Author response to Microstimulation in the Periaqueductal Gray Matter

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We have repudiated neither the methods nor the conclusions of the research undertaken by Dr. Pamela Davis and Dr. Shi Ping Zang. On the contrary we have duly cited their major works.[1,2] Our assertions neither contradict nor challenge any hypothesis, data, or results published by a wider scientific community on PAG control of vocal behavior, as wrongly claimed by Davis and Zhang. The main result of the work of Davis and Zhang[1] is *"two fundamental patterns of patterned respiratory and laryngeal muscle activity evoked following stimulation of discrete regions of the PAG,"* which, according to them, may or may not be associated with vocalization *(page 11346)*. Zhang et al[1,2] did not undertake any specific electromyography (EMG) analysis, except providing integrated waveforms to show patterns of muscle activity. The records they provided are also based on cohorts of D, L, Homocysteic Acid (DLH) injections, as well as muscle recordings obtained in subsets of cats (genioglossus (GG)/digastric (DG) data based on 2 experiments, 11C/IO data based on 4 experiments and so on, as tabulated in their results section). With such subset of recordings for each muscle, normalization of their data across all animals to undertake various quantitative analyses is not possible. That is probably the reason why Zhang et al[1] classified vocalizations as type A and type B sounds and mainly focused on elucidation of patterned muscle activity following stimulation of the PAG.

In our decerebrate cat studies we examined the total system physiology following stimulation of the PAG and the NRA. Our study involved a large number of glutamate agonist microinjections, including repetition and dose-response. In each animal these micro-injections were investigated with simultaneous EMG/BP/HR recordings (up to 16 channels of physiological data recordings) in order to elucidate the role of the PAG and NRA in emotional motor control. Extensive datasets obtained from these eight cats were published over a series of papers about the PAG and NRA control of respiration[3,4] and the PAG control of the crural and costal diaphragm.[5] The PAG induced vocal behavior[6] provides an exploratory dataset of different vocalizations, their precise orchestration (via raw EMG signals) and limited quantitative analysis to adjudicate vocal behavior. A future paper is envisaged with full population analysis towards modeling laryngeal and respiratory motor control during PAG and NRA induced vocal behavior.

The usual methods for quantification of the EMG signal employ visual estimation of the amplitude/magnitude of the rectified and smoothed "integrated" EMG signal.[1,2] This method provides only a semiquantitative measurement of the signal. In 2009, a neurolaryngology study group, convened a workshop focusing on the use of laryngeal electromyography to address both basic sciences and clinical aspects of neurolaryngology. A multidisciplinary panel of experts included scientists in neuromuscular physiology, electromyography, physical medicine and rehabilitation, neurology, laryngology, and members from the American Academy of Otolaryngology Head and Neck Surgery. The study group publication[7] observed that; "A lack of agreement exists on methodology, interpretation, validity, and clinical application of laryngeal electromyography." The study group further observed that it is NOT always possible to normalize population data owing to the uniqueness of each individual's phonation and/or their associated laryngeal function. The neurolaryngology study group's observations[7] apply to normalization concepts in decerebrate cat studies for two prime reasons.

1) Stimulation the vocalization center in the PAG produces "vocalization episodes," however within the episodes, no two vocalizations would be identical (ie, it is not possible to suggest that mew episode of cat-1 is "exactly" same as mew episode of cat-2). Thus, each vocal expression is taken as distinct within an episode[6] and subjected to any numerical analysis.
2) Most laryngeal muscles are not phasically active during eupnea in the decerebrate preparation.[6] They are activated following stimulation of the PAG,[6] which triggers vocalization. They remain active during vocal episodes and become inactive when vocalization ceases.[6] Hence lack of control data of laryngeal muscle function during eupnea warrants selective sampling of vocal episodes in singular animals and population averaging.

Earlier studies have also examined PAG induced vocalization based on two to four vocal episodes[8,9] obtained from a small number of cats. In fact, Zhang et al[1] seemed to have used principally two episodes out of 47 injections to benchmark their data representation and analysis.

Vocalization induced laryngeal, abdominal and diaphragmatic motor patterns should NOT be assumed as stereotypical motor patterns.[10,11] Our study[6] shows that every vocal phase in an episode is distinct and each vocalization shapes the laryngeal/abdominal/diaphragmatic muscle function in a specific way even in a single trial.[6] Even when the baseline breathing of two decerebrated cats is similar (aka 40 breaths/min), the mews following stimulation of the PAG (using similar neurochemical injection volumes) are not the same in their duration.[6] This makes it very difficult to normalize vocalizations and their associated EMG's across animals. In order to overcome this challenge, in our study we analyzed up to four vocalization episodes (such as a mew episode or a howl episode) in each animal for any relevant quantitative analysis. We have used a combination of decibel evaluation, activation cascades, intensity vs frequency evaluation, power density spectral analysis, respiratory (Ti, Te, & RF) modulation and Turns & Amplitude (T/A)

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measurements associated with specific vocal episodes to further adjudicate the mew, howl, cry and hiss. Our approach is probably the most comprehensive to classify cat vocalization induced from the PAG to date.

Response to specific points raised by Davis and Zhang

Point 1: In our opinion loudness indeed cannot be used to differentiate between a mew and a howl. Zhang et al[1] themselves describe two types of howls, loud howl and intense howl. What is the difference between the two? They are still a howl! Zhang et al[1] have neither provided quantitative variation for the two nor differentiated the howl from the mew they denote based on loudness factor.

Point 2: We have not classified vocalizations purely based on decibel measurements. The decibel and peak frequency analysis of the sound only provides an additional angle for quantitative laryngology. As we have stated in our paper, in order to connect the sounds generated in the PAG with specific emotional expressions, their muscle activation patterns are required. The fact that positive and negative vocal expressions possess distinct/different laryngeal, oral, and respiratory motor pattern is the major finding of our study.[6]

Point 3: There are no inconsistencies (as incorrectly claimed by Davis and Zhang) in data represented in the two papers of Subramanian et al.[3,6] Anatomy sections represented in Figure 2 in both papers show that mews are evoked predominantly in the lateral PAG, while the hisses are in the medial part of the ventrolateral PAG. In the 2008 paper,[3] we focused mainly on the respiratory modulation during a mew, as compared to motor characterization of the mew/howl/hiss/cry in the current paper.[6] We did not observe a growl in our study.[6] According to our hypothesis,[11] growling behavior requires the presence of forebrain and its connectivity to the midbrain which makes it impossible to evoke growling in a decerebrate preparation.

Zhang et al[1] statements on growling are inconsistent. On page 1139, they say "After a single DLH microinjection, PAG evoked vocalization usually consisted of 10−60 episodes (0.4−4.5 seconds) of expiratory sound which could be described as mewing/howling, hissing or growling," whereas in page 1341 they state, "growling was rarely evoked." Not only that, Zhang et al[1] did not provide any distinct motor pattern that could be established as a signature of a growl evoked in the PAG in a decerebrate cat. Bandler and Carrive[12] showed that PAG stimulation induced growling but that was through stimulation of the PAG in freely moving animals. Subramanian et al[3] were focused on PAG control of respiration. Subramanian et al[6] focused on vocal behavior and thus examined the mew, howl, hiss, and cry in greater depth with precise compartmentalization within the PAG. The questions or concerns thus raised by Davis and Zhang do not have any scientific merit.

The chief author and supervisor of the study (GH) has over 120 papers published on cat model research on the periaqueductal gray. In particular his publication "Anatomical study of the final common pathway for vocalization in the cat"[13] is often seen as the foundation for neurophysiological research on PAG/NRA control of vocalization, studies undertaken not only by Davis and Zhang but also by wider neuroscience community! GH has followed this with extensive research on the organization of the PAG and its descending projections to specific neuronal groups in the medulla and the spinal cord.[14,15]

Summing up, our paper[6] is neither a re-review nor re-analysis of our data. Subramanian et al[6] presents original research with a strong focus on emotional vocal behavior triggered from the PAG and an "attempt" to quantitatively model its motor features.

Thus, the scientific concern raised by Pamela Davis and Shi Ping Zhang on our methodology, data analysis and conclusions of our paper[6] is erratic, unsubstantiated, without evidence, and scientifically immature!

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