PROJECT NARRATIVE

The proposed research aims to determine brain abnormalities in SD and SD/VT patients as the basis for characterization of central mechanisms underlying symptom improvement following the use of sodium oxybate, a novel oral medication for the treatment of ethanol-responsive dystonia. The proposed research is *relevant to public health* because the elucidation of disorder-specific mechanistic aspects of brain organization in SD vs. SD/VT is ultimately expected to lead to establishment of enhanced criteria for clinical management of these disorders, including differential diagnosis and treatment. Thus, the proposed research is *relevant to the part of NIH's mission* that pertains to developing fundamental knowledge that will help to reduce the burdens of human disability.

FACILITIES & OTHER RESOURCES – MOUNT SINAI SCHOOL OF MEDICINE

Environment – Contribution to Success

The facilities and other resources available to the PI and her research team at the Mount Sinai School of Medicine include everything needed to undertake and complete the proposed research projects successfully. When she set up her office and adjacent neuroimaging laboratory, they were equipped with this project in mind. These resources are complemented by state-of-the-art core facilities for brain imaging, the General Clinical Research Center (GCRC), which provides a supportive environment for clinical research and the intellectual milieu for the development of young clinical investigators, and the Computational Core, which assists researchers by supplying expertise in the conduct of large-scale computations entailed by research in modern biomedical science. The intellectual environment is rich with other extramurally funded investigators, who are conducting research in the field of movement disorders. These facilities, together with those described for the other project/performance site, collectively provide a scientific environment that is strongly supportive of the proposed research and, therefore, will contribute to the success of this project. Moreover, regular face-to-face interactions and video- and tele-communications maximize interactions between members of the research team, especially those who are at a distance.

Institutional Commitment to ESI

The PI qualifies as a New and Early Stage Investigator. There is extensive evidence of institutional commitment to her development as an academic researcher. Her 12-month tenure-track academic appointment includes a total of 11.4 person months (95% effort) dedicated to research. The institutional start-up package provided to her included laboratory space, equipment, and research funds needed to launch her research program. The package was sufficient to contribute to generation of the preliminary data for this R01 application. Initial support for a postdoctoral fellow was provided. Administrative support is provided to the PI by a department administrative core. In addition, she, as part of the research teams, has access to patients seen in the Bendheim Parkinson and Movement Disorders Center and the Eugen Grabsheid, M.D., Voice Center for recruitment into her research studies. The PI's research area in dystonia is one of the priority research areas designated by her Department and School of Medicine, which will help to ensure continued institutional commitment to her research program.

Facilities

Neuroimaging Laboratory: The PI has a 220 sq ft neuroimaging laboratory, which is located in the departmental space, adjacent to her office on the 2nd floor of the Annenberg building. It is subdivided into individual workstations for present and incoming staff. All workstations have image processing computers (PC/Mac and Linux), which are equipped with software necessary for image processing (e.g., AFNI, SUMA, FSL, FreeSurfer, SPM, PMOD) and are linked via a network with two 10.5T Thecus N7700 ultimate network attached storage (NAS) servers for archiving neuroimaging data, a color laser printer, and a scanner. In addition, the laboratory equipment includes two Dell laptops equipped with software for conduct of neuroimaging experiments (E-prime, Chart Pro); two 16-analog input PowerLab (AD Instruments) data acquisition systems for behavioral recordings during the scanning sessions and in offline setting; Cintriq Wacom tablet for graphical analysis of neuroimaging data (e.g., drawing of regions of interest).

Imaging Science Laboratories (ISL) are shared facilities that include Image Processing, Electronics, MR Microscopy and Small Animal Laboratories. In addition, the Computational Biology Shared Research Facility provides researchers with access to high performance computing systems. The ISL hosts 2 research MRI systems – a 1.5 T Siemens Sonata and a 3.0 T Philips Achieva, which are specially equipped to perform high-resolution functional and structural brain imaging. Both scanners are equipped with standard T/R and 8-channel SENSE head coils.

The 3.0 T Philips scanner will be used to acquire functional and structural images in the studies under this proposal. This scanner is equipped with the highest-performance head gradient system on the market with maximum gradient amplitude of 80 mT/m and a slew rate of 200 mT/m/ms. This will reduce susceptibility-based distortions in single-shot EPI due to ultra-short echo-spacing and will benefit the acquisition of BOLD imaging. This system is running the latest Philips software release and has all standard clinical pulse sequences as well as the latest research pulse sequences for BOLD and high-resolution MRI/DTI. The 3T scanner is equipped with all the peripherals necessary for functional imaging: a visual system with built-in eye tracking (NordicNeuroLab), Serene Sound Audio system (Resonance Technology, Inc.) for delivery and

recording of voice samples, E-prime software for delivery of experimental paradigms (Psychology Software Tools), subject response recording using a fiberoptic button system (Psychology Software Tools), an in-house built olfactometer, a Biopac data acquisition system with an electrodermal activity (GSR100C), a constant current/voltage stimulator (STM100C), an oxygen saturation sensor (OXY100C), a respiratory effort transducer (RSP100C), and a physiological monitoring system for respiration and pulse oxygenation recordings. In addition, the MRI scanner is integrated with a time-of-flight PET system based on Philips Gemini TF. This PET system is integrated with the 3T MRI for coregistration and attenuation correction. Subjects can be scanned in both modalities on the same bed without having to move.

The scanner suite is equipped with its own waiting area, changing room, and examination room in addition to the dedicated isotope uptake rooms hosting computer systems for administering uptake tasks to research subjects. It has a shielded dose room with dose calibrator, gamma counter and centrifuge for immediate analysis of plasma samples. The ISL also provides dedicated MR Physics support for image acquisition and image analysis. Two certified technicians and a research assistant are always available to assist the investigators with MRI protocols (see the Letter of support from Dr. Fayad). *Availability of the ISL contributes greatly to the potential success of this project, which includes MRI as a main research approach in studies of Specific Aim #1 and #2.*

Clinical facilities: The Department of Neurology at MSSM is situated in 5,000 sq ft of office space and 8,000 sq ft of clinical space. Mount Sinai Hospital has a dedicated 40-bed neurology inpatient ward located in the Guggenheim Pavilion and a neurology intensive care unit located in the Annenberg Building. The Bendheim Parkinson and Movement Disorder Center of the Department of Neurology is a comprehensive program for treatment of movement disorders, such as Parkinson's disease and dystonia. The Movement Disorder Program has three interrelated components: patient care, teaching, and research. Therefore, in addition to caring directly for patients, the responsibilities of the Center include developing new research therapies, evaluating the collected results, and sharing those results with students, colleagues and other researchers through meetings, publications, and lectures.

The Eugen Grabscheid, M.D., Voice Center of the Department of Otolaryngology is a multidisciplinary program for the management of patients who suffer from voice disorders. The interdisciplinary team of otolaryngologists, speech/language pathologists, neurologists, and voice coaches use the most advanced technology and methods to provide comprehensive evaluation and voice care. The Center is equipped with the software-based KAY videostroboscopy/laryngoscopy systems and voice and speech recording unites for patient evaluation.

General Clinical Research Center (GCRC) provides a supportive environment for clinical research and the intellectual development of young clinical investigators. The GCRC has been continuously funded by the NIH for over 45 years since 1963. The GCRC is housed in approximately 8000 sq ft, where it has 8 inpatient beds, including 2 double rooms, which can be used as isolation rooms. It also has several areas for infusions, a dedicated exam room, and a private consultation room. There are facilities for studying ambulatory patients with the duration of the study less than 24 hours. The GCRC has its own 24-hour research nursing staff, nutritionists, and a dedicated core laboratory. The GCRC has both inpatient and outpatient facilities. The GCRC is contiguous to newly renovated hospital areas and provides access to intensive care units and resident coverage. *The availability of this Center is important for data collection (e.g., urine pregnancy test prior to MRI) under this research program.*

Other Resources

Computer: The PI has four research computers (Mac Pro, Boxx PC, Linux, and MacBook Air laptop), which are equipped with image processing software and linked via network with the color laser printer and database storage/imaging servers. Each member of the research team has an individual computer; those involved with image processing have additional dedicated workstations. Skype telecommunication software and equipment is on hand for all members of the research team. *The combination of these information technologies contributes to the potential for success by assuring both efficient data handling and optimal communication among the members of the research team.*

Office: The PI's 120 sq ft office is adjacent to her laboratory. It is equipped with a desk, credenza, two book cabinets, three 2-drawer filing cabinets, and hardwired high-speech Internet access. There is also an access to the Internet through the University's wireless network. The staff's shared office space has similar setting.

These facilities assure that the PI and her immediate research team will have the necessary space to formulate experiments, analyze results, and prepare manuscripts for publications.

Intellectual/Collaborative Resources: In addition to the established collaborations with the members of the research team on this proposal, the PI has several ongoing research collaborations with other investigators who are conducting research that is complementary to the proposed studies here.

- Kenneth Altman, M.D., Ph.D. (Department of Otolaryngology, Mount Sinai School of Medicine), Christy Ludlow, Ph.D. (James Madison University), and Barry Horwitz, Ph.D. (NIDCD/NIH) are experts in central mechanisms underlying voice and speech perception and production in normal and diseased individuals.
- Michele Tagliati, M.D. (Department of Neurology, Cedars Sinai Hospital), and Mark Hallett, Ph.D. (NINDS/NIH) are experts in movement disorders, with a special emphasis on primary focal dystonias.
- Cheuk Tang, Ph.D. (Department of Radiology, Mount Sinai School of Medicine), Matilde Inglese, M.D., (Department of Neurology, Mount Sinai School of Medicine), Barry Horwitz, Ph.D. (NIDCD/NIH), Ziad S. Saad (NIMH/NIH), Robert Cox (NIMH/NIH), Peter Hescovitch, M.D. (CC/NIH) consult the PI on neuroimaging data acquisition and analysis.

The PI has regular face-to-face interactions with colleagues at the Mount Sinai School of Medicine and regular email, telephone, and teleconferencing with colleagues at other institutions. *All collaborators provide invaluable constructive criticism and informal intellectual input, without which the proposed project would have far less chance of success.*

FACILITIES & OTHER RESOURCES – NEW YORK HEAD & NECK INSTITUTE

Environment – Contribution to Success

The mission of the New York Head & Neck Institute is to provide integrated care for all aspects of Head and Neck Surgery and Medicine at a single location in Manhattan, New York. With this integral structure, patient diagnosis is enhanced and treatment is expedited, leading to optimal recovery even for the most difficult clinical problems. Another important mission of the Institute is to advance research on the causes, diagnosis and treatment of diseases affecting the head and neck. The New York Center for Voice and Swallowing Disorders is one of the nine Centers of Excellence of the Institute. This Center, which was established in 1994, has been internationally recognized as a leader in evaluation, diagnosis, treatment, education, and research in the majority of disorders that affect voice and swallowing. Dr. Andrew Blitzer, M.D., D.D.S., F.A.C.S., is a director of New York Center for Voice and Swallowing Disorders. He is an internationally known otolaryngologist with expertise in voice and swallowing disorders, nasal and sinus surgery, and head and neck reconstructive surgery. Dr. Blitzer is a pioneer and leading authority in the use of botulinum toxin treatment for SD, other forms of primary focal dystonia, and other conditions with excessive muscle function, muscle pain, and tremor. *The outstanding availability of resources to identify, diagnose, and recruit SD patients for the proposed studies contribute exceptionally to the overall success of this project.*

Facilities

Clinical: The New York Center for Voice and Swallowing Disorders is a comprehensive program for treatment of laryngeal disorders, including SD. The Center has one of the largest databases of SD patients in the country. It serves approximately 1,000 SD patients annually, drawing patients not only from the New York Metropolitan Area but also nationwide. Besides clinical activities, the Center is actively involved in training the next generations of laryngologists and in research on identifying new therapies for patients with laryngeal problems. The highly recognized team of otolaryngologists and speech/language pathologists use the most advanced technology to provide comprehensive patient care. The Center is equipped with all major and minor equipment necessary to successfully conduct the selection, examination, and recruitment of subjects into the proposed studies. Specifically, the facilities include ten flexible fiberoptic and two rigid endoscopes, which can be used with either strobe, distal chip, or triple chip cameras for the performance of nasolaryngoscopy; video and audio recording equipment (digital camcorders, microphones, equalizers) necessary for archiving the laryngeal examination, voice and speech recordings for offline analysis; computers for data storage, and patient information databases. *The participation of the New York Center for Voice and Swallowing Disorders in the proposed studies has a major positive impact because of the unique clinical setting and the large number of SD patients seen annually.*

Office: Dr. Blitzer has a 200 sq ft office that is adjacent to the Clinic. It is equipped with desk, credenza, filing cabinets and hardwired high-speech Internet access. Dr. Blitzer has a personal computer, which is connected to a printer and a scanner. The staff's shared office space has a similar setting with individual computers connected to Internet. The New York Center for Voice and Swallowing Disorders is within a short ride (about 20 min) from the MSSM, where the PI's office is located. *The facilities of this Center assure that the subcontract Co-Investigator and his immediate research team will have the necessary space and office resources as well as communication means with other members of the research team for successful collaboration on the proposed studies.*

SPECIFIC AIMS

Spasmodic dysphonia (SD) is a task-specific primary focal dystonia characterized by selective loss of voluntary voice control during speech production due to uncontrolled spasms in the laryngeal muscles. SD is a chronic debilitating condition, which often extends beyond vocal communication impairment and causes significant occupational disability and life-long social isolation. SD becomes even more incapacitating when it is associated with voice tremor (VT), characterized by rhythmic alterations in pitch and loudness during vowel production and by inability to sustain a vowel for more than a few seconds. Action-induced VT, which is present in about 1/3 of SD patients, often resembles SD symptoms, complicating the differential diagnosis and treatment of these disorders. At present, SD and VT are diagnosed using their voice and speech characteristics, while the outcome of “gold standard” treatment with botulinum toxin injections into the laryngeal muscles helps confirm the initial diagnosis. However, response to botulinum toxin does not reliably separate SD and VT. Moreover, it cannot be used for successful treatment of all SD and VT patients because it is mostly beneficial for the adductor type of SD (ADSD), but not for the abductor type (ABSD) or VT. Current limitations in the clinical management of SD and VT stem from our limited understanding of the pathophysiology underlying these disorders and the lack of biomarkers for their diagnosis and treatment. There is, therefore, a *critical need* to determine the neural correlates contributing to the pathophysiology of SD and VT in order to establish disorder-specific biomarkers and to elucidate the mechanism of action of alternative treatment options in these patients.

To this end, an interesting observation in about 30% of SD and almost all VT patients is the marked improvement of their symptoms after alcohol ingestion. SD and VT are not unique in this regard, as several other movement disorders are well known to dramatically improve with alcohol ingestion, including essential tremor, myoclonus-dystonia, and posthypoxic myoclonus. Because of this feature, patients with SD and VT frequently consume alcohol to manage their voice problems prior to participating in social events. This approach, however, has obvious limitations, including the development of alcohol addiction and the rebound of SD and VT symptoms with increased severity when the effects of alcohol wear off. Our preliminary studies demonstrate that sodium oxybate (Xyrem®), which mimics the effects of ethanol but has no tolerance or rebound effects, significantly improves voice symptoms in ethanol-responsive symptomatic SD patients, due, in part, to attenuation of abnormally increased brain activation. The positive effects of sodium oxybate are even greater in SD/VT patients due to a greater extent of modulation of their abnormal brain function. Thus, sodium oxybate may be a promising pharmacological agent for treatment of ethanol-responsive SD and SD/VT patients, especially those patients whose symptoms are not fully managed with botulinum toxin injections. Sodium oxybate might also be useful in combination with botulinum toxin treatment to specifically target the VT component of SD.

Our *long-term goal* is to determine the pathophysiology of SD and related disorders, such as VT, and develop new diagnostic and treatment options for these patients. The *objective of this application* is to identify brain abnormalities in SD and SD/VT patients as the basis for characterization of central mechanisms underlying symptom improvement following the use of sodium oxybate. Our *central hypothesis* is that, compared to SD patients, SD/VT patients will have additional brain abnormalities within the sensorimotor brain circuits controlling voice production, which are being modulated to a greater extent with sodium oxybate treatment. We further postulate that the clinical efficacy of sodium oxybate treatment will correlate with its central modulatory effects. The *rationale* for the proposed research is that identification of distinct brain mechanisms underlying SD and SD/VT clinical manifestations would provide the necessary insights into the pathophysiology of these disorders, while understanding the neural correlates of sodium oxybate action would allow establishment of a scientific rationale for the use of a novel treatment in these disorders.

We propose the following two *specific aims*:

1. Determine disorder-specific brain abnormalities in SD and SD/VT patients. Based on our preliminary data, our *working hypothesis* is that distinct brain abnormalities will be present in SD and SD/VT patients, who also share common abnormalities within the sensorimotor brain networks controlling voice production.

2. Characterize the central effects of sodium oxybate treatment in ethanol-responsive SD and SD/VT patients. Based on our preliminary data, our *working hypothesis* is that the greater beneficial effects of sodium oxybate treatment in SD/VT patients compared to SD patients are due to a greater extent of modulation of their abnormal brain activation.

At the completion of these studies, it is our *expectation* that we will have (*i*) identified characteristic profiles of brain abnormalities in SD and SD/VT patients, (*ii*) determined the extent of their modulation with a novel treatment, sodium oxybate, both of which will be critical for (*iii*) establishment of neural markers and a new therapeutic option for patients who do not benefit from botulinum toxin treatment alone. These results are expected to have an important *positive impact*, because they will fundamentally advance our knowledge of the pathophysiology of SD and VT as well as allow for improved treatment.

RESEARCH STRATEGY

Significance. SD in isolation or in combination with VT is a relatively rare disorder with a prevalence of 5.9 per 100,000 in the general population (Asgeirsson et al., 2006), although a significant portion of patients remains undiagnosed (Muller et al., 2002; Lo et al., 2009). SD develops spontaneously in midlife and progresses to become a chronic debilitating condition, which impacts nearly every aspect of a patient's life, causing emotional stress, loss of employment, social embarrassment and isolation. Thus, SD presents a significant, life-changing health problem for those who have it. In the course of their disorder, about 1/3 of SD patients develop action-induced VT, which additionally challenges their already impaired vocal communication. Clinically, the presence of VT in SD patients complicates the accuracy of diagnosis due to similarities between symptoms of these disorders, often leading to misdiagnosis and mistreatment, which are burdensome, both financially and psychologically. On the other hand, treatment of VT is not established. While SD symptoms can temporarily be managed with botulinum toxin injections, VT has a poor response to this treatment. No other pharmacological or surgical treatments have been specifically designed to provide long-term relief of symptoms of VT in SD patients. Based on a trial-and-error methodology, a minority of SD/VT patients receive such oral medications as propranolol, primidone, clonazepam and lorazepam, which, however, provide only mild benefits (Adler, 2000).

One of the main reasons for the poor clinical management of SD and VT patients is the limited understanding of the pathophysiology underlying these disorders. Contributing to this knowledge gap is the lack of information about brain abnormalities in VT patients, either in isolation or in combination with SD. While recent neuroimaging studies have identified brain functional and structural alteration in SD (Haslinger et al., 2005; Ali et al., 2006; Simonyan et al., 2008; Simonyan and Ludlow, 2010; Simonyan et al., 2010; Simonyan and Ludlow, 2011), no literature is available on possible brain changes contributing to the development of VT. Further, understanding of the mechanisms of action of existing treatment options in SD and VT patients is even more limited. While two recent studies of botulinum toxin treatment on brain function in SD patients have yielded conflicting results, no studies have examined the central effects of oral medications, which are empirically prescribed for some SD and SD/VT patients. Collectively, the lack of such knowledge significantly hinders our ability to develop and employ novel pharmacological agents for successful treatment of SD and VT.

In this proposal, we aim to 1) determine specific brain abnormalities contributing to the development of the additional VT component in SD patients, and 2) identify disorder-specific alterations that are being modulated with a novel pharmacological intervention, sodium oxybate, used alone or in combination with botulinum toxin treatment. Collectively, this knowledge would lead to the enhanced treatment of SD and SD/VT patients, especially those, who have limited, if any, benefits from botulinum toxin injections. Thus, *the proposed research contribution is significant because it will advance our understanding of the pathophysiology of SD and VT as well as have potential direct impact on improved clinical management of patients with these disorders.* Furthermore, the results of the proposed studies are expected to facilitate establishment of enhanced criteria for (*i*) identification of imaging biomarkers in SD and VT, which can be used for (*ii)* their accurate differential diagnosis, and (*iii*) assessment of efficacy of newly developed treatment strategies.

Innovation. As noted above, the current lack of understanding of SD and VT pathophysiology renders us unable to develop better treatment options for these patients. On the other hand, uncertainties about the central mechanisms of action of both existing and novel therapeutic options further contribute to this challenge. We propose a series of multi-modal neuroimaging studies that will (1) identify functional and structural brain abnormalities in SD vs. SD/VT patients to characterize disorder-specific brain changes, which will provide a scientific framework for (2) elucidating the central mechanisms of action of a novel oral medication, sodium oxybate, as a treatment of SD and VT. Direct comparisons between SD and SD/VT patients and between the central effects of sodium oxybate and botulinum toxin treatments *in the same experimental setting* have clear benefits for successful identification of characteristic patterns of abnormal brain function that may be modulated by a targeted treatment. Furthermore, the proposed correlations between central effects of sodium oxybate and symptom improvement will enhance our assessment of this drug as a novel treatment option for SD and SD/VT patients, many of whom have failed available therapies.

The proposed research is innovative because it focuses not only on identification of distinct pathophysiological mechanisms underlying SD and VT but also because it holds the promise for characterizing the central effects of a novel oral medication, sodium oxybate, as a potential treatment for refractory symptoms in SD and SD/VT patients. The use of advanced multi-modal neuroimaging methodologies together with detailed and extensive analyses of brain functional and structural organization in SD and SD/VT patients is anticipated to yield potentially novel results, which will be critical for the establishment of criteria for improved clinical management of these disorders.

Approach

Specific Aim # 1: ***Determine disorder-specific brain abnormalities in SD and SD/VT patients.***

1.1 Introduction. Although several lines of evidence suggest that abnormal brain function and structure in the key brain regions controlling sensorimotor aspects of speech production play an important role in the development of SD (Haslinger et al., 2005; Ali et al., 2006; Simonyan et al., 2008; Simonyan and Ludlow, 2010, 2011), the central mechanisms underlying the contribution of VT in SD are largely unknown. This gap in knowledge represents an important problem because it hinders the identification of improved treatment options for these patients. The *objective of this aim* is, therefore, to identify abnormalities of brain functional and structural organization in patients with SD vs. SD/VT. Supported by our strong preliminary data, our *working hypothesis* is that distinct brain abnormalities will be present in SD and SD/VT patients, who also share common abnormalities within the sensorimotor brain networks controlling voice production. We will test this hypothesis using the *experimental approach* of multi-modal neuroimaging, including functional MRI (fMRI), high-resolution MRI, and diffusion tensor imaging (DTI) to determine the organization of functional and structural speech-controlling networks, respectively, in SD vs. SD/VT vs. healthy controls. The *rationale* for these studies is that identification of VT-contributing brain changes in SD will allow us to develop neuroimaging biomarkers for the differential diagnosis of SD and SD/VT, as well as help identify treatment opportunities targeting specific brain abnormalities in these patients.

1.2 Justification and Feasibility

1.2.1 Review of Relevant Literature. Considerable progress has been made recently in the identification of brain functional and structural alterations in SD patients using neuroimaging techniques, such as fMRI, positron emission tomography (PET), DTI and high-resolution MRI (Haslinger et al., 2005; Ali et al., 2006; Simonyan et al., 2008; Simonyan and Ludlow, 2010, 2011). These studies have consistently found functional abnormalities in the laryngeal sensorimotor cortex, basal ganglia, thalamus, and cerebellum as well as structural white matter alterations within the corticobulbar, basal-ganglia-thalamo-cortical and cerebello-thalamo-cortical circuits. Further, using a combination of high-resolution MRI and fMRI in SD patients, a significant relationship has been identified between gray matter structural and functional alterations in the key structures of the speech control system, including the laryngeal sensorimotor cortex, inferior frontal (IFG), superior temporal and supramarginal gyri, and cerebellum (Simonyan and Ludlow, 2011). The severity of SD voice breaks has been reported to positively correlate with white matter alterations in the ventral thalamus (Simonyan et al., 2008) and with gray matter changes in the IFG and cerebellum (Simonyan and Ludlow, 2011). Substantiating these findings, postmortem tissue examination found focal axonal degeneration and demyelination in the genu of the internal capsule and mineral accumulations in the basal ganglia, thalamus, and cerebellum in one ADSD patient (Simonyan et al., 2008) as well as clusters of microglial activation in the brainstem nuclei associated with laryngeal control in two ADSD patients (Simonyan et al., 2010). The results of these studies indicate that functional and structural abnormalities do exist in SD patients and that they are coupled with functional abnormalities at different levels within the speech production system, possibly contributing to SD symptom elicitation. However, these studies do not report any direct comparisons between SD and SD/VT patients, hence, they contribute little to complete understanding of VT, which is a prominent feature in about 1/3 of SD patients.

Although the central mechanisms of VT either in isolation or in combination with SD are unknown, studies in other forms of essential tremor (ET) have revealed several brain abnormalities, which may also be present in VT. Specifically, studies in animal models of action-induced tremor have shown abnormal functioning of the central oscillator in the Guillain-Mollaret triangle located in the brainstem and involving the inferior olivary nucleus (Elble, 1998). In a series of neuropathological studies in ET, degenerative changes have been found with predominant cerebellar pathology, such as Purkinje cell loss and an abundance of Purkinje cell axonal torpedoes without Lewy bodies inclusions (Louis et al., 2006; Louis et al., 2007; Axelrad et al., 2008; Louis and Vonsattel, 2008; Louis et al., 2009). Neuroimaging studies have further disclosed abnormal function and structure in the prefrontal cortex, parietal lobule, thalamus and cerebellum (Boecker et al., 1996; Berg et al., 2000; Nitschke et al., 2001; Benito-Leon et al., 2009). Some have also pointed to deficient GABAergic transmission as a contributing factor in the development of ET (Sinton et al., 1989; Boecker et al., 1996; Mally et al., 1996).

Collectively, these studies demonstrate the presence of alterations in functional and structural brain organization in SD and possibly VT patients. Further research is needed to better understand the co-occurrence of these disorders and their pathophysiological mechanisms in order to develop more efficient pharmacological treatments.

1.2.2 Preliminary Studies

I. Functional activation abnormalities can be differentiated between SD and SD/VT patients.

Building on our prior knowledge of abnormal brain activation in SD patients (Haslinger et al., 2005; Ali et al., 2006; Simonyan and Ludlow, 2011), in our pilot study, we used a sparse-sampling event-related blood oxygen level dependent (BOLD) fMRI design to demonstrate that patients with SD and SD/VT exhibit both shared and distinct brain function abnormalities during symptomatic speech production. Specifically, compared to age- and gender-matched healthy subjects, both patient groups (7 subjects in each group) showed *commonly* increased activation in the bilateral laryngeal sensorimotor cortex, IFG, dorsolateral prefrontal cortex (dlPFC), supplementary motor area (SMA), cingulate cortex, precuneus, left angular and supramarginal gyri, putamen and cerebellum ($p \leq 0.01$, corrected) (**Fig. 1**, *left panel*). Compared to each other, *distinct* patterns of brain abnormalities were present in each SD and SD/VT group, encompassing different segments of the speech sensorimotor networks. Compared to SD, brain changes in SD/VT patients had a greater extension within the motor cortical regions, basal ganglia, thalamus, and cerebellum (**Fig. 1**, *right panel*).

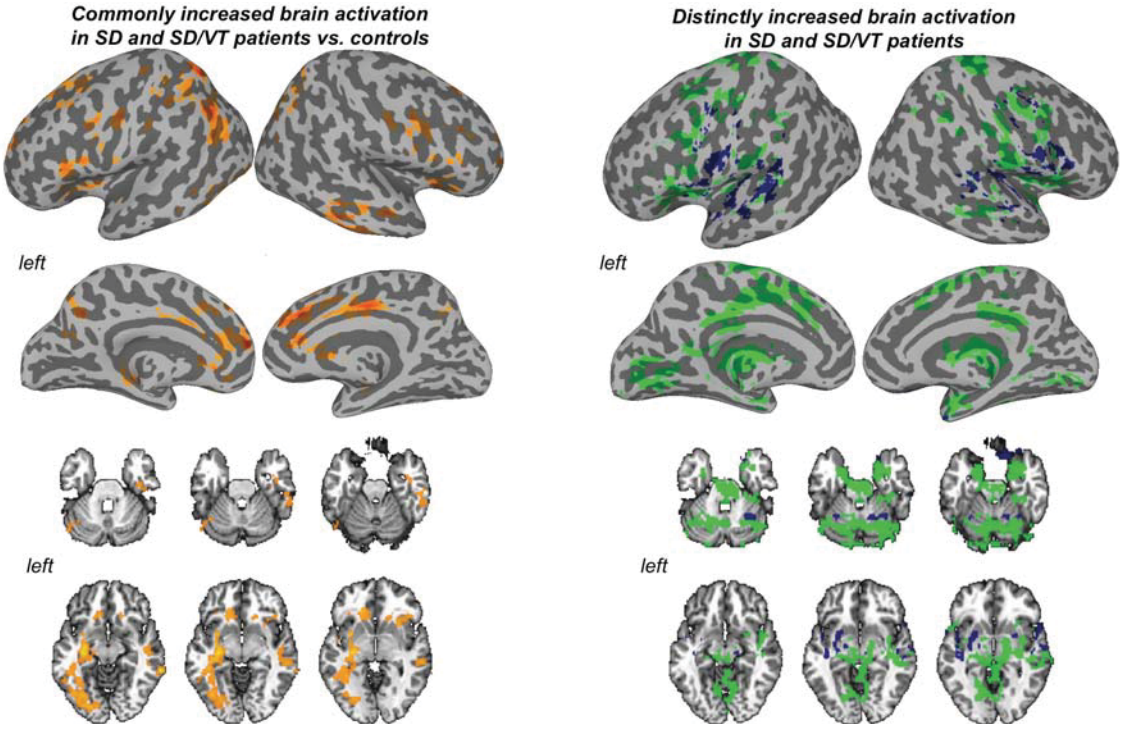

Figure 1 shows additionally increased brain activation, compared to healthy subjects, that is *common* to both SD and SD/VT patients (*left panel*) and *disorder-specific* differences in functional increases in SD (*in blue*) compared to SD/VT (*in green*) patients ($p \leq 0.01$, corrected for multiple comparisons).

II. Structural abnormalities are shared and distinct between SD and SD/VT patients.

In a pilot study, we examined the structural brain organization in SD and SD/VT patients compared to age- and gender-matched healthy control (10 subjects in each group) as well as between SD and SD/VT patients. We used DTI with tract-based spatial statistics (TBSS) to assess the white matter integrity and high-resolution MRI with voxel-based morphometry (VBM) to examine the gray matter organization in these patient groups.

Compared to controls, the patterns of regional abnormalities were *similar* in both SD and SD/VT patients. Decreased fractional anisotropy (FA), a marker of decreased white matter integrity and coherence, was found in the genu of the internal capsule, cerebral and cerebellar peduncles, and in the segment of the superior longitudinal fasciculus underlying the IFG and premotor cortex (**Fig. 2-I,** *upper left panel,* see next page). Commonly increased gray matter volume (GMV) in both SD and SD/VT patients, compared to controls, was observed in the primary laryngeal sensorimotor cortex, supramarginal gyrus, posterior cingulate cortex, ventral anterior thalamus, and cerebellum (**Fig. 2-II,** *lower left panel).*

Despite these similarities in brain structural abnormalities, SD and SD/VT patients exhibited somewhat *distinctive* patters of brain alterations. SD/VT showed *additional* FA decreases in the right premotor cortex and cerebellum (**Fig. 2-I,** *upper right panel, in green*) and GMV increases in the dorsal premotor cortex, ventral thalamus and cerebellar vermis (**Fig. 2-II,** *lower right panel, in green*). On the other hand, SD patients showed

additional FA decreases within the superior longitudinal fasciculus (**Fig. 2-I**, *upper right panel, in blue*) and GMV increases in the supramarginal and angular gyri (**Fig. 2-II**, *lower right panel, in blue*).

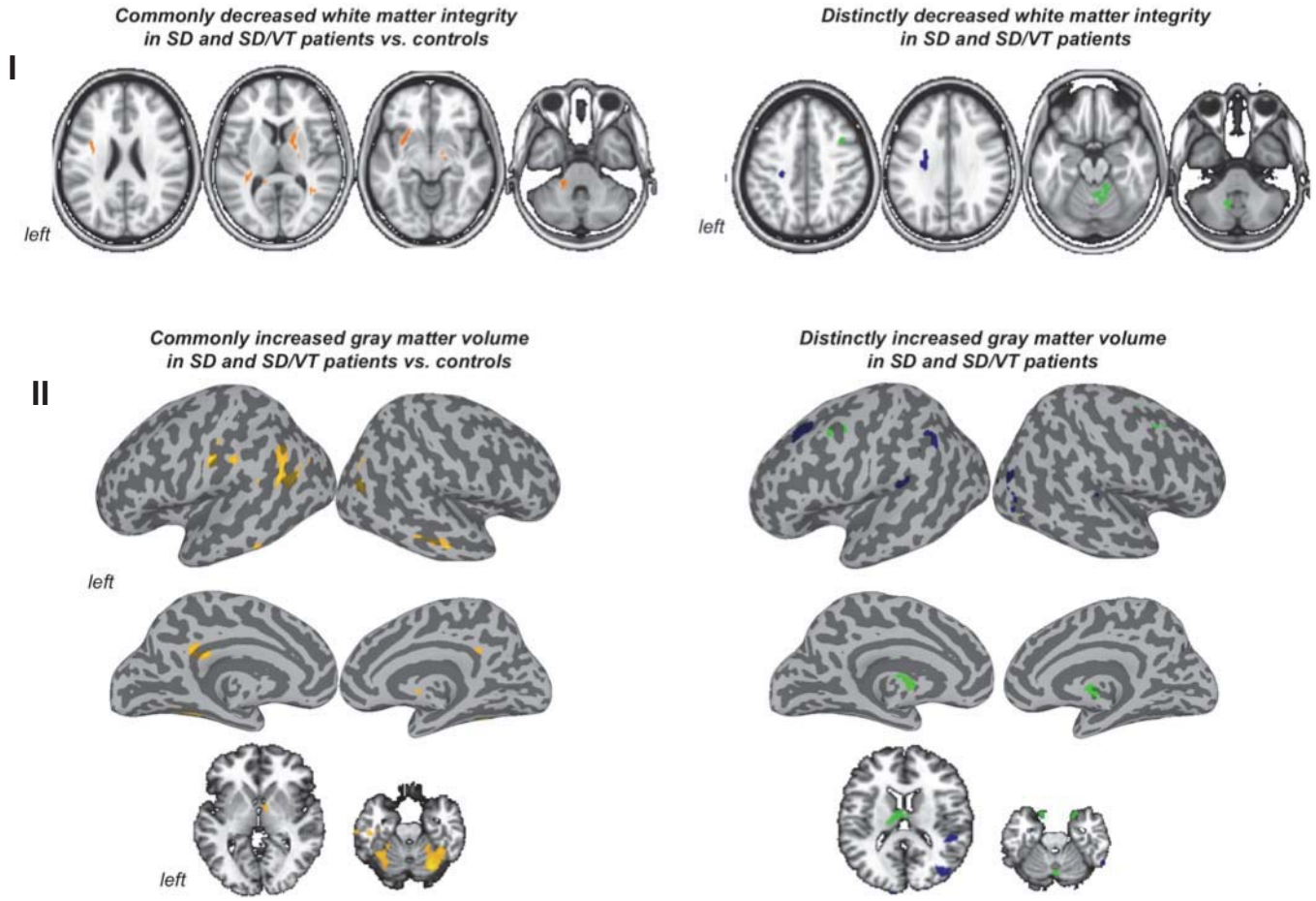

Figure 2 shows *common* (in yellow) and *distinct* (blue in SD and green in SD/VT) white matter (I) and gray matter (II) changes in SD and SD/VT patients ($p \leq 0.01$, corrected for multiple comparisons).

The results of our pilot neuroimaging studies, thus, strongly indicate the presence of both *shared and distinct* functional and structural alterations in SD and SD/VT patients, which can be found within the cortical and subcortical regions controlling sensorimotor aspects of voice and speech production. Compared to SD, SD/VT exhibits additional brain changes, which may potentially be targeted with novel treatment options. *Collectively, our data show that imaging techniques are capable of identifying differences in brain abnormalities between SD and SD/VT and support the feasibility of the below-proposed approach in our hands.*

1.3 Research Design

The following 3 groups of subjects will participate in all proposed studies of the Specific Aim #1: (1) SD patients; (2) SD/VT patients, and (3) healthy controls, who have no history of any neurological, psychiatric or laryngological problems. Because SD is a rare disorder, both ADSD and ABSD patients will be included to closely match with ADSD/VT and ABSD/VT patients, which will ensure the homogeneity between patient populations. Based on the reported SD clinical prevalence (Ludlow et al., 2008) and our own experience, we estimate that about 80% of the participating patients will have ADSD and 20% will have ABSD. SD patients only with VT without ET in other body parts will be recruited. Because the majority of VT patients report improvement of their voice symptoms after alcohol intake, we predict that at least 60% of recruited SD/VT group will also be ethanol-responsive. To further assure homogeneity between patient populations, we will carefully match SD and SD/VT patients according to their report of alcohol effects, in addition to their age, gender and an SD type match. Eligibility of patients will be established based on a three-tiered approach, including initial screening interview, detailed voice/speech, laryngological and neurological examinations (see *Appendices 1 and 2*), as previously described (Ludlow et al., 2008). Any subject with neurological (other than SD and SD/VT), psychiatric, or laryngological problem will be excluded from the study. All recruited patients must be symptomatic; if they are treated with botulinum toxin injections, they may be included only if they are symptomatic, that is at the end of their botulinum toxin treatment cycle. Controls will be carefully matched with SD and

SD/VT populations by their age and gender. Although we have a large neuroimaging database of SD patients and controls, those data were acquired by the PI on a different MRI scanner several years ago and, therefore, will not be used in the proposed studies to ensure the consistency in the scanner use and study design.

1.3.1 Study #1: Mapping disorder-specific brain activation in patients with SD vs. SD/VT using fMRI during symptomatic voice and speech production. We will use a sparse-sampling event-related BOLD fMRI design on a 3.0 Tesla Philips MRI scanner. We already successfully used this design for the investigations of voice and other laryngeal behaviors (Loucks et al., 2007; Simonyan et al., 2007; Simonyan et al., 2009; Simonyan and Ludlow, 2010). Brain activity will be studied during production of 10 symptomatic speech sentences (e.g., “We eat eels every day”, “Sam has a rabbit in his hand”) and syllables (repetitive vowel /i/ and continuous vowel /i/). In the scanner, subjects will listen to an auditory example of the task presented in a pseudorandomized order and reproduce it within the 5-s interval of the ‘silent’ period of scanning, only after which a 2-s image acquisition will follow, while the subjects remain silent and motionless. A resting condition without auditory presentation will serve as a baseline. Four scanning sessions will be acquired with a total of 36 experimental trials and 24 baseline conditions. Structural MRI will be acquired for an anatomical reference.

Data analysis will follow the similar processing pipeline as previously employed by us using AFNI software (Simonyan et al., 2007; Simonyan et al., 2009; Simonyan and Ludlow, 2010). Following initial data processing, the task-related responses will be analyzed using multiple linear regression with a single regressor for each task convolved with a canonical hemodynamic response function. Baseline drifts will be modeled using quadratic polynomials in time for each separate imaging run, and motion parameter estimates will be used as additional regressors of no interest in the multiple regression analysis. To account for disorder-specific vocal motor differences between SD and SD/VT groups, such as adductor and abductor voice breaks and shakiness, respectively, we will include the number of voice breaks and severity of voice tremor as variables in a within-group statistical analysis. Quantitative measures of SD and VT symptoms will be derived from the *Study 1.3.3* within this Aim. The correction of individual statistical parametric maps for multiple comparisons will be performed using Monte-Carlo simulations (Forman et al., 1995), which will identify a minimum cluster size at a voxelwise threshold of 0.005 at overall significance level of a corrected $p \leq 0.025$.

For group analysis, the 3-D anatomical datasets of each subject will be spatially normalized and converted to the standard anatomical space. The resulting normalization parameters will be applied to the 4-D time series datasets. To estimate the main effect of a task per group, the group analysis will be carried out using a three-way analysis of variance (ANOVA) with subject as a random factor and the task and group as fixed factors ($p \leq 0.025$, voxelwise corrected) to identify (1) shared abnormalities in all patients vs. controls, and (2) distinct abnormalities between SD vs. SD/VT patients. Statistical differences in brain activation extent will be assessed using the subtraction method within the three-way ANOVA design. This univariate data analysis will provide information about the extent and intensity of brain activation in different groups (i.e., SD and SD/VT patients vs. controls and SD patients vs. SD/VT patients).

To further our knowledge of functional brain abnormalities in SD and VT, we will performed multistage brain network analysis, which will allow for identification of abnormal brain networks and the relationship between the key network regions, possibly contributing to the pathophysiology of these disorders. For this, we will first perform a correlation analysis between functional and structural datasets in SD and SD/VT patients to determine the brain regions of abnormal function with underlying structural changes. The structural data will be derived from the *Studies 1.3.2 and 1.3.3.*

Once such regions are identified, the group peaks of activation in these regions will be used as 4-mm seed regions for functional connectivity analysis (Horwitz, 2003) using AFNI software to determine the *trait characteristics* of voice-controlling networks in SD and SD/VT. For this, we will use psychophysiological interactions (PPI) analysis, which refers to significant changes in the contribution of one brain region’s activity to that in another region due to changes in the experimental or psychological context (Friston et al., 1997). Time series during task production and silent fixation will be extracted from each seed region in each subject and multiplied by the task vector with the follow-up regression with the time series from the entire brain. The results of regression analysis will indicate the changes in interaction of the seed region with the entire brain relative to the examined condition (e.g., speech vs. silent fixation). A significant change in interaction will mean that the functional connectivity between the seed region and a given region in the brain is significantly different when the subjects perform one task compared with another. The subject-specific functional connectivity maps will be submitted to group analyses to identify differences within and between the groups at a corrected $p \leq 0.025$ to account for multiple comparisons. To further correct for multiple comparisons across the voxels in the whole brain at a group $p \leq 0.025$, we will esti-

mate the voxelwise probability of a false positive detection using Monte Carlo simulation with the individual voxel probability threshold of $p \leq 0.005$ and data-dependent minimum cluster size threshold.

As the last step in network analysis, we will examine effective connectivity between the brain regions of interest (ROIs) showing abnormal PPI network(s) in order to identify direct and indirect influences between the regions within the network(s). For this, we will use dynamic causal modeling (DCM) in SPM8 software, which is a hypothesis-driven neurodynamics model that uses a bilinear state equation to characterize an experimentally perturbed cognitive system using a hemodynamic forward model (Friston et al., 2003). The 4-mm radius sphere ROIs will be used to extract time series from each individual's scanning sessions. DCM estimates 3 different sets of parameters: (1) input parameters that quantify how brain regions respond to external stimuli; (2) intrinsic parameters reflecting the effective connectivity that characterizes the coupling between regions, and (3) modulatory parameters that measure changes in effective connectivity induced by some experimental conditions. Based on the results of correlation analysis between functional and structural datasets, the model will be specified with a specific hypothesis of how the affected regions would likely interact with each other. Speech production will be used as a modulatory input to estimate the change in connection strength as a function of the speaking task relative to other conditions in patients with SD and SD/VT and controls. All parameters (intrinsic and modulatory) of the DCM model and their posterior probabilities will be assessed with Bayesian inversion by means of expectation-maximization algorithm. Within-group analyses will be conducted using both the local and global approaches. The local approach will estimate the significance of the parameters at the local connection level, whereas the global approach will use the Bayesian model selection to identify the set of modulatory connections that is most plausible in the context of speech production. While the local approach guarantees that the connections considered for the model selection are consistent across the subjects, the global approach finds the optimal combination of these consistent local. Statistical comparisons between groups (SD and SD/VT vs. controls; SD vs. SD/VT) will also be conducted at a corrected $p \leq 0.025$.

The proposed studies, from univariate analysis to network modeling, are expected to provide full characterization of shared and distinct functional brain abnormalities between SD and SD/VT patients. This approach is feasible in our hand because we have already used similar analysis techniques in our previous studies (Simonyan et al., 2009; Simonyan and Ludlow, 2010, 2011).

Study population and Sample size. To estimate the number of subjects to be included in this study, we reviewed our recent fMRI study in SD patients, which used the similar fMRI experimental design (Simonyan and Ludlow, 2010). Significantly increased activation intensity was found in the primary somatosensory cortex during production of symptomatic syllables in SD patients compared to controls (mean % signal change ± s.d.: SD vs. controls: 0.63 ± 0.16 vs. 0.39 ± 0.23; $p \leq 0.01$). Using these values, results of two-tailed *t*-test calculations suggest that a sample size of 15 subjects per group would have 80% power to detect group differences at a 0.025 significance level. The power for network analyses is the same. We propose to recruit 20 subjects per group to account for the subjects' dropout possibilities and to increase statistical power for detection of differences between groups.

1.3.2 Study #2: Mapping disorder-specific gray matter abnormalities in patients with SD vs. SD/VT using high-resolution MRI. In this study, we propose to identify GMV abnormalities that are common and distinct between SD and SD/VT patients. VBM is the most commonly used technique to identify changes in both cortical and subcortical gray matter in response to physiological and pathological conditions. To obtain VBM measures, we will acquire whole-brain high-resolution MR images (voxel size ≤ 1 mm isotropic) on a 3.0 Tesla Philips scanner within the same session of fMRI study described above.

Data analysis. Simialr to our recent study in SD (Simonyan and Ludlow, 2011), brain volume in each subject will be segmented into gray matter, white matter, and CSF. VBM analysis will follow the standard procedure using VBM8 toolbox in the SPM8 software. Spatial normalization will be performed using a diffeomorphic nonlinear registration tool (DARTEL) to improve inter-subject registration (Ashburner, 2007). Voxelwise statistical differences in GMV between groups will be examined using independent *t*-tests with age, gender, total intracranial volume, number of SD-specific voice breaks, severity of tremor and disorder duration as nuisance effects (Ashburner and Friston, 2000). The cluster-level significance will be assessed using a conservative non-stationary cluster extent correction (Hayasaka and Nichols, 2004) at $p \leq 0.025$.

Study population and Sample size. The same 3 groups of SD, SD/VT and healthy control subjects recruited for the fMRI study will participate in this study. To determine the sample size for this study, we reviewed our previous study in 40 SD patients and 40 controls (Simonyan and Ludlow, 2011). That study found increased GMV in the laryngeal primary sensorimotor cortex (HV vs. SD: 0.22 ± 0.04 vs. 0.26 ± 0.04). Using these values, *t*-test indicated that a sample size of 17 subjects per group will provide 80% power to detect group differ-

ences at a 0.025 significance level in the present study. To increase statistical power of the proposed studies and account for subjects' dropout possibilities, we will recruit the same 20 subjects per group, who participate in the *1.3.1. Study #1.*

1.3.3 Study #3: Mapping disorder-specific white matter abnormalities in patients with SD vs. SD/VT using DTI. In this study, we propose to identify white matter abnormalities contributing to SD and SD/VT using DTI on a 3.0 Tesla Philips scanner. Whole-brain brain images will be acquired using spin-echo EPI sequence with paired gradient pulses for diffusion weighting; diffusion will be measured along at least 33 non-collinear directions. We successfully used a similar scanning protocol in our previous studies (Simonyan et al., 2008; Simonyan et al., 2009).

Data analysis. Diffusion-weighted images will be corrected for eddy current distortions and head motion using affine registration to a reference volume and an individual's T1-weighted image. The diffusion tensor for each voxel will be calculated based on the eigenvectors and eigenvalues using multivariate fitting and diagonalization. Statistical analyses of FA and mean diffusivity (MD) changes between patients and control will be conducted using unbiased whole-brain voxelwise TBSS analysis (Smith et al., 2006) using FSL software as previously described (Simonyan et al., 2008). Within- and between-group statistical differences in FA and MD for each point of the white matter skeleton will be assessed using univariate GLM design at an adjusted $p \leq 0.025$ to correct for multiple comparisons.

To examine white matter connectivity and its abnormalities in SD and SD/VT patients, we will further use probabilistic tractography (Behrens et al., 2007) in order to identify anatomical connectivity between the regions of the abnormal network as determined in *Study 1.3.1.* To generate a probabilistic streamline and build up the connectivity distribution, the distribution probabilities of each fiber direction will be sampled in each voxel. These calculated local diffusion directions will allow for modeling of multiple fiber orientations in each voxel starting from the seed voxel. To identify the connections between the ROIs, probabilistic tractography will be performed with the following parameters: 5000 streamline samples, 2000 steps with 0.5 mm step length and 0.1-curvature threshold. All resulting probabilistic tractography maps will be averaged to create the group maps normalized to the standard space and thresholded at a corrected $p \leq 0.025$.

Study population and Sample size. To estimate the number of subjects in this study, we reviewed our recent DTI studies, which used a similar experimental design to examine white matter abnormalities in SD patients and controls (Simonyan et al., 2008; Simonyan et al., 2009). Significantly decreased FA was found in the right genu of the internal capsule in SD (patients vs. controls: 0.55 ± 0.05 vs. 0.61 ± 0.07). Using these values, a power of 80% at a 0.025 significance level will be obtained with 18 subjects per group for the proposed study based. Again, to increase the statistical significance and allow for between-study comparisons, we propose to recruit the same 20 subjects per SD, SD/VT and control group for the DTI study. The statistical power for tractography is the same.

1.3.4 Study #4: Relationship between brain abnormalities and clinico-behavioral characteristics in SD and SD/VT. The role of neuroimaging studies in elucidation of pathophysiological mechanisms of SD would be considerably enhanced if coupled with strategies that yield information about their relevance to symptom elicitation. Additionally, identification of disorder-specific brain abnormalities that are correlated with disorder symptoms can prove useful for assessment of efficacy of existing and newly developed therapeutic interventions. Our approach here will be to examine the relationships of functional brain activity and structural brain organization with clinico-behavioral correlates (i.e., disorder severity scores). For all 20 subjects per group (both patients and controls), the behavioral measures will be obtained during initial intake screening, history and physical examination, and three-tiered diagnostic questionnaire (see *Appendices 1 and 2*). Patients' voice and speech will be digitally recorded during scanning for offline acoustic analysis to assess the SD and VT severity.

Data analysis. Blinded analysis of patients' voice samples will be performed to quantify the disorder severity, including the frequency of SD-specific voice breaks, voice harshness, breathiness, and voice tremor (see *Appendix 2*). In each patient group, unbiased whole-brain voxelwise Pearson's correlation coefficients will be computed to determine the relationships among brain activation (measured as percent BOLD signal change) and symptom severity at a corrected $p \leq 0.025$ using AFNI software (Simonyan and Ludlow, 2011). Similarly, whole-brain voxelwise Pearson's correlation coefficients will be used to assess relationships among measures of structural abnormalities (i.e., derived from VBM and DTI analyses) and quantitative measures of SD and VT symptoms (i.e., number of voice breaks, voice harshness, breathiness and voice tremor derived from voice and speech acoustic analysis). The alpha level will be set at $p \leq 0.025$ to correct for multiple comparisons.

1.4 Expected Outcomes. Upon the successful completion of the proposed studies in Specific Aim #1, it is our expectation that each disorder will have been found to exhibit a unique pattern of functional and structural al-

terations within the cortico-basal ganglia-thalamo-cortical and cerebello-thalamo-cortical networks. These findings will significantly advance our knowledge of SD and VT pathophysiology by establishing the concept that functional brain abnormalities in SD follow distinctive patterns when combined with VT. We expect that these findings will be fundamental in defining the neuroimaging markers of these disorders, which can be used for prediction and better differential diagnosis of SD and VT.

1.5 Potential Problems and Alternative Strategies. A potential problem may arise from insufficient availability of patients who are symptomatic, have no history of brain and/or laryngeal surgery, do not take any drugs affecting the central nervous system, and have the last botulinum toxin treatment at least 3 months prior to the study participation. Such a problem seems unlikely, however, because using these same eligibility criteria in the past, we were successful in recruiting required number of SD patients for our recent imaging studies. We will make every effort to enhance our recruitment process by advertising the studies through the National Spasmodic Dysphonia Association (NSDA) newsletter and website and by contacting over 1,500 patients in our combined databases, especially those who previously participated in our studies.

A potential problem in the fMRI studies involving orofacial behaviors is the susceptibility to motion-induced artifacts, which may lead to false signal changes and spatial misalignments because of movement-induced field changes and head drift along the scanning session (Birn et al., 1999). To overcome this potential limitation, we propose to use the event-related sparse-sampling fMRI design, which is much less susceptible to speech-induced motion artifacts. Furthermore, we will immobilize the subject's head during the scanning session using memory-foam pillows and a forehead strap as well as remove any residual head movements as the first step in data analysis. A similar study approach was used successfully in our previous studies (Loucks et al., 2007; Simonyan et al., 2007; Simonyan et al., 2009; Simonyan and Ludlow, 2010).

During data analysis, the registration procedure between functional and reference anatomical MRI datasets may present a limitation due to error introduction in some subcortical structures. To minimize this possible error, we will (*i*) acquire all datasets within the same scanning session, and (*ii*) use the most up-to-date image registration tools, which have already been validated for accurate registration between datasets of different modalities. In the unlikely event we experience problems with data processing, we will consult our collaborators, Drs. Horwitz and Inglese, who are well-known experts in neuroimaging field (see their Letters of Support).

Specific Aim # 2: Characterize the central effects of sodium oxybate treatment in ethanol-responsive SD and SD/VT patients.

2.1 Introduction. Treatment options of SD and VT are limited and directed to short-term symptom management, usually employing medications such as botulinum toxin injections into the laryngeal muscles. However, botulinum toxin treatment is not beneficial in all SD patients. It is estimated that 90% of ADSD patients receive 90% benefit from botulinum toxin treatment, while only 10% of ABSD patients receive 70% benefits (Blitzer, 2010). The benefits of botulinum toxin treatment are even much less, if any, for patients with VT or combined SD and VT. In addition, botulinum toxin treatment is burdensome both psychologically and financially because injections are relatively expensive and must be repeated every 3-4 months throughout a patient's life. Furthermore, the "gold standard" treatment of SD with botulinum toxin is based on an empirical rationale, as the mechanisms of action by which botulinum toxin relieves SD symptoms remain unclear. Studies of central effects of botulinum toxin treatment in SD patients are limited and so far produced conflicting results. An fMRI study found no effects of treatment on cortical activation but decreased subcortical activation within the thalamus and basal ganglia during vowel production in ADSD patients (Haslinger et al., 2005). Another study, using PET during narrative speech production in ADSD patients, found decreased activation in the motor cortex, cerebellum, thalamus and basal ganglia and increased activation in the sensory association areas, operculum and midbrain after treatment with botulinum toxin (Ali et al., 2006).

To date, oral medications are known to provide little relief from SD and VT symptoms, and, thus, these patients are not systematically prescribed any medications (Adler, 2000). However, some patients report anecdotal benefits from taking lorazepam, clonazepam, diazepam, gabapentin, as well as some receive antidepressants and anti-anxiety drugs to cope with their disorder-associated depression and anxiety. Recently, a series of clinical trials have reported significant beneficial effects of sodium oxybate on symptom improvement in treatment-refractory hyperkinetic movement disorders, including dystonia and ET (Frucht et al., 2005a; Frucht et al., 2005b; Arpesella et al., 2009). This medication seems to target more efficiently the tremor symptoms. Hence, it is possible that it may represent an alternative treatment option for SD/VT patients, whose symptoms are not fully responsive to botulinum toxin treatment.

However, despite remarkable improvement of symptoms in patients with movement disorders, the central effects of sodium oxybate also remain unknown. The absence of such information is a significant problem because it prevents the development of a scientific rationale for the use of a potentially beneficial novel pharmacological agent for the treatment of SD and VT. The *objective of this aim* is, therefore, to identify the mechanisms of central action of a promising novel medication, sodium oxybate, in SD and SD/VT patients as well as to determine the extent of central effects of a combined use of sodium oxybate and botulinum toxin treatments. Our *working hypothesis* is that that the greater beneficial effects of sodium oxybate treatment in SD/VT patients compared to SD patients are due to a greater extent of modulation of their abnormal brain activation. This hypothesis has been formulated based on our preliminary data and will be tested using the *experimental approach* of fMRI during symptomatic speech production before and after treatment in SD and SD/VT patients, who report improvement of their symptoms after alcohol intake. The *rationale* for these studies is that the explanation of central mechanisms of action of sodium oxybate in SD and VT patients would help establish a scientific rationale for this drug as a new treatment option for patients who have failed other therapies.

2.2 Justification and Feasibility

2.2.1 Review of Relevant Literature. A long-standing observation in patients with primary dystonias, myoclonus and tremor is the improvement of their symptoms with ethanol ingestion. This feature led to recent explorations of sodium oxybate (Xyrem®), which mimics the therapeutic effects of ethanol, as an alternative treatment option for patients with ethanol-responsive movement disorders. Sodium oxybate is chemically identical to gamma-hydroxybutyric acid (GHB), a naturally occurring inhibitory neurotransmitter (Waszkielewicz and Bojarski, 2004). When ingested orally, sodium oxybate is quickly absorbed, crosses the blood-brain barrier and is converted into gamma-aminobutyric acid (GABA) within the brain (Crunelli et al., 2006). In 2002, the FDA approved Xyrem® for the treatment of catalepsy in narcoleptic patients and instituted the Xyrem Success Program® (xyrem.info/healthcare-professionals). The Xyrem Success Program® is a central registry that controls the distribution and administration of Xyrem® (Fuller De Fau - Hornfeldt et al., 2004). Physicians who prescribe Xyrem®, and all patients who receive the drug are registered with the program. After a prescription is written, the central pharmacy contacts the patient and sends the drug directly to the patient. Follow-up phone calls to the patient and regular contacts with the prescribing physicians have ensured that nearly 10,000 patients have received the drug in the United States without known episodes of drug diversion or illicit use.

Recent tolerability and efficacy trials of sodium oxybate in patients with ET, myoclonus-dystonia and post-hypoxic myoclonus have established satisfactory tolerability and improvement of disorder symptoms over 50% as measured by blinded videotape review (Frucht et al., 2005a; Frucht et al., 2005b; Arpesella et al., 2009). Our *preliminary data* further demonstrate that sodium oxybate significantly improves symptoms of SD and VT, with SD/VT patients receiving greater benefits compared to SD patients. Patients with movement disorders usually take 1-2 gm of the drug given three times daily compared to patients with narcolepsy, who take between 4.5 and 9 gm of the drug at night (Frucht et al., 2005a; Frucht et al., 2005b; Arpesella et al., 2009). Sodium oxybate works quickly, within 45 minutes of oral administration and its effects are visible in the office. Each dose typically lasts 3½-4 hours. Possible side effects include dose-dependent sedation and dizziness, which, however, have marked individual variability. In summary, sodium oxybate may hold promise of a potentially new treatment option for SD and SD/VT, especially for those who have limited symptom improvement following botulinum toxin injections.

2.2.2 Preliminary Data.

I. Sodium oxybate is effective and well tolerated in SD and SD/VT patients. In a pilot study, we evaluated 2 patients with SD and 4 patients with SD/VT. All patients were symptomatic at the time of examination. Three patients were treated with botulinum toxin injections with the last injection received at least 3 months prior to the study participation; botulinum toxin treatment in the other 3 patients was not successful. All patients reported significant relief of their symptoms with ingestion of alcohol.

Sodium oxybate was administered in the office by mouth. Pre- and one hour post-drug voice and speech examinations were performed and videotaped. Initial dose was 1 gm three times daily. It was increased individually every two weeks, until a dose was achieved that improved symptoms without undesirable side effects. The target dose was 1.25 gm T.I.D., although the dose required to achieve benefits varied between patients and correlated with the dose of alcohol required to achieve a similar result. Sodium oxybate was well tolerated by all patients without tolerance and rebound. Based on blinded acoustical analysis of voice and speech samples, SD-related symptoms (breaks, harshness and breathiness) improved over 36% from baseline, whereas voice tremor improved over 71% from baseline. Some patients continued sodium oxybate treatment together

with botulinum toxin injections after study completion. These patients reported that such combined use helped prolong the botulinum toxin injection cycle in addition to relieving VT symptoms.

II. Sodium oxybate modulates abnormally increased activation in SD and SD/VT patients. Using a sparse-sampling BOLD fMRI design and symptomatic speech production, we examine the effects of sodium oxybate on brain activation abnormalities in 3 SD and SD/VT patients to evaluate the mechanisms of central action of this novel pharmacological agent. Compared to healthy controls, before treatment, all patients exhibited typically increased brain activation during symptomatic speech production ($p \leq 0.05$, corrected) (**Fig. 3-I**) similar to abnormalities described before (Haslinger et al., 2005; Ali et al., 2006; Simonyan and Ludlow, 2010) and in our *1.2.2 Preliminary Studies* above. After treatment, patients' brain activation was significantly attenuated and followed a nearly normal pattern present during normal speech production, with only few regions remaining hyperactive ($p \leq 0.05$, corrected) (**Fig. 3-II**). A two-tailed paired *t*-test calculation using the measure of the total number of significantly active brain voxels before and after treatment in patients showed that the drug-induced attenuation of abnormal functional activation was in average 49% ($p = 0.017$) (**Fig. 3-III**). Specifically, sodium oxybate treatment significantly decreased and normalized abnormally elevated brain activity in the prefrontal, premotor and auditory cortices as well as in the basal ganglia, thalamus, and cerebellum. In *1.2.2 Preliminary Studies* presented above, we found these regions to have a greater extent of abnormal activation in SD/VT patients compared to SD patients.

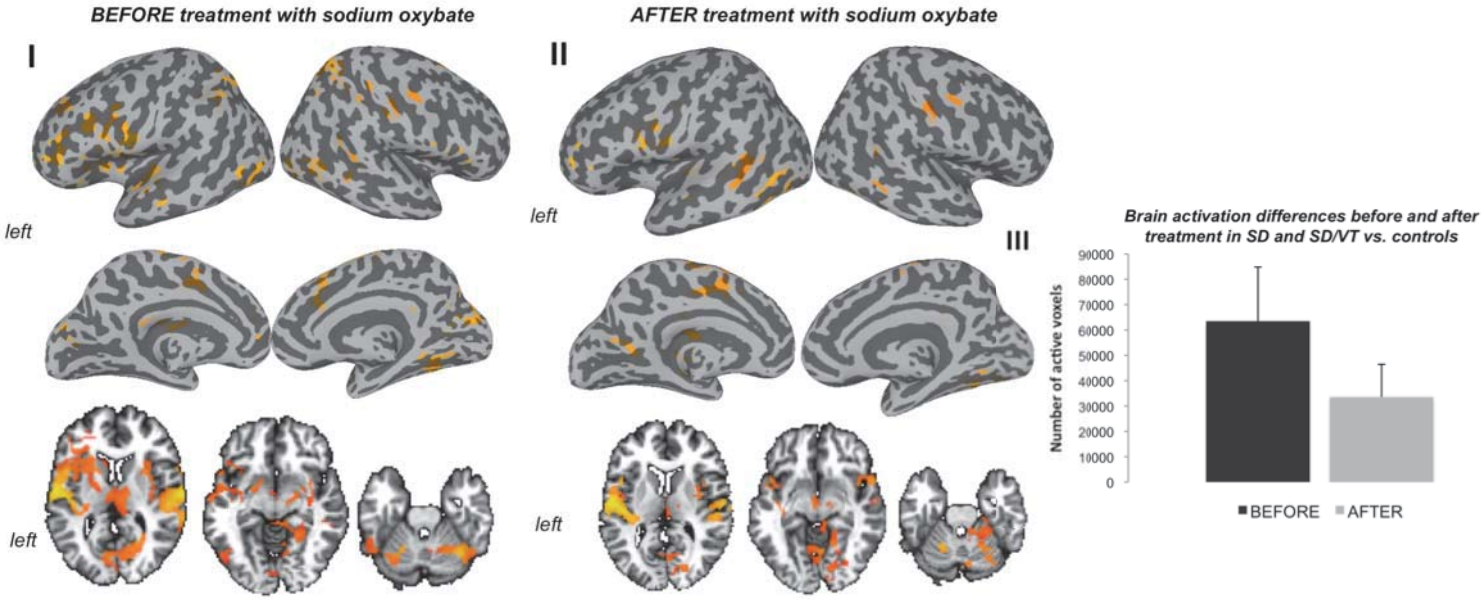

Figure 3 depicts differences in brain activation in SD and SD/VT compared to healthy controls before (**I**) and after (**II**) treatment with sodium oxybate (voxelwise $p \leq 0.05$, corrected). Panel (**III**) shows significantly decreased number of hyperactive brain voxels after the treatment compared to the before treatment baseline in patients ($p = 0.017$).

Collectively, our preliminary data indicate that sodium oxybate is effective in SD patients and especially in those who present with a VT component. Furthermore, our data suggest that the effects of sodium oxybate treatment are exerted through the modulation of overly increased brain activation.

2.3 Research Design

The following 2 groups of patients will participate in the proposed studies of Specific Aim #2: (1) SD patients, and (2) SD/VT patients. Because sodium oxybate has been shown to be effective in patients with movement disorders who self-report symptom improvement after alcohol intake, only ethanol-responsive patients will be recruited for the purposes of these studies. We estimate that 60% of ethanol-responsive patients per group, who participated in the studies in Specific Aim #1, will also participate in the studies in Specific Aim #2. Both ADSD and ABSD patients will be included and closely matched with ADSD/VT and ABSD/VT patients. Patients will be identified according to the screening and detailed examinations as described in Specific Aim #1. In this Specific Aim, we will (1) evaluate the central effects of sodium oxybate in SD and SD/VT patients to determine the relationship between improved clinical symptoms and modulated brain function, and (2) examine the extent of central effects from the combined use of sodium oxybate and botulinum toxin treatments as an alternative treatment schedule for patients with limited benefits.

2.3.1 Study #1: Mapping central effects of sodium oxybate treatment in patients with SD and SD/VT using fMRI during symptomatic speech production. Similar to the fMRI study under Specific Aim #1, we will use a sparse-

sampling event-related BOLD fMRI design during symptomatic voice and speech production on a 3.0 Tesla Philips MRI scanner. Each patient will participate in 2 fMRI sessions, before and after treatment with sodium oxybate. All recruited patients will have to be symptomatic; if they are treated with botulinum toxin injections, they may be included only if they are symptomatic at the end of their botulinum toxin treatment cycle. During each scanning session, the patient will be asked to perform short English sentences as well as repetitive and continuous vowel /i/, designed to elicit SD and VT symptoms. Four scanning sessions will be acquired with a total of 36 experimental trials and 24 baseline resting conditions. Structural MRI will be acquired for an anatomical reference.

Following the initial (baseline) fMRI session, patients will receive an individualized tolerable and efficacious single dose of sodium oxybate, given orally in the office. Patients' symptoms will be evaluated and videotaped one hour after receiving this dose with the immediately followed-up second fMRI session, during which the patients will be asked to produce the same tasks performed during the pre-treatment fMRI study. Patients' post-treatment scans will be compared to their pre-treatment scans to identify differences in brain activation abnormalities due to the effects of sodium oxybate treatment. Voice and speech production during both scanning sessions will be digitally recorded for acoustical analysis and correlations with the central effects of the treatment.

Data analysis. Using AFNI software, fMRI data analysis will include initial pre-processing followed by analysis of the task-related responses using multiple linear regression with a single regressor for each task convolved with a canonical hemodynamic response function, as described above in *1.3.1 Study* of Specific Aim #1. For group analysis, a two-way ANOVA will be conducted with subject as a random factor and task as fixed factor (corrected $p \leq 0.025$). Conjunction analysis at a cluster significance threshold of $p < 0.005$ will determine common and distinct regions of activation within the patient group during task production in the pre- and post-treatment fMRI sessions. Finally, pre- and post-treatment neuroimaging findings will be correlated with the behavioral data derived from acoustical analysis of voice and speech recordings.

Additionally, to determine the extent of sodium oxybate effects on speech network dynamics, we will re-examine functional and effective network connectivity using the same PPI and DCM procedures as described in the *Study 1.3.1*.

Study population and Sample size. To our knowledge the central effects of sodium oxybate has not been reported. Therefore, to estimate the number of patients to be included in this study, we reviewed our pilot fMRI study in SD and SD/VT patients. Compared to baseline, we found an average of 49% reduction of overly increased brain activation after treatment with sodium oxybate. Power analysis determined a sample size of 22 patients in each group to have 80% power at 0.025 to detect changes in brain activation when comparing before and after treatment conditions. We propose to recruit 25 ethanol-responsive SD and SD/VT subjects per group to account for the subjects' dropout possibilities and to increase statistical power.

2.3.2 Study #2: Evaluation of combined central effects of sodium oxybate and botulinum toxin treatments in patients with SD and SD/VT using fMRI during symptomatic speech production. In this study, we will use the same fMRI design as used in the Similar to *Studies 1.3.1* and *2.3.1* to examine the combined effects of sodium oxybate and botulinum toxin on brain activation changes due to the treatment.

We estimate that about 40% of all recruited patients (mostly with ADSD) will report some benefits from botulinum toxin injections. These patients will be enrolled in this study and will undergo the third scanning session after receiving their botulinum toxin treatment. They will be asked to take their individualized dose of sodium oxybate T.I.D. after the completion of the *Study 2.3.1* and until the end of this study. We predict that about 60% of SD (typically ABSD) and SD/VT patients recruited to the *Study 2.3.1* will report limited, if any, benefits from botulinum toxin treatment. These patients will, therefore, be replaced with newly recruited patients, whose symptoms do response to both alcohol intake and botulinum toxin treatment. Thus, at the time of this study, all patients will be treated with both sodium oxybate and botulinum toxin injections. The decision of whether sodium oxybate should be continued after the completion of all studies in this proposal will be made by the patient and his/her referring physician.

Because more than 50% of patients experience breathiness with mean duration of 10.3 days and 14% of patients develop dysphagia to liquids with mean duration of 2 days as most common side effects following botulinum toxin injections (Novakovic et al., 2011), we will acquire the fMRI data at least 14 days after the botulinum toxin treatment.

We will use the same *fMRI design* as in the *Studies 1.3.1 and 2.3.1. Data analysis* will follow the same pipeline as described in *Study 2.3.1*. The *power* for this study is the same as in *Study 2.3.1.*

2.4 Expected Outcomes. Upon the successful completion of the studies in Specific Aim #2, we expect to have determined the brain mechanisms of action of a novel pharmacological agent, sodium oxybate, as a potentially alternative treatment for symptom management in SD and SD/VT patients. Furthermore, we expect to identify the extent of central effects from the combined use of sodium oxybate and botulinum toxin. This treatment strategy may be useful in prolongation of the effects of botulinum toxin injections in those patients who receive limited benefits, mainly due to presence of the VT component. Collectively, we expect that outcome of the proposed studies will establish a strong scientific basis for the use of newly developed treatment strategies in SD and SD/VT patients.

2.5 Potential Problems and Alternative Strategies. Similar to the studies under Specific Aim #1, a potential problem may arise from an insufficient availability of patients who must be symptomatic and ethanol-responsive. However, such a problem seems unlikely for reasons described under subsection 1.5.

A different strategy for the evaluation of central effects of sodium oxybate might be the initial conduct of large-scale clinical trials using this drug in SD and VT and then exploration of its central mechanism of action in these patients. We, however, decided against this strategy because the establishment of a scientific basis for the use of sodium oxybate in SD and VT patients is of a more importance than the empirical test of yet another medication in these patients. Furthermore, the conduct of a large-scale clinical trial from the statistical point of view may be difficult as the recruitment of large numbers of eligible patients with these rare disorders may present a significant challenge.

Timetable

The studies under both Specific Aims will be conducted in *parallel* throughout the funding period, according to the timeline outlined below.

AIMS	Year 01	Year 02	Year 03	Year 04	Year 05
Specific Aim #1	◄	—	—	—	►
Study #1: Mapping disorder-specific brain activation in patients with SD vs. SD/VT using fMRI during symptomatic voice and speech production	◄	—	—	►	
Study #2: Mapping disorder-specific gray matter abnormalities in patients with SD vs. SD/VT using high-resolution MRI		◄	—	—	►
Study #3: Mapping disorder-specific white matter abnormalities in patients with SD vs. SD/VT using DTI		◄	—	—	►
Study #4: Relationship between brain abnormalities and clinico-behavioral characteristics in SD and SD/VT					
Specific Aim #2		◄	—	—	►
Study #1: Mapping central effects of sodium oxybate treatment in patients with SD and SD/VT using fMRI during symptomatic speech production		◄	—	—	►
Study #2: Evaluation of combined central effects of sodium oxybate and botulinum toxin treatments in patients with SD and SD/VT using fMRI using symptomatic speech production			◄	—	►

Summary and Future Directions

Studies in Specific Aims #1 and #2 have been designed to assess the distinct pathophysiology underlying SD and SD/VT development as well as to determine the central effects of a potentially promising novel pharmacological agent, sodium oxybate, as an alternative treatment option for these patients. The outcome of the proposed studies is expected to provide missing information about the brain functional and structural changes in SD and VT patients. This knowledge will be fundamental for our *long-term goal* of determining the pathophysiology of SD and VT necessary to develop the scientific framework for establishment of therapies for these disorders. Hence, having defined the pathophysiological interactions within the voice motor control system in SD and SD/VT in this proposal will provide a strong scientific basis for the series of experiments to identify the effects of pharmacological agents on modulation of damaged links in the pathophysiological brain networks in these patients. The information derived from these studies would be critical to the ultimate enhancement of clinical care of these patients. Furthermore, the results of the proposed studies will advance our knowledge of other primary dystonias and essential tremor.

PROTECTION OF HUMAN SUBJECTS

1. Risks to the subjects

a. Human Subjects Involvement, Characteristics, and Design. As described in the *Approach* section, we will recruit SD, SD/VT and healthy control subjects for participation in the proposed studies. Both ADSD and ABSD patients as well as ADSD/VT and ABSD/VT patients will be included and matched with each other in their age, gender and SD type (AD or AB). Clinically healthy subjects will be those who do not have present or past history of any neurological, psychiatric or laryngological problems. Controls will be carefully matched with SD and SD/VT populations by their age, gender, and ethnicity.

For all studies in Specific Aim #1, we propose to recruit the same 20 subjects per SD, SD/VT and control groups. Because the majority of VT patients report improvement of their voice symptoms after alcohol intake, we predict that the at least 60% (12 patients) of recruited SD/VT group will also be ethanol-responsive. To assure homogeneity between patient populations, we will carefully match SD and SD/VT patients according to their report of alcohol effects, in addition to their age, gender and an SD type match. A total of 60 subjects will undergo 2-hour scanning session for the studies in Specific Aim #1.

For all studies in Specific Aim #2, we will recruit 25 ethanol-responsive SD and SD/VT patients 2. We estimate that 12 ethanol-responsive patients per group, who participated in the studies in Specific Aim #1, will also participate in the studies in Specific Aim #2. Thus, this will require us to recruit additional 13 SD and 13 SD/VT patients for the proposed studies in Specific Aim #2. A total of newly recruited patients will be 26. The patients will participate in the fMRI studies of this Specific Aim according to the following schedule:

(1) Because the 12 patients/group, who already underwent neuroimaging studies in Specific Aim #1, will not need a pre-treatment baseline scanning session, they will only complete two 2-hour post-treatment scans with sodium oxybate and botulinum toxin + sodium oxybate, respectively;

(2) the remaining 13 patients/group will complete three 2-hour scanning sessions, which will include one pre-treatment baseline scan and two post-treatment scans with sodium oxybate and botulinum toxin + sodium oxybate, respectively.

A total number of subjects recruited for studies under both Specific Aims will be 86.

All recruited subjects will be outpatient. To establish their eligibility for participation, *all* subjects will be first screened through the administration of the intake screening questionnaire, which will contain general questions about their health. SD patients will additionally be questioned about their disorder (e.g., onset, site, treatment, effects of alcohol of voice symptoms) (see **Appendix 1**).

Diagnosis of SD will be established based on the three-tiered approach as follows:

(i) *the patients' self-screening questionnaire*, which will include questions regarding the amount of the effort required for speaking (e.g., Does it take a lot of work for you to talk?) and the presence of symptoms during different vocal activities (e.g., Can you do any of the following normally? Shout, Cry, Laugh, Whisper, Sing, Yawn) (**Appendix 2, Tier 1**);

(ii) *voice and speech recording and evaluation*, which will include the acoustic analysis of 20 adductor sentences with a high number of glottal stops and vowels and 20 abductor sentences with a high number of voiceless consonants (/p/, /t/, /k/, /s/, /h/, /f/) produced in normal speaking voice and in a whisper (**Appendix 2, Tier 2**);

(iii) *detailed laryngological examination*, which will include fiberoptic nasolaryngoscopy to observe the presence of vocal fold spasms during voice and speech production (**Appendix 2, Tier 3**);

(iv) *detailed neurological examination*, which will be performed to exclude other neurological conditions, especially other forms of dystonia and tremor (**Appendix 2, Tier 3**).

Diagnosis of VT in SD will be established based on the following additional criteria:

(i) Vocal tremor during vocalization that primarily involves laryngeal structures;

(ii) Exclusion of other laryngeal pathologies based on fiberoptic nasolaryngoscopic examination.

We have successfully used this approach of subject selection in our previous studies. Furthermore, a recent pilot study of 30 patients with SD, VT and muscle tension dysphonia found 97% accuracy in the classification of these disorders using the above-described three-tiered approach.

Eligible subjects will be male and female adults of diverse racial and ethnic backgrounds. However, because SD usually occurs between 40 and 55 years of age and predominantly in women and in Caucasians, a higher percentage of Caucasians and women are expected to be recruited. Because the onset of SD is in mid-life, children are not expected to participate. Vulnerable subjects, e.g., cognitively impaired, institutionalized individuals, prisoners, will not be eligible for these studies.

Eligibility for participation will be based on the following criteria.

Inclusion criteria:

1. SD and SD/VT patients will have clinically documented ADSD or ABSD with and without positives effects of alcohol on their symptoms;
2. Healthy controls will be healthy volunteers with a negative history of laryngeal, neurological, or psychiatric problems;
3. Age from 21 to 80 years.
4. Native English speakers.
5. Right-handedness (based on Edinburgh Handedness Inventory).

Exclusion criteria:

1. Subjects who are incapable of giving an informed consent.
2. Pregnant or breastfeeding women until a time when they are no longer pregnant or breastfeeding. All women of childbearing potential will have a pregnancy test performed, which must be negative for participation in the imaging studies.
3. Subjects with past or present medical history of (*a*) neurological problems, such as stroke, movement disorders (other than SD and VT in the patient groups), brain tumors, traumatic brain injury with loss of consciousness, ataxias, myopathies, myasthenia gravis, demyelinating diseases, alcoholism, drug dependence; (*b*) psychiatric problems, such as schizophrenia, major and/or bipolar depression, obsessive-compulsive disorder; (*c*) laryngeal problems, such as vocal fold paralysis, paresis, vocal fold nodules and polyps, carcinoma, chronic laryngitis.
4. Patients who are not symptomatic due to treatment with botulinum toxin injections into the laryngeal muscles. The duration of positive effects of botulinum toxin vary from patient to patient but lasts on average for 3-4 months. All patients will be evaluated to ensure that they are *fully symptomatic* prior to entering the study, except the Study 2.3.2, which will examine the effects of combined botulinum toxin and sodium oxybate treatments on abnormal brain function in SD and SD/VT patients.
5. To avoid the possibility of confounding effects of drugs acting upon the central nervous system, all study participants will be questioned about *any* prescribed or over-the-counter medications as part of their initial intake screening (see **Appendix 1**). Those patients who receive medication(s) affecting the central nervous system (except sodium oxybate as part of the studies under Specific Aim #2) will be excluded from the study.
6. The patients will be asked whether they have undergone any head and neck surgeries, particularly any brain surgery and laryngeal surgeries, such as thyroplasty, laryngeal denervation, and selective laryngeal adductor denervation-rennervation (see **Appendix 1**). Because both brain and laryngeal surgery may potentially lead to the brain structure and function re-organization, all patients with history of brain and/or laryngeal surgery will be excluded from the study.
7. Subjects who have tattoos, ferromagnetic objects in their bodies (e.g., implanted stimulators, surgical clips, prosthesis, artificial heart valve, etc.) that cannot be removed for the purpose of study participation.

Subjects accepted into the study will require up to four visits for patients and up to two visits for healthy controls, including one screening visit (intake questionnaire, self-screening questionnaire, voice and speech recording, nasolaryngoscopy, neurological exam) and one to three scanning session depending whether healthy control or patient. Time commitment for the screening visit may be up to 3 hours for patients and 1 hour for healthy volunteers; fMRI, MRI and DTI will be combined into the same scanning session of up to 2 hours. Scanning sessions will be scheduled according to the treatment schedule.

b. Sources of Material. Every effort will be made to respect and maintain the confidentiality of study participants according to the current legal requirements and as required by the Federal Privacy Act. The TBR Research Associate will be directly involved in the maintaining of subject databases, ensuring that strict standards of confidentiality are upheld at all times. Only the research team will have access to the research

data. Subject identifying information will be removed from neuroimaging data, nasolaryngoscopy, voice and speech recordings. A unique research code will be assigned to all data for confidentiality purposes. The list linking the codes to the subjects will be stored on the PI's password-secured computer. Only the PI will have an access to the list linking the code to the subject. The cross-reference will be available only to the investigators associated with this project.

Hard copies of the results of the physical exam, medical history, questionnaires and evaluations will be kept in the secured cabinets and password-protected computers located in the PI's office.

Neuroimaging data will be kept on the secured password-protected servers and image processing workstations in the PI's laboratory. Data will be backed up on the laboratory's protected storage servers.

All digitalized data from patients' nasolaryngoscopy, voice and speech recordings will be kept on the password-protected server accessible only by the members of the research team.

c. Potential Risks. Participation in these studies will entail minimal risk.

1. There may be some discomfort associated with *fiberoptic nasolaryngoscopy*. Prior to nasolaryngoscopy, the decongestant may be applied through the nares to widen the nasal passages. The subjects may experience mild discomfort in the nose or pharynx. The taste may be unpleasant. During the procedure, most people have a vague awareness that something is in their nose and brief feelings of nasal pressure or an urge to sneeze. When the nasolaryngoscope reaches the back of the nose, subjects may feel a slight pinch for a few seconds. Sometimes, the examination triggers a cough or gag, which stops when the nasolaryngoscope is repositioned. Discomfort is more common in people with a narrow nasal passages or a deviated septum. In these cases, a smaller nasolaryngoscope will be used. Problems that sometimes occur with nasolaryngoscopy may include coughing, gagging, vomiting, or a slight drop in pressure causing faintness. In these cases, the examination can be stopped, restarted, cancelled, or re-scheduled. Other problems that may occur but are rare include a nosebleed, choking, infection, spasm of the larynx, or an allergic reaction. Sudden or unexpected head movement not requested by the examiner during the nasolaryngoscopy can cause bleeding or other injury. After the examination, some people may have a mild sore throat for a few hours.

2. *fMRI, MRI* and *DTI* do not involve radiation and are safe when used on individuals who are appropriately screened for the procedure (FDA. Magnetic resonance diagnostic device: panel recommendation and report on petitions for MR reclassification: Food and Drug Administration; 1988. Report No.: 53; Shellock FG. Safety, Magnetic Resonance Imaging: Mosby; 1992). The FDA has classified these procedures as a class II risk.

The 3 Tesla scanner has identical safeguards as the 1.5 Tesla scanner. However, the gradients for the 3.0T scanner have higher strength and slew rate than the 1.5T clinical scanners. Gradient strength allows for higher resolution. The higher radio frequencies (RF) associated with the 3 Tesla scanner (125 KHz at 3 Tesla vs. 63 KHz at 1.5 Tesla) allow for deposition of larger amounts of energy into tissue during scanning. A higher maximum slew rate allows for faster imaging. To avoid tissue overheating, the operating system of the scanner has automated safeguards that keep the specific absorption rates (SAR) of RF energy well below the guidelines set by the Bureau of Radiological Health, FDA. The operating system limits RF deposition in the head to an average rate of 10 Watts, < 4 W/Kg, which has been shown to raise the average core temperature approximately 0.3° Celsius. These temperature changes are within the normal diurnal rhythms (±1°C) found in human core temperatures or a change in temperature associated with a brisk 20-minute walk (±1°C). No studies have documented any detrimental effect of gradient magnitude on human health.

Subjects are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips, metallic prostheses (including metal pins and rods, heart valves, cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metalworkers are also at risk for injury because of possible presence of small metal fragments in the eye, of which they may be unaware. Individuals with one or more of the MRI exclusion criteria will be excluded from the study. Each individual's health history will be evaluated relevant to these criteria.

Although there is no documented risk from MRI to pregnant women, this population will be excluded from MRI/fMRI/DTI studies. There are no known long-term risks or consequences of MRI scans.

3. There may be remote risk of *loss of data*. We will make every attempt to prevent the privacy and confidentiality risk by replacing personal identifying information with codes and by keeping encrypted digital data on the password-secured computer.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Patient volunteers will be recruited through the New York Voice and Swallowing Center (collaboration with Dr. Blitzer), Bendheim Parkinson and Movement Disorders Center and the Eugen Grabsheid, M.D., Voice Center at the Mount Sinai Hospital (the PI has appointments in both Centers), advertisements in the NSDA, Dystonia Medical Research Foundation (DMRF) newsletters and on their websites, advertisements in the local patient support groups, contacts with referring physicians and from the large pools of 1,500 patients who have previously participated in our studies or have been seen for treatment by our research team. A control group of research volunteers, including spouses and non-blood relatives of patients, will be recruited through the advertisement of the studies in the local newsletters and flyers posted in the Mount Sinai Hospital and New York Institute of Head & Neck. Participants will be recruited regardless of their race, sex, age, religion, or national origin.

During the first contact, the purposes, procedures, and risks of the protocol will be described in non-technical language. If the subject expresses an interest in study participation, then he/she will be interviewed to review background medical information, including voice, speech, language, swallowing, hearing, breathing, neurological, psychiatric diseases and disorders (see **Appendix 1**, Intake Screening Form). If eligible, an appointment will be scheduled for the informed consent, after which the self-rating questionnaire, voice and speech recording, laryngological and neurological evaluations (see **Appendix 2**) as well as the neuroimaging studies will be performed.

The study procedures will be arranged as follows:

- Initial screening to establish eligibility by Dr. Simonyan, TBD Research Associate, Ms. Pretorius
- Review of medical information by Drs. Simonyan, Blitzer or Frucht
- Informed Consent administered by Drs. Simonyan, Blitzer or Frucht
- Patient's self-rated questionnaire
- History, physical and neurological examination performed by Drs. Frucht and Taneja
- Laryngological examination performed by Drs. Blitzer and Sinclaire
- SD patients' voice and speech recording performed by Dr. Simonyan and TBD Speech-Language Pathologist
- Sodium oxybate administration by Dr. Frucht
- Botulinum toxin injections by Dr. Blitzer or by patient's primary laryngologist
- fMRI, high-resolution MRI and DTI, performed by Dr. Simonyan and her research team
- Neuroimaging data analysis and interpretation by the research team

There will be no scheduled follow-up hospital visits unless deemed necessary by Drs. Simonyan, Blitzer or Frucht. The subjects will be instructed to contact the PI with any questions they may have regarding the study.

b. Protection Against Risk

Steps to minimize risk during fiberoptic nasolaryngoscopy. Nasolaryngoscopy session will be ended immediately if subject presents with factors that could place him/her at unnecessary risk before, during or after the procedure. The potential sensations during nasolaryngoscopy will be always addressed in pre-procedure screening and consent. Information about allergic reaction to any medications will be obtained from the subject before the examination. If the subject would feel lightheaded, sweaty or weak during the examination, the examination will be stopped and the subject will be laid back with his/her feet raised until the feeling passes. The risk of infection will be minimized by using disposable tips on decongestant nasal spray bottles and by carefully cleaning and disinfecting the nasolaryngoscopes after each use.

Steps to minimize risk during fMRI/MRI/DTI. During the scanning, participants will be instructed not to cross their arms, hands, or legs as this could create a circuit, in which current flow is induced. An experimental session will be ended immediately if subject feels uncomfortable within the scanner or develops condition(s) that could place him/her at unnecessary risk before, during or after the study. Subjects will be screened for these conditions prior to the study, and if they have any of these conditions, they will not receive an MRI scan.

Although there is no documented risk from MRI to pregnant and breastfeeding women, these populations will be excluded from MRI/fMRI/DTI studies until a time when they are no longer pregnant or breastfeeding. All women of childbearing potential will have a pregnancy test performed, which must be negative for participation in the imaging studies.

Although SD and VT patients have difficulty speaking because of their voice breaks, they usually do not report problems associated with symptomatic task performance in the MRI environment. Based on our

extensive experience in imaging patients with SD and VT, no patient dropped from the study due to inability to perform vocal tasks or due to aggravation of SD symptoms over the extended period of scanning. Thus, performance of vocal tasks over the time period proposed in this application will be feasible in SD and SD/VT patients. In this application, the task-production fMRI study is designed to have resting periods between vocal tasks, which will help avoid exhaustion and boredom during scanning sessions.

3. Potential Benefits of the Proposed Research to Human Subjects and Others

There may be direct benefits to patients who participate in the study involving sodium oxybate treatment. Furthermore, through this research we will obtain critically missing knowledge about the relevance of brain changes to the development of VT in SD, which will advance our understanding of SD differential diagnosis and pathophysiological mechanisms. This knowledge will potentially lead to the establishment of new treatment opportunities in these patients. In some cases, the diagnostic information may be helpful for treatment suggestions or referrals made by Drs. Blitzer or Frucht.

4. Importance of the Knowledge to be Gained

The research studies in this application are expected to provide a detailed knowledge about the brain abnormalities in patients whose SD symptoms are combined with VT. Only by studying patients with different SD manifestations within the same study, we will be able to fully characterize the range of shared and distinct brain abnormalities in these patient populations and further correlate our findings with the results of their treatment effects. The knowledge gained from these studies is important because we will be able to identify neuroimaging biomarkers differentiating SD and SD/VT, which will be critical for improvement of accuracy of diagnosis of these related and often misdiagnosed disorders. Collectively, the expected results of the proposed studies will be critical for elucidation of pathophysiological mechanisms underlying SD and VT, which will serve as basis for the development of new criteria for detection, diagnosis and therapy of these patients. It is also anticipated that the potential novelty of the findings will facilitate understanding of the pathophysiological mechanisms of other forms of dystonia and tremor.

INCLUSION OF WOMEN AND MINORITIES

SD is reported to occur predominantly in women (up to 79%) and in Caucasians. Therefore, a higher percentage of Caucasian women are expected to participate in the proposed studies.

Some minority groups (Asians and American Indians) are poorly represented because limited information is available on the frequency of SD in these groups. For that reason, these minority groups may not be common in the patient populations involved in these studies. Every effort will be made to recruit minorities through the advertisements at the National Spasmodic Dysphonia Association and through the otolaryngologists serving areas of large minority populations.

INCLUSION OF CHILDREN

SD is a disorder of adult onset, usually first occurring after 40 years of age. Therefore, children will not be included in this study.

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RESOURCE SHARING PLAN

We will make neuroimaging data available to the scientific and clinical communities through publications and scientific meeting presentations. Raw neuroimaging data will also be available upon request to other researchers in the field of movement disorders on a collaborative basis.