap). Southern Illinois University Edwardsville's Institutional Animal Care and Use Committee (IACUC) approved all procedures. Rats were placed into separate cages 24 hours prior to surgery without food, and ad libitum water. Fasting the animals ensured robust masticatory behavior during data collection. The rats were placed into two surgery treatment groups: masseter-digastric (n=8), and masseter-temporalis (n=8).

1.2. Surgery

Rats were placed in a clear plastic chamber with Isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) liquid inhalation, 5% at 0.2L/min O2 (E-Z Anesthesia, Palmer, PA) until deep anesthesia was induced. Afterwards, the rats were removed from the anesthesia chamber to a heat pad and we administered 3% isoflurane during the surgical procedure via a nosecone. The surgical area was shaved with electrical clippers (Oster Mini Max Model 7849-100, Jarden Corporation,-ye, New York). Two silver fine-wire electrodes 0.076 mm in diameter (perfluoroalkoxy alkane (PFA) insulated, A-M Systems, Sequim, WA) with 0.5 mm exposed tips inserted into the belly of the muscles. The animals were divided into paired groups masseter/temporalis and masseter/digastric. We attempted to record from the right masseter (n=16), temporalis (n=8), and anterior digastric (n=8) muscles respectively. The electrodes were placed subcutaneously to and secured with Graphite-Filled Conductive Wire Glue (RadioShack, Fort Worth, TX) to E363 stainless steel gold-plated sockets (Plastics One, Roanoke, VA). The sockets were installed into an electrode pedestal MS363 (Plastics One, Roanoke, VA) with Ethyl Cyanoacrylate adhesive (World Precision Instruments, Sarasota, FL). During surgery, the rats were secured in a stereotaxic instrument (World Precision Instruments, Sarasota, FL). 1 mm deep holes were drilled into the skull by hand with a D#56 drill bit (Plastics One, Roanoke, VA). Four 0-80 X 1/8 screws (Plastics One, Roanoke, VA) were installed 1.0 mm deep into the frontal and parietal bones of the skull. We attached the ground wire onto the anterior right skull screw with Graphite-Filled Conductive Wire Glue. The electrode pedestal was glued to the screws with methyl methacrylate dental cement (Pearson Dental, Sylmar, CA). A buildup of dental cement formed a custom headcap. Similar methods have been used previously [12].

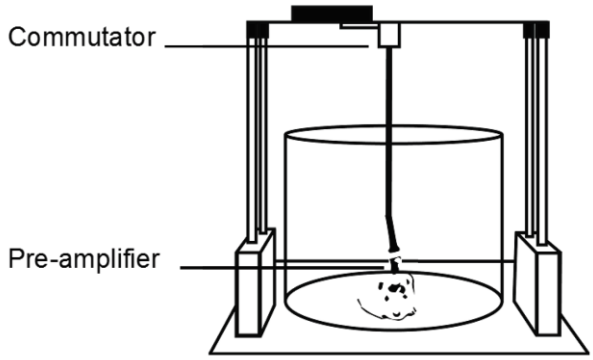

Fig. (1). The rats were tested in a clear Plexiglas rat cage for observation. The custom miniaturized preamplifier was screwed on to a headcap attached to the skull, and the signals were fed through a commutator (used with permission from Taylor et al.) [12].

1.3. Data Acquisition

We used a testing apparatus that utilized a commutator and a pre-amplifier that allowed unrestrained locomotor movement and natural, spontaneous oral behaviors [12]. The rats were placed in a Plexiglass testing chamber (Pinnacle Technology, Lawrence, KS) 1 hour after recovery from anesthesia (see Fig. **1**). The electrode pedestal MS363 was attached to a custom 8442 miniaturized preamplifier (Pinnacle Technology, Lawrence, KS) with a built in x100 gain and 10 Hz high pass filter on all channels. This preamplifier was connected to a 4-channel commutator (Plastics One, Roanoke, VA) mounted above the cage. EMG signals were sent to a 1700 Differential AC Amplifier (A-M Systems, Sequim, WA) for further amplification (x500). The secondary amplifier was set with low and high frequency cut-offs of 10 Hz and 10 kHz. We used an analog-to-digital (DAQ) converter Digidata 1440 (Molecular Devices, Sunnyvale, CA, USA), connected to a computer running Windows XP (Microsoft, Seattle, WA). We used a sampling rate of 1000 Hz. The apparatus was contained within a faraday cage (AutoMate Scientific, Inc., Berkeley, CA) grounded to the secondary amplifier. The electrophysiology equipment received power from a LCR2400 AC line spike and line noise filter (Tripp-Lite, Chicago, IL). The rats would periodically produce brux-like motor patterns for several hours after placement into the novel Plexiglass rat cage. Brux-like cycles were sometimes

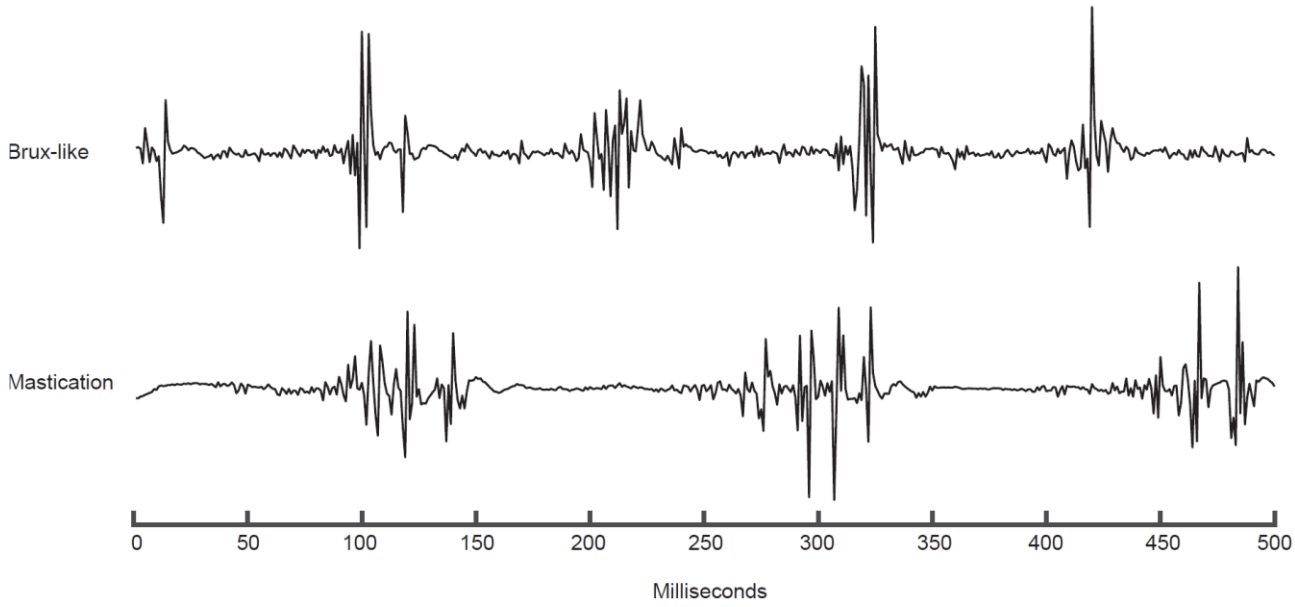

Fig. (2). Raw EMG traces of the temporalis muscle from the same animal (jt36) during brux-like behavior and mastication.

Table 1. Summary of descriptive statistics.

	n	Range	Minimum	Maximum	Mean	Std. Error	Mean EMG Bursts (n)
	Animals	RMS	RMS	RMS	RMS	RMS	Analyzed /Animal
Mastication Digastric	8	.24	.37	.61	.4697	.03033	60
Brux-like Digastric	8	.21	.24	.45	.3644	.02322	47
Mastication Masseter	14	.25	.37	.61	.4664	.02170	70
Brux-like Masseter	14	.28	.17	.46	.3487	.02423	58
MasticationTemporalis	6	.21	.43	.64	.5006	.02963	94
Brux-like Temporalis	6	.28	.22	.50	.3658	.04096	74

provoked by tapping two No. 3 scalpel handles above and behind each rat's head until the desired behavior was produced by the anxious rat [5, 12]. When the brux-like episodes were no longer easily evoked, individual rat chow pellets (Mazuri, Arden Hills, MN) were given to the rats for the masticatory recordings. We continued to record masticatory EMGs until the rats became satiated or the electrodes failed. At the end of the experiment, the rats were deeply anesthetized with Isoflurane and sacrificed via decapitation. The electrode placements were confirmed by post mortem dissections after the testing was completed.

1.4. EMG Amplitude Data Analysis

EMG amplitudes were analyzed using Bioproc2 Data Processing software (Robertson, G. University of Ottawa, Canada). Thresholds were calculated with Bioproc2 (default magnitude of standard deviation: 2) for each trace to determine the onset and offset of the EMG bursts. EMG bursts with movement artifacts or noise were eliminated. Previous investigators have demonstrated that computer-assisted methods allow the experimenter to reject recordings with movement artifacts or spurious noise [15]. In the current study, we occasionally observed some electrical noise that appeared related to locomotor activity of the animal inside the cage. Root mean square (RMS) has been shown to represent the amplitude of the EMG signal [16]. For each rat, we identified the single maximum RMS amplitude for each muscle across all trials. Afterwards, we calculated the normalized RMS amplitudes by dividing all burst amplitudes by the maximum burst amplitude for each muscle. The normalized mean RMS values for each muscle were compared for both masticatory and brux-like behaviors. Statistics were conducted with IBM SPSS statistics software version 24 (Armonk, New York, United States). The Wilcoxon Matched-Pair Signed-Rank test (2 sided) was used to compare masticatory and brux-like behaviors for each respective muscle.

RESULTS

Two animals were eliminated from the analysis due to electromagnetic interference, and/or electrode failure (masseter/temporalis group). In Fig. (**2**), we can appreciate clear differences between the raw EMG traces recorded from the same muscle (temporalis), electrode placement, and animal (jt36). After we calculated the RMS values for each muscle, we were able to compare them (see Table **1**).We found that the mean RMS is greater during mastication versus brux-like behavior for the masseter ($Z=1$, $p<.001$), temporalis ($Z=1$, $p<.046$), and digastric ($Z=2$, $p<.025$) (see Fig. **3**).

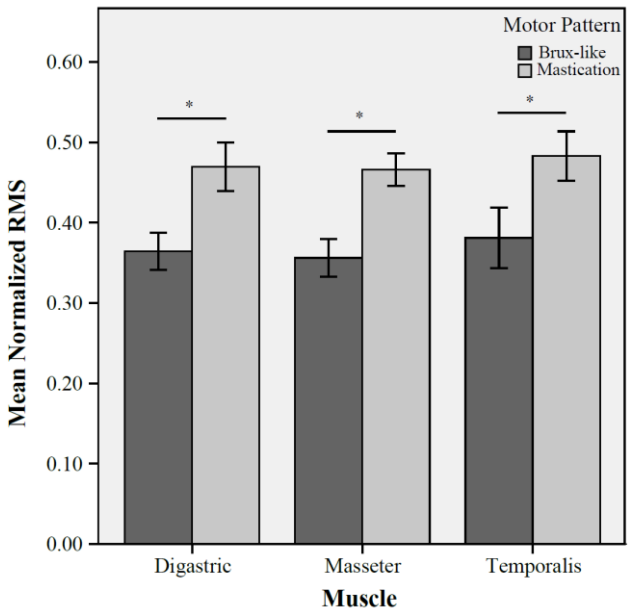

Fig. (3). The mean EMG burst amplitude (mean normalized RMS) is greater for Mastication verses Brux-like behavior for the Diagastric (n=8), Masseter (n=14), and Temporalis muscles (n=6).

DISCUSSION

Our primary objective was to compare EMG activity in muscles that produce motor patterns of the jaw. Our main results demonstrate greater task-dependent RMS values quantified from masticatory versus brux-like episodes. However, we should be cautious with our interpretation of EMG intensity. While it might provide a reliable estimate of the volume of recruited muscle, it may not necessarily be an accurate indicator of the developed force. Numerous factors, such as muscle length, velocity, and activation-deactivation kinetics help de-termine the force an active motor unit can produce [17]. How-ever, in isometric contractions, the relationship between force and EMG amplitude had been reported as close to linear and predictable in the masseter muscle of the monkey Macaca fascicularis [18].

Rats naturally exhibit brux-like motor behavior when startled [5] or under stress. Previous investigators suggest that "thegosis" (tooth grinding) might prevent the overgrowth of the continuously growing incisors, as well as sharpening teeth that become dull [19]. As such, we are currently using the term "brux-like" to describe the motor pattern we have observed. So far, we have not determined if bruxism (a maladaptive re-sponse) produces different motor patterns, amplitudes or se-quences versus "thegosis" (tooth sharpening). So far, we know that during mastication we can observe an alternation of jaw opening and jaw closing muscles; and during a brux-like event, the temporalis and masseter exhibit co-contraction [12]. When we compare the brux-like motor patterns of the rat to human bruxism, we also observe co-contraction [20]. It has been ar-gued, that the human nervous system might exhibit co-contraction in response to injury or threat of injury, and can has been referred to as "protective muscle splinting" [20].

We must always be concerned with translating results of this rat bruxism model to humans since most of the recordings occurred around an hour after awakening from surgery. How-ever, we were able to obtain consistent recordings for up to 8 hours until the electrode signals started vestigators limited their examination of brux-like behavior to the temporalis muscle at 1 hour after recovery using an intramuscular injection of Ketamine, Xylazine, and Acepromazine [5]. Our results were consistent, and we were able to expand on those previous results by examining both jaw opening (digastric), and jaw closing muscles (temporalis and masseter). We also improved upon the analysis by quantifying the EMG amplitudes with a detailed RMS analysis.

CONCLUSION

Establishing an animal model for bruxism will help us investigate the underlying circuits that might produce pathology in human beings. We used EMG recordings to indirectly characterize motor neuron activity of the trigeminal motor nuclei. The trigeminal motor nuclei receive and integrate inputs from numerous parts of the nervous system. The trigeminal nuclei serve as the final destination of all nervous system signals before innervating the muscle fibers. As such, characterizing the activity of the muscles of mastication is a vital first step to understanding the underlying neurological processes that precede it. The results from the current study also provides us with information that could help us further investigate CPG circuits in a reduced fictive preparation (e.g. isolated brainstem). A major question remaining is whether there are separate masticatory and brux-like CPGs [5] in the brainstem or multifunctional interneurons that comprise a single CPG that form a core, multipurpose circuit as found in the spinal cord [21].

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by Pilot Project grant (#PP-13-01) Southern Illinois University School of Dental Medicine, and Vaughnie Lindsay New Investigator Grant from Southern Illinois University Edwardsville awarded to DBW.

REFERENCES

[1]. Lobbezoo F, Ahlberg J, Glaros AG, et al. Bruxism defined and graded: an international consensus. J Oral Rehabil 2013; 40: 2-4.
[2]. Lavigne GJ, Khoury S, Abe S, Yamaguchi T, Raphael K. Bruxism physiology and pathology: an overview for clinicians*. J Oral Rehabil 2008; 35: 476-94.
[3]. Pearson NL. A grinding issue: drug-induced bruxism. Can Pharm J Rev Pharm Can 2008; 141: 300-1.
[4]. Pohto P. Experimental aggression and bruxism in rats. Acta Odontol Scand 1979; 37: 117-26.
[5]. Byrd KE. Characterization of brux-like movements in the laboratory rat by optoelectronic mandibular tracking and electromyographic techniques. Arch Oral Biol 1997; 42: 33-43.
[6]. Sano R, Tanaka E, Korfage JAM, et al. Heterogeneity of fiber characteristics in the rat masseter and digastric muscles. J Anat 2007; 211: 464-70.
[7]. Budtz-Jørgensen E. A 3-month study in monkeys of occlusal dysfunction and stress. Scand J Dent Res 1980; 88: 171-80.
[8]. Nakamura Y, Katakura N. Generation of masticatory rhythm in the brainstem. Neurosci Res 1995; 23: 1-19.
[9]. Grillner S. Neurobiological bases of rhythmic motor acts in vertebrates. Science 1985; 228: 143-9.
[10]. Lund JP. Mastication and its control by the brain stem. Crit Rev Oral Biol Med 1991; 2: 33-64.
[11]. Luschei ES, Goldberg LJ. Neural Mechanisms of Mandibular Control: Mastication and Voluntary Biting. In: Terjung R, editor. Comprehensive Physiology [Internet]. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2011 [cited 2018 Aug 21]. Available from: http://doi.wiley.com/10.1002/cphy.cp010227

[12]. Laylor J, Wall D, Welch D. Masticatory and brux-like motor patterns in the freely behaving rat: electromyography and phase analysis. Trans Ill State Acad Sci 2017; 110: 1-7.

[13]. Faulkner KD. Preliminary studies of some masticatory characteristics of bruxism. J Oral Rehabil 1989; 16: 221-7.

[14]. Fridlund AJ, Cacioppo JT. Guidelines for human electromyographic research. Psychophysiology 1986; 23: 567-89.

[15]. Barrett G, Shibasaki H, Neshige R. A computer-assisted method for averaging movement-related cortical potentials with respect to EMG onset. Electroencephalogr Clin Neurophysiol 1985; 60: 276-81.

[16]. Robertson G, Caldwell G, Hamill J, Kamen G, Whittlesey S. Research methods in biomechanics-2nd Edition. 2 edition. Champaign, Illinois: Human Kinetics; 2013. 440 p.

[17]. Roberts TJ, Gabaldón AM. Interpreting muscle function from EMG: lessons learned from direct measurements of muscle force. Integr Comp Biol 2008; 48: 312-20.

[18]. Hylander WL, Johnson KR. The relationship between masseter force and masseter electromyogram during mastication in the monkey Macaca fascicularis. Arch Oral Biol 1989; 34: 713-22.

[19]. Every RG. Significance of tooth sharpness for mammalian, especially primate, evolution. Contrib Primatol 1975; 5: 293-325.

[20]. Bell WE. Temporomandibular disorders: classification, diagnosis, management. Year Book Medical; 1986. 352 p.

[21]. Berkowitz A, Roberts A, Soffe SR. Roles for multifunctional and specialized spinal interneurons during motor pattern generation in tadpoles, zebrafish larvae, and turtles. Front Behav Neurosci 2010; 4: 36.

This is an open access article licensed under the term of the Creative Commons Attribution-NonCommercial 4.0 International License
(Attribution-NonCommercial 4.0 International-CC-BY-NC 4.0)
© 2019 Mesford Publisher INC