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TITLE: Screening Therapeutic Agents Targeting Neuromuscular Junctions in ALS

PRINCIPAL INVESTIGATOR: Dr. Sandrine Da Cruz

CONTRACTING ORGANIZATION: LUDWIG INSTITUTE FOR CANCER RESEARCH, LTD.
La Jolla, CA

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

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8. PERFORMING ORGANIZATION REPORT

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14. ABSTRACT

One of the earliest events in ALS is the loss of the connections between motor neurons and muscles, which are critical for our most basic motor functions such as breathing. The disruption of this connection leads to the destruction of sophisticated structures called neuromuscular junctions (NMJs), the contacts that transfer the motor neuron commands onto the muscles. Importantly, the preservation of the motor neurons is not sufficient to prevent the loss of the NMJs, thus pointing to NMJs as potential sites of toxicity. Therefore, targeting NMJs represents an unexplored therapeutic strategy to treat ALS.

This project represents a synergistic collaboration between two academic investigators proposing an innovative strategy which combines structural and functional analyses of NMJs in multiple mouse and human *in vitro* and *in vivo* models for muscle denervation induced by ALS-causing mutations (SOD1, TDP-43 and C9orf72). We proposed to identify small molecules that prevent the disconnection or stimulate (re)connection between motor neurons and muscles in ALS. We have assembled a comprehensive set of approaches that will allow us to screen a large number of drugs to identify those that can help maintain NMJs structure and functions. The power of our approach is to then use models that recapitulate the disease at NMJs to more stringently select the most efficient drugs. These models include genetic mouse and human models, including ones from sporadic forms of ALS. This approach, if successful, may identify one or more drugs with the potential to be repurposed for the treatment of ALS. The system can also be used more broadly for identifying completely new drugs that can encourage maintenance of nerve attachment to muscle or stimulate its reattachment.

Recognizing that NMJ loss is an early hallmark of disease and that efforts targeting the initial step - muscle denervation are scarce, our multi-pronged approach has the potential to uncover new insights into the unknown mechanisms underlying toxicity at the NMJs and establish the preclinical proof of concept for targeting NMJs as a therapy for ALS patients. Thus, our findings are likely to be widely applicable to most ALS forms including sporadic cases and have the potential to be directly translated into effective therapeutics that may delay muscle denervation and improve patient's autonomy and quality of life.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	17	

Standard Form 298 (Rev. 8-98)

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by initial muscle denervation and subsequent loss of motor neurons, leading to paralysis and death within 2-5 years after diagnosis. As no cure exists, this disease is in desperate need of effective therapeutics. A major hallmark of ALS is the ***denervation of neuromuscular junctions (NMJs)***, which is one of the ***earliest events in ALS*** patients and murine models. However, investigative therapeutic approaches aiming at improving NMJ and muscle functions are scarce. Thus, it is critical to develop therapeutic strategies ***targeting NMJ dysfunction*** and denervation in ALS. The Da Cruz and Robitaille teams proposed to utilize an ***innovative multi-pronged screening approach*** that combines *in vitro, ex vivo* and *in vivo* ***multiple genetic*** (mutant SOD1, TDP-43 and C9orf72) ***mouse and human models recapitulating functional NMJs*** as integrated tools to ***identify and validate potential therapeutic compounds targeting muscle innervation***. Ultimately, this multi-pronged collaborative effort may allow elucidating some of the yet unknown molecular targets contributing to NMJ (re)innervation. Furthermore, it may durably impact ALS by identify new sets of therapeutics for ALS, linked to combating a key degenerative mechanism underlying familial and sporadic ALS pathogenesis.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Amyotrophic lateral sclerosis (ALS)
Neuromuscular disorders
Neuromuscular junctions (NMJs)
Denervation
Reinnervation
Screening
Non-cell autonomy

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Screening of small molecules to identify potential therapeutics that stimulate innervation/re-innervation or prevent/delay denervation induced by ALS-mutants

- **Major Task 1:** Screen of 404 drugs/compounds (FDA-approved or related to NMJ biology)
  - Projected completion date: Month 1-10
  - Percentage of completion: 40-50%: subtask 1 completed, subtask 2 initiated (10%), subtask 3 not started

- **Major Task 2:** Initiate of screen of a >1,200 small molecule library (LOPAC)
  - Projected completion date: Month 24
  - Percentage of completion: 0.5%
- **Major Task 3:** Exchange of ALS mouse models between the two labs and maintenance of the new line
  - Projected completion date: Month 12
  - Percentage of completion: 0%
- **Major Task 4:** Generate mouse experimental cohort ($SOD1^{G37R}$) to accomplish Aim 2A and early phase of Aim 3
  - Projected completion date: Month 12
  - Percentage of completion: 75%

Milestone #1 (combining Task #1 and #2): Selection of candidate compounds for secondary assays (Month 6-8)- percentage completed: 20%

Specific Aim 2: Validate functional potency and efficacy of lead compounds

- **Major Task 5:** Validate the candidate compounds using ex vivo nerve/glia/muscle preparations
  - Projected completion date: Month 21
  - Percentage of completion: 0%
- **Major Task 6:** Validate the candidate compounds using co-cultures of human motor neurons (from iPSCs) and differentiated C2C12 cells
  - Projected completion date: Month 22
  - Percentage of completion: 0%
- **Major Task 7:** Generate mouse experimental cohorts ($SOD1^{G37R}$ and $TDP\text{-}43^{Q331K}$) to accomplish late stages of Aim 3
  - Projected completion date: Month 12-24
  - Percentage of completion: 10% for $TDP\text{-}43^{Q331K}$ and 30% for $SOD1^{G37R}$

Milestone #2: identify/ranking of lead compound(s) from ex vivo validation (Month 8-12): percentage completed: 20%

Milestone #3: Ranking/prioritization of lead compound for in vivo pre-clinical trials (Aim 3) (Month 10-12): percentage completed: 0%

Specific Aim 3: Execute pre-clinical trials of lead compounds in ALS mouse models

- **Major Task 8:** Test compound(s) in early symptomatic $SOD1^{G37R}$ and $TDP\text{-}43^{Q331K}$ mice: pre-clinical assay
  - Projected completion date: Month 24
  - Percentage of completion: 0%

Milestone #4: Identification of a potential therapeutic agent improving motor function in ALS mice (Month 22-24): percentage completed: 0%

Milestone #5: Preparation of manuscript (Month 22-24): percentage completed: 0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Given that NMJ alterations are an early event in ALS and strategies counteracting such deleterious defects are lacking, we are combining *in vitro*, *ex vivo* and *in vivo* multiple genetic (mutant SOD1, TDP-43 and C9orf72) mouse and human models recapitulating functional NMJs to perform ***an integrated, multi-pronged screening approach to identify and validate potential therapeutics targeting neuromuscular innervation***. This project should enable the development of therapeutic strategies targeting muscle innervation with the discovery of small molecules that restore NMJ functions.

We proposed to screen FDA-approved drug/neuromuscular disease clinical candidate libraries in three complementary models of NMJs: ***1)*** Spinal cord motor neurons (MNs) isolated from ALS mouse models ($SOD1^{G93A}$ and $TDP\text{-}43^{Q331K}$) co-cultured with differentiated C2C12 skeletal myotubes, ***2)*** *ex vivo* nerve/glia/muscle preparations isolated from a mouse model of ALS ($SOD1^{G37R}$) and iPSC-derived MNs harboring ALS mutations ($TDP\text{-}43^{Q331K}$, $TDP\text{-}43^{M337V}$ and C9orf72 expansion; all are already available in the Da Cruz lab) or from sporadic patients and ***3)*** ultimately *in vivo* testing of therapeutic effectiveness of lead compound(s) in mice expressing ALS-linked mutations ($SOD1^{G37R}$ and $TDP\text{-}43^{Q331K}$) which develop age-dependent progressive muscle denervation prior MN death. Period 1 has been devoted predominantly to achieving aim 1 (since aim 2 and 3 depend on its progress) so we provide below in details our progress towards achieving this goal.

Aim 1 (Year 1): Screening of small molecules to identify potential therapeutics that stimulate innervation/re-innervation or prevent/delay denervation induced by ALS-mutants

- *Optimization of the screening platform in motor neuron/C2C12 co-cultures using fast automated confocal microscopy imaging and analysis with in-house algorithms.*

Using an established miniaturized co-culture system of MNs (from ALS mouse spinal cords or iPSCs) and muscle cells (differentiated C2C12 cells) modeling NMJ formation, maintenance and loss. Normal and ALS-causing mutant motor neurons maintain innervation of muscle cells and mediate muscle contractions. However, with time, NMJs established by mutant ALS-expressing MN are progressively and reproducibly lost, as *in vivo* setting. To identify small molecules that stimulate innervation or re-innervation and/or inhibit NMJ loss induced by ALS causing mutants, we proposed to optimize this miniaturize system to enable the screening of small molecules in collaboration with experts in small molecule screening and drug development (Dr. Andrew Shiau).

Since no small molecules are known to increase the functional interaction between MNs and muscle cells, a necessary step is to develop *biological* positive controls to validate the screening assay. We tested a range of previously molecules known to alter NMJ innervation or axonal branching including agrin and neurotrophic factors such as NT3 and bFGF. While a modest effect of agrin is observed leading to a 30% increase in innervation of human wild-type NMJs (accompanied by an increase in the number of Acetycholine receptors AChRs), no significant effects on innervation was measured with the neurotrophic factors. The experimental paradigm we use is as follows: a 48h treatment of the given compound at day 6 of co-cultures (when the number of NMJs is stable). However, for the molecules which may serve as potential positive control a dose response was performed using this paradigm.

Recently, the neuroleptic pimozide has been found to stabilize the NMJ in SOD1 mice in Dr. Robitaille's hands (Patten et al, 2017, JCI) and in a small clinical trial of sporadic ALS patients, with a larger Phase IIb trial under way in Canada. Thus, we tested pimozide in our human wild-type co-cultures using the same experimental paradigm as previously outlined and find that pimozide reproducible induces a 2-fold increase in innervation in a dose-dependent manner (Figure 1) in our human iPSC-derived motor neuron/muscle co-cultures (after one week of co-culture). This astounding effect has never been reported before and not only provides us with a positive control which is key for our screening platform, but also validates the relevance of our co-culture system.
Importantly, pimozide has been reported to cause advert deleterious effects at higher dose (Parkinson's like syndrome, dyskinesia, mostly neuromuscular extrapyramidal reactions etc.) in patients with Tourette's disorder, and we find that a higher concentration of pimozide (2.5µM and 10µM (not shown) is toxic leading to NMJ loss due to MN death (Figure 1).

Given the strong effect on promoting innervation in wild-type NMJs, we have initiated preliminary efforts testing derivatives of pimozide obtained from our collaborator Dr. Shiau (SMD department, Ludwig). The goal is two-pronged: 1) identify a derivative molecule with similar effect on innervation but with reduced toxicity at higher doses (to avoid severe side effects reported in patients), and 2) pinpoint to the molecular target(s), through which pimozide derivative is acting. We have five derivative compounds that we started testing right before lockdown and as soon as experiments can resume we will be testing those in human and mouse ALS co-cultures. The next priority once experiments can resume will also be to test the effects of pimozide on ALS co-cultures, but given the effects on wild-type NMJs and the evidence from Dr. Robitaille in mutant $SOD1^{G37R}$ mice, it is likely that ALS-induced denervation will be improved by pimozide, and possibly one of its derivatives.

Finally, to achieve our goals to optimize data acquisition and analysis, efforts have been devoted to reduce intra- and inter-plate variability which has been achieved (15-25% and 10-20%, respectively) by using a BioTek EL406 washer/dispenser for automated cell plating and antibody staining protocols. Furthermore, with the additional algorithm we developed which accurately scores NMJs identified by immunofluorescence and confocal microcopy, the analysis time per 96-well plate has been reduced to 5 hours.

Altogether, the efforts devoted to the optimization of the assay during this first period of funding have led to conditions that allow for screening. Indeed we were aiming for a Z-factor (positive control) $\geq$0.3 and the coefficient variability (CVs) $\leq$ 20% based on three independent experiments. Using human wild-type iPSC-derived motor neurons co-cultured with differentiated C2C12 muscle cells using non-treated condition (after 8days) as a negative control and as a positive control treatment with pimozide (1µM) for 48hrs, we obtain a Z-factor of 0.48 and intra and inter CVs that are in that range as outlined above.
Once we can resume our experimental effort, we will start screening in mouse MN/muscle co-cultures as originally proposed.

Once we can resume our experimental effort, we will start screening in mouse MN/muscle co-cultures as originally proposed and pursue with our aims.

The Robitaille lab spent their effort on generating the required number of mice for the aim 2 of the projects, awaiting the derivative compounds to be tested. Also, owing to the slow progression of the symptoms in SOD1^{G37R}, the production of mice colonies for aim 3 have already been initiated. A postdoctoral fellow, Dr Roberta Piovesana have been recruited to perform experiments of Aim 2. We are hoping that she will be joining the lab in the fall, provided that the COVD-19 situation allows it. She already has all her visa and paperwork all completed.

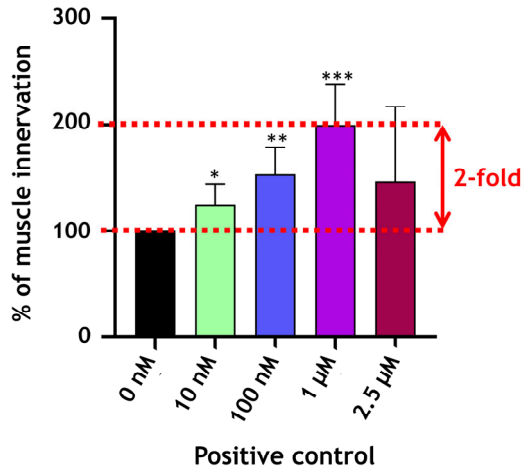

Figure 1. Identification of positive control promoting muscle innervation. Graph represents average ± standard deviation.

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.

Dr. Da Cruz presented the overall approach to the Department of Neurosciences at the Mayo Clinic (Florida) in October 2019. Dr. Robitaille presented the overall strategy at the Packard Center Annual symposium in March 2020.

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

As soon as experiments are allowed to restart, we will resume all our efforts as originally outlined. A no cost-extension will be requested to enable completion of the project once we have clarity on timing.

4. **IMPACT:** D*escribe 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).

The identification of a molecule which enables a 2-fold increase in innervation in our motor neuron/muscle co-cultures is not only a critical finding to validate our screening platform (using it as a positive control) but also provides key insight into the mechanisms that govern muscle innervation. Thus, we will be testing additional derivative compounds to elucidate the identity of potential targets, but also to possibly identify a new compound that more specifically targets NMJs without the known current side effects of our positive controls. These findings may significantly impact therapy development to treat ALS and other neuromuscular disorders.

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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

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.

Problem 1 encountered in the first semester: unsuccessful breedings (timed-matings). Few/No pregnancies could be obtained during the fall/early winter 2019.
Why? We believe this was due to renovation work being done in the mouse rooms in preparation of an annual accreditation inspection.
Measures taken: we changed colony to a quieter room, changed rack, order of new female breeders (using now CD1 females instead of C57Bl6 to increase the size of our litters once pregnant). Performed co-cultures with human iPSC-derived motor neurons and tested compounds to avoid wasting time towards achieving our goals). This problem was solved right before the pandemic.

Problem 2 encountered in the first semester: mouse protocol approval for collaborator Robitaille at the University of Montreal was substantially delayed (Approval only on February 7, 2020) so no mouse work could not be initiated.

Problem 3 encountered mid-March 2020: experiments had to be stopped due to the World-wide COVID-19 pandemic, and no new mouse or human motor neuron/muscle co-culture has been allowed yet to be restarted since. This had, and still has a tremendous impact on achieving our milestones.
1. The mouse colony had to be kept minimal (no experimental mating has been allowed other than for maintenance of the lines) until July 2020 (although given the increase in the number of covid cases, I worry this may revert again).
2. Only 10% of the personnel was allowed to be on site (and only to enable to most urgent work i.e. maintaining mouse lines or finish on going work) until June 30, 2020. Since July, about 25% of the personnel is now allowed to be on site but still under very restricted conditions.
3. Given the multi-step complex nature of our experimental procedure which requires i) setting up timed-matings of the ALS mouse line, ii) a nearly full-day of bench work for the embryos dissections (the number of hours a researcher can currently be in the lab is very limited), genotyping and ultimately motor neuron purification and plating, iii) every-day commitment (for a couple of hours) for the co-cultures and iv) imaging in fast high resolution microscope it has not been possible to perform any experiment and the screening is on hold.
Note that the Small Molecule Discovery unit led by collaborator Dr. Shiau at the Ludwig Institute (which hosts the fast high resolution Yokogawa confocal microscope that is required for our primary screening) has been shut down due to the covid crisis. Only recently a few users have been authorized to resume imaging under very limited conditions.

The impact of this temporary 'shutdown' is not trivial and meeting our milestones as initially outlined in our Statement of work is a real challenge. Even more so, because the downstream ex vivo and in vivo tests of the candidate drugs identified in the co-culture system to be done in my collaborator's lab rely on the outcome of the screening which cannot currently be done. So, the impact on the goals to be achieved by Dr. Richard Robitaille's team at the University of Montreal is heavy as they depend on this work which is now on hold.
Given the current situation with the pandemic it is not possible to estimate when experiments will fully resume. Only data analysis has been possible during this lockdown period and the maintenance of the ALS mouse lines, with some recent imaging since access to the microscope has resumed under limited conditions.

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.

Due to the world-wide COVID-19 pandemic and the lockdown measures implemented at the Ludwig/UCSD starting mid-March 2020, all new experiments could not be started to take care of the co-cultures until drug testing and ultimately immunofluorescence is achieved as outlined above. Therefore, expenses on consumables have been nearly null.
Personnel costs remained unchanged as during this period, personnel devoted their time to the analysis of data previously obtained until the lockdown, as well as the maintenance of the mouse lines. More recently, since access to the microscope has been allowed for a limited period of time, imaging of pending experiments stopped during lockdown has been performed.

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

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

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).*

Nothing to report

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).*

Nothing to report

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.*

See above for presentations.

- **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.

Not applicable. None of the results are listed on our respective websites.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Not applicable

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

Not applicable

- **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;*
  - *physical 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.*

Nothing to report

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.)*

Ludwig Institute:

- Name: Sandrine Da Cruz
  No Change

- Name: Carlos Chillon-Marinas
  No change

- Name: Noe Govea-Perez
  No Change

University of Montreal:

- Name: Richard Robitaille
  No Change

- Name: Danielle Arbour
  No change

- Name: Joanne Vallée
  No Change

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.

Changes in other support since June 2019:

- Da Cruz lab:
  - The Muscular Dystrophy Association. (#628227) Sandrine Da Cruz (PI). 2019-2022. "Mechanisms and therapy underlying FUS-mediated ALS disease". This project is to determine the contribution of FUS aggregation and cell-to cell spreading to ALS pathogenesis. $300,000 total costs.

- Robitaille lab:
  - ALS Canada, Richard Robitaille PI (Angela Genge, Benoit Coulombe & Mathieu Lavallee-Adam co-PI). 2020-2021; total costs $100 000 CND
    Profiles and biomarkers of neuromuscular junctions of ALS patients. The goal is to study the morphological and molecular profiles of neuromuscular junctions from ALS patients.

  - CIHR Geroscience demonstration grant; (Richard Robitaille co-PI, Gilles Gouspillou PI); 2019 - 2022; total costs $450 000 CND
    Investigating the contributions of mitochondrial dysfunction and impaired neuromuscular junction integrity to the aging-related loss of muscle mass and function in humans. The goal of this grant is to perform a longitudinal study of the neuromuscular and mitochondrial changes during normal aging in human.

  - NSERC discovery grant (R. Robitaille PI); 2021 - 2025; total costs $345 000 CND
    Heterogeneous glial regulation of inhibitory hippocampal synapses. The goal of this grant is to study the heterogeneity of astrocytes and its implication in the differential regulation of different types of inhibitory interneurons and their impact on the hippocampal network.

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.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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.*