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TITLE: Activation of Central Pattern Generator for Respiration Following Complete High Cervical Spinal Cord Interruption

PRINCIPAL INVESTIGATOR: Vitaliy Marchenko,MD,PhD

CONTRACTING ORGANIZATION

DREXEL UNIVERSITY
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The original hypothesis was that an important role of intraspinal inhibitory circuits (GABA- and Glycine-ergic) in control of spinal central pattern generator (CPG) for breathing According our SOW spinal respiratory neurons (cervical C3-C5 and C1-C2 levels) were characterized by their neurotransmitter profile (GABAa- and Glycine-ergic). Epidural stimulation applied at frequencies 100-300 Hz to the area of phrenic nucleus location (C4 cervical segment) in spinal (C1-transected) rats induced time-frequency depended facilitation of phrenic nerve activity and was able to maintain life-supporting paced breathing for at least 30 min. After injection of blockers GABAa and glycine receptors (GABAzine and strychnine) phrenic nerve facilitation was drastically increased. For the first time our experiments demonstrate the high effectiveness of combining pharmacological and electrical (epidural stimulation) modulation of spinal circuits at the level of phrenic nucleus in complete spinal cord injury animal model. These results can be used to develop a new therapeutic strategy to help paraplegic patient to wean from artificial ventilation. These newest findings are being prepared for publication in peer-review scientific journals and presentation at the upcoming annual meeting for the Society for Neuroscience.

15. SUBJECT TERMS
Spinal cord injury, epidural stimulation, respiration, phrenic nerve, GABA, Glycine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

The overall objective of this new research program is to develop strategies to promote restoration of voluntary breathing in people with cervical spinal cord injury. The focus of the present proposal will be to: 1) investigate spinal inhibitory circuits controlling phrenic motoneurons and thus diaphragm (the primary inspiratory muscle) activity before and after acute high cervical spinal cord transection, and 2) optimize combined drug delivery (GABAa / Glycine receptors blockers) and epidural stimulation to improve treatment efficacy of respiratory disorders in patients with spinal cord injury (including weaning from artificial ventilation in tetraplegic).

2. KEYWORDS:

Spinal cord, spinal cord injury, C1 spinal cord transection, respiration, phrenic nerve, motoneurons, interneurons, GABA, Glycine, Strychnine, GABAzine, epidural stimulation

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

As outlined in the SOW the present research addresses the following two aims:
Aim1: Identify the location and neurotransmitter profile of spinal inhibitory interneurons related to respiratory motor control and initiating of spinal CPG follow C1Tx (1-14 months).
Aim2: Identify the optimal combination of intrathecally delivered blockers of fast inhibition (Glycine- and GABA-ergic) and epidural electric stimulation for improving respiratory recovery follow C1Tx (15-36 months).
For the 2nd year of project **Major Task 1.2 (13-14 months)** and **2.1. (15-24 months)** were planned:
Major Task 1.2. Characterize the location and neurotransmitter profile (GABA- and Glycine-ergic) of transsynaptically labelled spinal interneurons connected to phrenic motoneurons.
Major Task 2.1. Identify optimal parameters of epidural stimulation of C1-C6 (C2,C4) ventrolateral aspects with additional bilateral microinjections into phrenic nucleus (C3-C6) and upper cervical respiratory group (C1-C2) of GABAzine, Strychnine, GABAzine+Strychnine in decerebrate artificially ventilated, paralyzed and C1-transected animals.

What was accomplished under these goals?

Major task 1.2 (13-14 months):

Neuroanatomical tracing methods were employed using mono- and transynaptic tracers (cholera toxin-B and pseudorabies virus (PRV), respectively), to label spinal phrenic motoneurons and the pre-phrenic interneurons. Immunocytochemical methods were used to identify their neurotransmitter profile (GABA- or Glycine-ergic).

This task was completed. The anatomical distribution of spinal GABA- and glycinergic pre-phrenic interneurons at C3-C5 level was defined in details highlighting more dominant role of glycinergic pre-phrenic interneurons.

1) Topographical analyse of the representative populations of transsynaptically labeled interneurones (Fig. 1C: PRV-614, red color) reveals the most dense concentration of glycenergic inteneurons in Rexed area VII and X (Fig.1-B: green crosses and Fig. 1-C-D: green color) and more diffuse distrubution of GABA-ergic neurons (Fig. 1-B: blue asterisks and Fig. 1-F-D: blue color) at C3-C5 cervical level.

2) Glycinergic interneurones were also found among phrenic motoneurones (Fig.1-B – green crosses, and Fig. 1-D; green color), circled with red line (Fig. 1-B). We hypothesize that these cells serve as classic Renshaw cells in recurrent inhibitory circuits.

3) The number of labeled glycinergic neurons was in ~3 folds higher than GABA-ergic that confirm well-known statement about dominant role o glycinergic inhibition in spinal circuits (Aprison & Werman, 1965) (Fig. 1B).

4) We also identified interneurones containing both GABA and glycine (green arrows on merged panels 1-D and 1-F, Fig.1). Co-existance of GABA and glycine has already been demonstrated in the brainstem, but remains underinvestigated in the spinal cord (Todd et al., 1996).

Major Task 2.1: *Identify optimal parameters of epidural stimulation of C_1-C_6 (C_2,C_4) ventrolateral aspects with additional bilateral microinjections into phrenic nucleus (C_3-C_6) and upper cervical respiratory group (C_1-C_2) of GABAzine, Strychnine, GABAzine+Strychnine in decerebrate artificially ventilated, paralyzed and C1-transected (C1-Tx) animals.*

Phrenic nerve activity from both sides in paralyzed animals and tracheal pressure with end-tidal CO2 level in non-paralyzed rats were recorded and their responses to epidural stimulation were assessed. Epidural stimulation (EDS) was centered over the ventrolateral spinal cord at the C2 or C4 spinal segments. In addition, EDS was combined with local application of GABAzine and Strychnine (over C1-C2 and C3-C5 cervical segments).

This task was completed. The major finding in these experiments was that:

1) C4 cervical segment (the center of Phrenic Nucleus location) was the most sensitive to a single threshold pulse of EDS affecting tracheal pressure (TP) and end tidal CO2 level (Fig. 2A). The stimulation of C1-C2 segments resulted in 3 fold increasing of threshold current with simultaneous activation of neck muscles. Based on these results the biphasic EDS of C4 segment (C4-EDS) with pulse length of 0.2 ms was chosen as an optimal paradigm for central pacing of diaphragm innervated by phrenic nucleus. This pulse length also was used as an optimal for EDS of upper thoracic segments in rats by DiMArco et al. (2013).

2) The minimum current intensity (one threshold, 1T) of a single EDS pulse to elicit phrenic response in paralyzed animals was 77.96±11.59 µA (Fig. 2B-1T), which is very close to the threshold current (81.6±35.9 µA) affecting TP and end tidal CO2 level in non-paralyzed animals (Fig. 2A). Stimulus intensity of 3 thresholds (3T) elicited two components in phrenic nerve response – early N1 (direct) response with latency 1.08 ±0.02 ms and delayed N2 (synaptic) response (10.14±1.01 % of N1 amplitude) with latency 2.54 ±0.05 ms (Fig. 2B-3T).

3) EDS with frequencies of 200-300 Hz (0.2 ms of pulse length during 0.33 s every sec) successfully paced the stable breathing at 3 thresholds (3T) (220-240 µA) of current strength for more than 30 min in 11 (200 Hz) or 12 of 13 (300 Hz) trials in non-paralyzed C1-Tx animals (Fig.3). The current intensity of 1.5T- (~100-120 µA) resulted in non-effective support of paced breathing with progressive decreasing in tidal volume and unstable end-tidal CO2 in almost all rats (11 of 13).
4) C4-EDS applied at frequencies 100-300 Hz in paralyzed C1-Tx animals induced time-frequency depended facilitation of N2 synaptic phrenic nerve component (Fig.4). N1 and N2 components were changed in a reciprocal way (Fig. 4 - blue traces). While N1 was depressed by increasing stimulation frequency, the N2 component demonstrated a significant increase in amplitude starting from the 2nd half of the 100 Hz train. The significant depression of the N1 component was observed in the 2nd half of the 200 ($p=0.0095$) and 300 Hz ($p=0.003$) trains. The N2 component increased by 5.77 fold - from 10.14±1.01 (N2-control) to 58.49±5.98 % during the 2nd half of the 300 Hz train ($p=0.00003$). It was also observed that total phrenic nerve output (N1+N2) significantly changed by 132% on average ($p=0.01$) during the 300 Hz train.
5) Injection of GABAzine (GABAz, GABAa inhibitory receptor selective blocker, 25 µMol) and strychnine (STR, glycine inhibitory receptor selective blocker, 25 µMol) at subthreshold doses for initiation of spinal central pattern generator into the phrenic nucleus at the C3-C5 segment (50 nl in each segment) significantly decreased threshold (from 87.1±20.1 to 60.6±14.9 µA, $p=0.037$) affecting TP and phrenic nerve responses (from 81.6±35.9 to 53.5± 21.4 µA, $p=0.043$) to single C4-EDS pulse. The current intensity of 3T elicited significantly higher N1 (+72.3%, $p=0.026$) and N2 (+86.7%, $p=0.015$) phrenic components (Fig. 1B - red phrenic response trace). Stable paced breathing after GABAz&STR injection was observed in all (13/13 animals) even at 100 Hz of EDS. Dynamic changes of phrenic nerve component revealed the same tendency of time- and frequency-depended reciprocal changes in amplitude of N1 and N2 components (Fig. 4 – red traces) described for control experiments. However, the N1-component was initially increased during 1st (+21.8%, $p=0.042$) and 2nd (+17.4%, $p=0.048$) half of 100 Hz EDS train. Synaptic N2 component reached the maximum amplitude at 200 Hz of C4-EDS (9.44 times higher of control, $p=0.000063$). The direct N1-component was maximally suppressed at 300 Hz (-33.9%, $p=0.00052$) but was still higher of control level amplitude before GABAz&STR application (+34.3%, $p=0.0017$).
6) W also detected significant increase in mean blood pressure (BP) from 67.84±7.31 mm Hg before to 82.57±9.04 mm Hg ($p=0.028$) during 300 Hz of C4-EDS. This increase of blood pressure may play an important role in gas compensation during EDS.

Therefore, for the first time our experiments demonstrate the high effectiveness of EDS in combination with partial blockade of fast inhibitory circuits at the level of phrenic nucleus in complete spinal cord injury animal model.

Fig.1: Cumulative distribution of labeled interneurons at the level of Phrenic Nucleus (C4 cervical segment)

[data from 4 triple-labeling experiments]

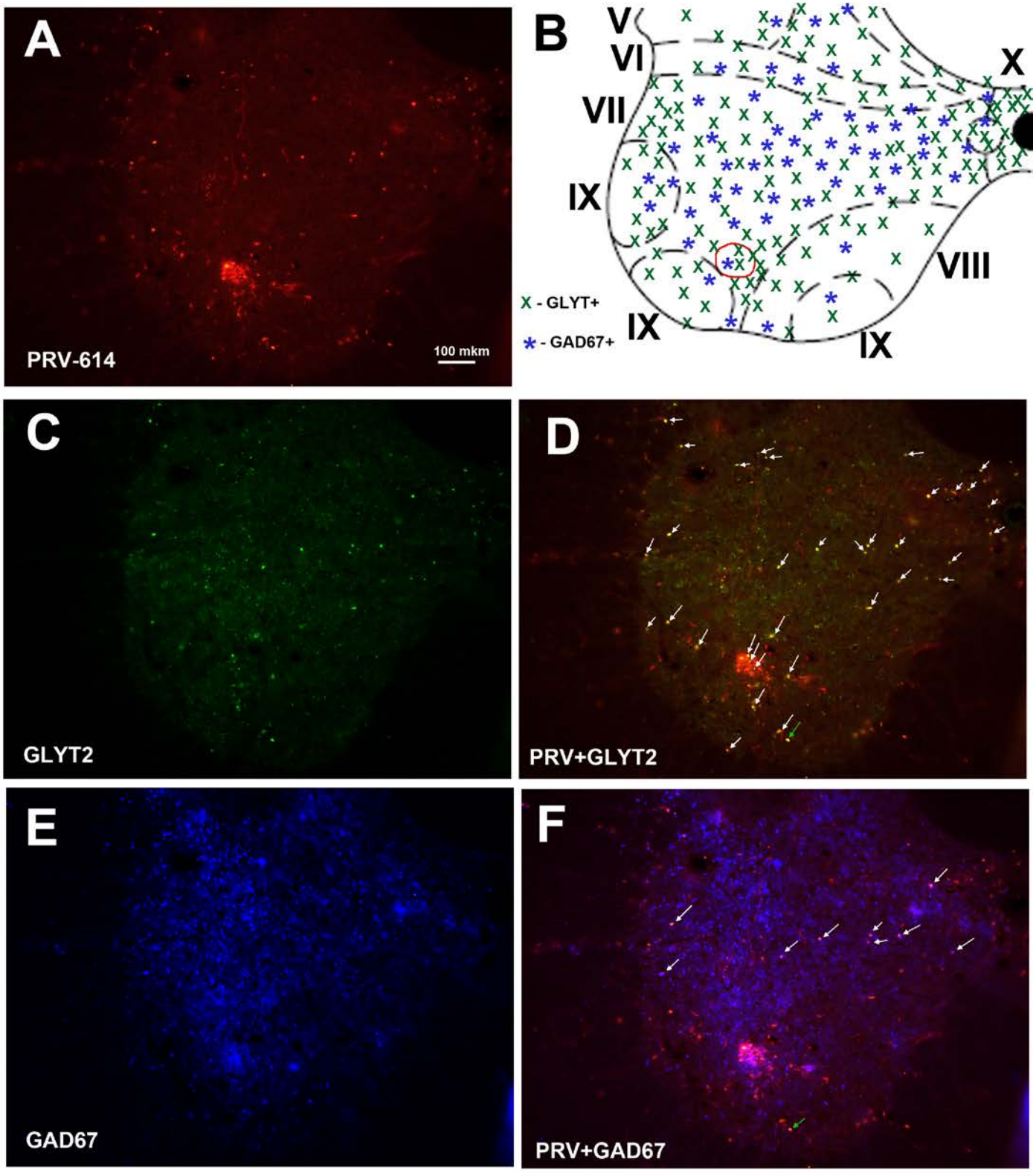


Fig.2: Treshold Responses of Tracheal Pressure and Phrenic Nerve to a single C4-EDS biphasic pulse (0.2 ms)

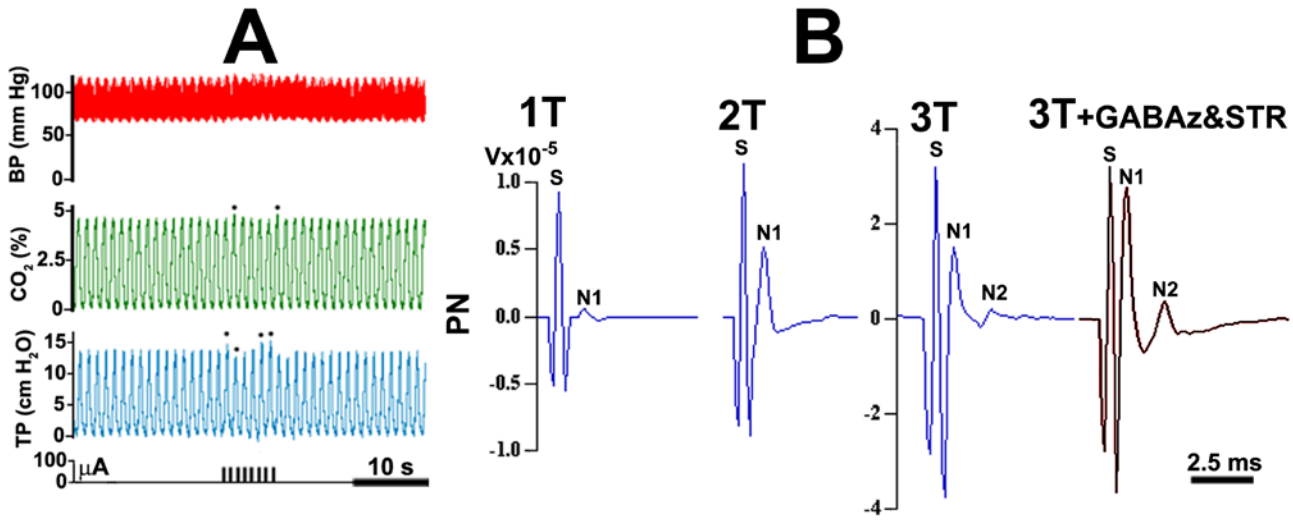


Fig.3: Succesful C4-EDS pacing of breathing

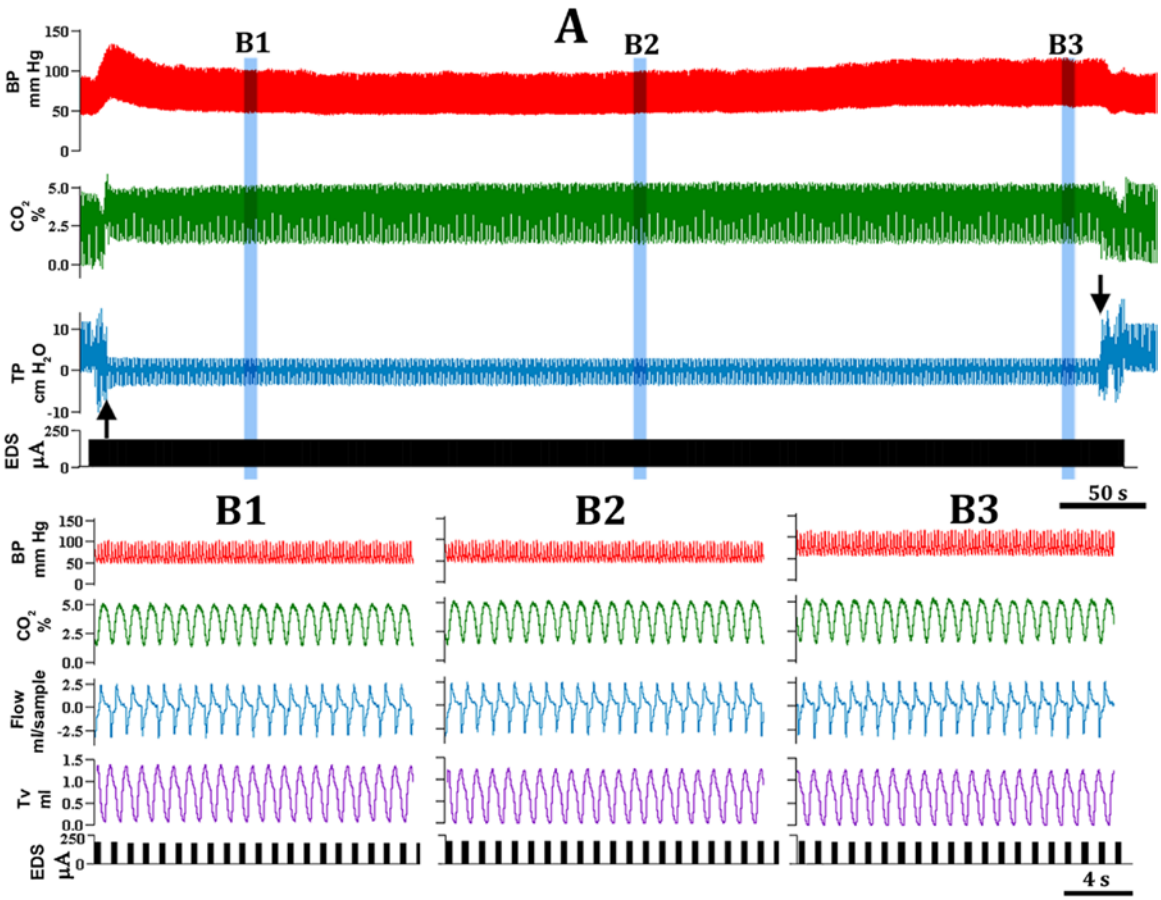
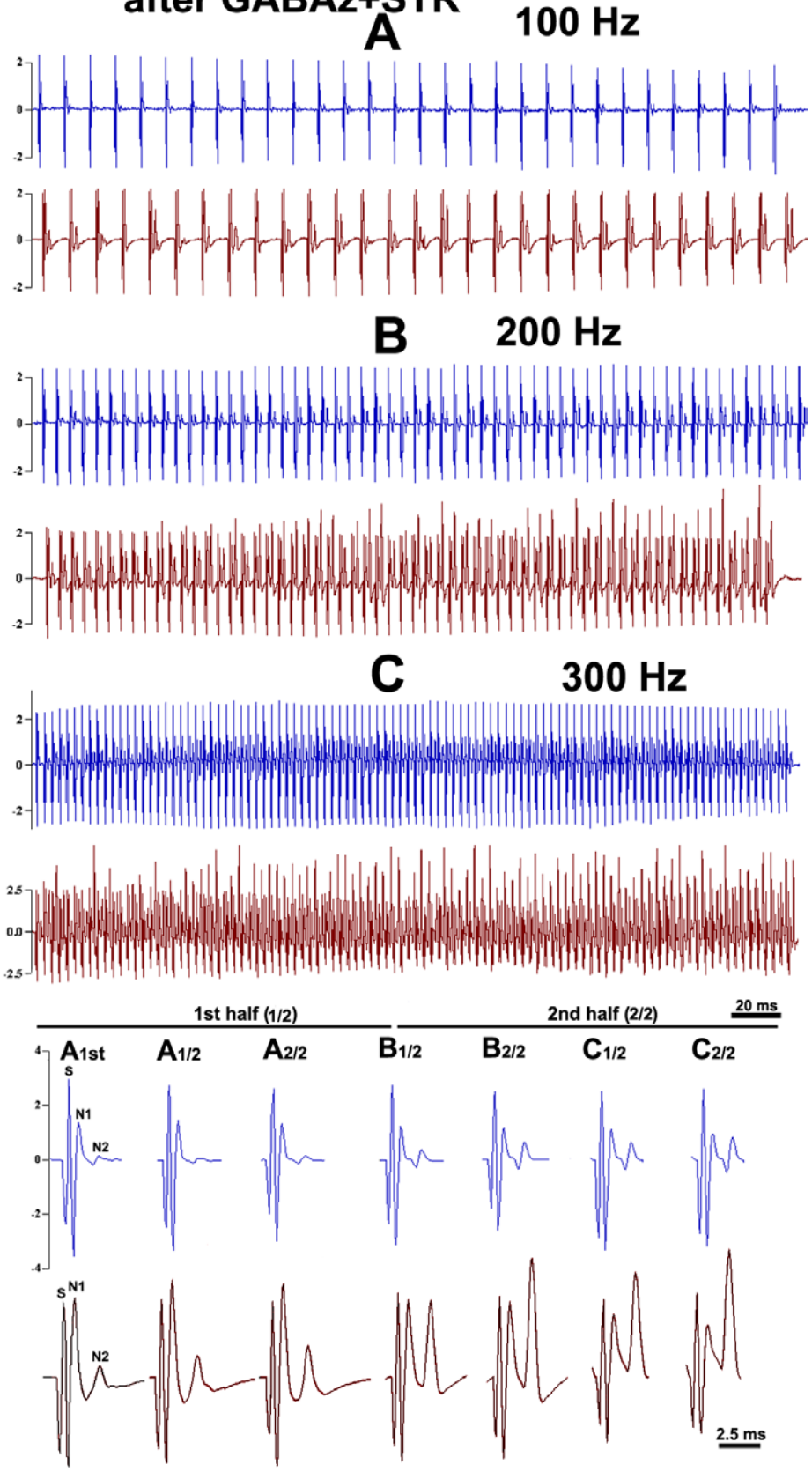


Fig.4: Phrenic nerve responses to C4-EDS before (blue traces) and after GABAz+STR



What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

According to the SOW we will find an optimal combination of intrathecal drug delivery (GABAzine and Strychnine) and epidural stimulation in high unanesthetized cervical transected animals.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal

disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

In the majority of cases people with tetraplegia are not able to breathe voluntarily, thus artificial ventilation is required. Our project is devoted to the development of treatments that harness the neuroplastic potential of spinal circuits (interneurons and motoneurons) that are capable of generating rhythmic breathing-like activity in respiratory muscles in an animal model of complete high cervical spinal cord injury. To date the role of spinal respiratory interneurons in respiratory motor pattern formation is almost ignored. The present work addresses this gap in research, bringing this clinically relevant concept to the forefront of this field of SCI research by proving the importance of intraspinal circuits in respiratory motor pattern formation.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Hormigo KM, Zholudeva LV, Spruance VM, **Marchenko V**, Cote MP, Vinit S, Giszter S, Bezdudnaya T, Lane MA. Enhancing neural activity to drive respiratory plasticity following cervical spinal cord injuryExp Neurol. 2017 Jan; 287(Pt 2):276-287. *Acknowledgement of federal support: yes.*

2. Bezdudnaya T, Lane M.A., Marchenko V.*
Paced breathing and phrenic nerve responses evoked by epidural stimulation following complete high cervical spinal cord injury.
Paper was submitted to J Applied Physiology (JAPPL-00895-2017). acknowledgement of federal support: yes.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

***V. MARCHENKO**, T. BEZDUDNAYA, M. A. LANE.
Epidural stimulation and pharmacological blockade of fast inhibition improve respiratory pacing following complete spinal cord injuryAbstract was submitted for SFN-2017 Symposia. Control/Tracking Number: 2016-S-13226-SfN. *acknowledgement of federal support: yes.*

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

- **Technologies or techniques**

  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name: Vitaliy Marchenko – no changes
Project Role: PI

Name: Michael Lane – no changes
Project Role: collaborator

Name: Tatiana Bezdudnaya
Project Role: Research-Instructor
Nearest person month worked: 1.05 (35% or 4.2 months per year), $ 1,750 monthly salary
Researcher Identifier: n/a
Contribution to Project: Dr. Bezdudnaya, PhD, works at our department as instructor and has a many year strong experiences in electrophysiology and programming. She was hired on part time (35% of salary support) from Sept 1st/2015 and performed work in electrophysiological experiments and data analyses.

Name: Kristiina Hormigo (Negron before marriage)
Project Role: Research Assistant II
Nearest person month worked: 50% (or 6 months per year), $ 18,750 yearly salary
Researcher Identifier: n/a
Contribution to Project: Dr. Negron, MS, works at our department as a Research Assistant II and has a strong experience in immunohistochemistry and maintaining the lab. She was hired on part time (50% of salary support) from May 1st, 2016 and has performed work in histological experiments and data analyses.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending

grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

1. Quad Chart.
2. Ghali Michael George Zaki and Marchenko Vitaliy*. Patterns of Phrenic Nerve Discharge after Complete High Cervical Spinal Cord Injury in the Decerebrate Rat. Journal of Neurotrauma. June 2016, 33(12): 1115-1127.
3. Hormigo KM, Zholudeva LV, Spruance VM, Marchenko V, Cote MP, Vinit S, Giszter S, Bezdudnaya T, Lane MA. Enhancing neural activity to drive respiratory plasticity following cervical spinal cord injury. Exp Neurol. 2016 S0014-4886(16)30267-9 – InPress

4. Bezdudnaya T., Lane M. A., Marchenko V.* Distribution of respiratory-related neurons in C3-C5 cervical segments and their responses to blockade of GABAa and Glycine receptors in decerebrate rats. Abstract: SFN-2016 Symposia. Control/Tracking Number: 2016-S-13226-SfN.

Activation of Central Pattern Generator for Respiration Following Complete High Cervical Spinal Cord Interruption

SC140038

W81XWH-14-1SCIRP-IIRA

PI: Vitaliy Marchenko **Org:** Drexel University **Award Amount:** $500,000 (direct)/ $ 775,235 (direct&indirect)

Study/Product Aim(s) (according to SOW)

- Local IACUC and HRPO/ACURO protocol approving (Sept-Oct/2015)

•**Aim 1.** Identify the location and neurotransmitter profile of spinal inhibitory interneurons related to respiratory motor control and initiation of spinal CPG follow a complete C1 spinal transection. **Major Task 1.2** (month 13-14). Characterize the location and neurotransmitter profile (GABA- and Glycine-ergic) of labelled spinal interneurons connected to phrenic motoneurons.

•**Aim 2.** Identify the optimal combination of intrathecally delivered blockers of fast inhibition (Glycine- and GABA-ergic) and epidural stimulation for improving respiratory recovery follow C1Tx.

•**Major Task 2.1.** (month 15-24). Identify optimal parameters of epidural stimulation with additional bilateral microinjections into phrenic nucleus and upper cervical respiratory group of GABAzine and Strychnine in C1-transected animals.

Fifty two (52) experiments were completed for 2nd year. According to approved SOW the Majors task 1.2 and 2.1 is completed by 100% .

Timeline and Cost

Activities	CY 15	16	17	18
Aim 1 Major Task 1.1Subtask 1.1.1				
Aim 1 Major Tasks 1.1 and 1.2				
Aim 2 Major Task 2.1				
Aim 2 Major Tasks 2.2 and 2.3				
Estimated Budget ($K)	**$89**	**$266**	**$266**	**$177**

Updated: 09/30/2017

Goals/Milestones

CY15 Goal – Major Task 1.1 - Identify spinal interneurons related to respiratory motor control and spinal CPG follow C_1-transection

☑ Electrophysiology and immunohistochemistry of spinal neurons

CY16 Goals – Major Tasks 1.1-1.2 (Transsynaptic labeling and immunohistochemistry of spinal respiratory-related neurons)

☑ Electrophysiology and immunohistochemistry of spinal neurons

CY17 Goal – Major Task 2.1: Identify optimal parameters of spinal cord epidural stimulation and intraspinal GABAa/Glycine antagonist injection in paralyzed and C1-transected animals

☐ Electrophysiology and immunohistochemistry of spinal neurons

CY18 Goal – Major Tasks 2.2-2.3: Identify optimal parameters of spinal cord epidural stimulation and intrathecal GABAa/Glycine antagonist injection in paralyzed and non-paralyzed C1-transected animals

☐ **Comments/Challenges/Issues/Concerns**

Budget Expenditure to Date

Projected Expenditure:$ 264,905 (Sept,1/2016–Aug/017) direct

Actual Expenditure: $ $243,417. (Sept,1/2016–Aug/2017) direct

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Control/Tracking Number: 2017-S-14454-SfN
Activity: Scientific Abstract
Current Date/Time: 5/7/2017 10:19:59 PM

Epidural stimulation and pharmacological blockade of fast inhibition improve respiratory pacing following complete spinal cord injury

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Abstract:
The use of epidural stimulation (ES) in patients with spinal cord injury (SCI) has gained increased media attention in the past decade with significant success demonstrated in human studies, including improvement of lower limb motor function in paraplegic patients. DiMarco, Kowalski and colleagues were first to investigate ES applied to T1-T2 ventral surfaces to restore respiratory function following high cervical spinal cord transection. In the current study we combine ES (at C3-C5 segments corresponding to area of phrenic motor pool) and pharmacological strategies (blocking of GABAa and Glycine inhibitory receptors) for more specific activation of spinal interneurons and motoneurons involved in the shaping of respiratory motor output following C1 transection (C1Tx). All experiments were performed in decerebrate, unanesthetized adult Sprague-Dawley rats, 5-6 h post C1Tx. ES was applied to ventrolateral surface of C3-C5 cervical segments bilaterally via teflon-coated silver (0.01'' bare and 0.013'' coated, AM-Systems) stimulating electrodes (0.2 ms biphasic stimulation, 100-200 Hz during 0.3 s, one train per sec). Prior to pacing procedure, the minimal thresholds (Tr) of current (87.4±10.3 for C3, 73.6±8.2 for C4 and 84.7±8.2 mkA for C5 segments) affecting tracheal flow and end-tidal CO2 level were detected. ES applied to the C4 level (~ 5 Tr, 350.7±41.2 mkA) produced non-fatigue contraction of chest and diaphragm muscles with stable tracheal flow (2.3±0.21 ml of tidal volume) and end-tidal CO2 (4.5±0.3 %) during 1 h. In contrast, ES applied to C3 or C5 segments required much higher current (~ 7 Tr, 633.8±84.5 mkA) with development of muscle fatigue in 5 out of 8 rats. Ten minutes after intrathecal administration of 30 mkl (25 mkMol) of GABAzine and strychnine (blockers of GABAa and glycine inhibitory receptors, respectively) the minimal thresholds were significantly decreased for all segments (62±7.9 for C3, 41.1±6.4 for C4 and 67.9±7.3 mkA for C5).