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TITLE: Evaluation of the King-Devick Test to Assess Eye Movements and the Performance of Rapid Number Naming in Concussed and non-Concussed Service Members

PRINCIPAL INVESTIGATOR: Dr. Michael Dretsch

CONTRACTING ORGANIZATION: The Geneva Foundation
917 Pacific Ave., Ste. 600
Tacoma, WA 98402

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MAJ Michael Dretsch, PhD

E-Mail: Michael.n.dretsch.mil@mail.mil

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The Geneva Foundation
917 Pacific Ave., Ste. 600
Tacoma, WA 98402

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project was aimed at investigating the sensitivity and specificity of different screening modalities to concussion in Army Soldiers attending combatives training and airborne school. The primary study assessed changes in oculomotor functioning as measured by the King-Devick (KD) test. Results showed that the KD tests has poor discriminant ability (AUC = .60, sensitivity/specificity) to distinguish between concussed and non-concussed Soldiers, and therefore, should only be used to supplement clinical evaluations. The use of salivary biomarkers shows promise as a potential screening tool in that when our data, from a subset of our Soldiers, were included with other samples to form much larger studies, had adequate discriminant ability (AUC = .84-.86). Brain imaging (7T MRI), resting-state functional connectivity of a subset of the sample showed promise, albeit major limitations due to sample size, for normative modeling analytic approach with sensitivity of 74% and specificity of 96%.

15. SUBJECT TERMS
MTBI, concussion, neurocognitive

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TABLE OF CONTENTS

1. INTRODUCTION:

The primary study objective is to determine the concurrent validity, sensitivity, and specificity of the King-Devick Test to cognitive impairment of attentional processes associated with acute mild traumatic brain injury (mTBI) in service members. The secondary objective it to explore the neurophysiological and neurostructural changes in the brain associated with both combatives training and acute concussion. In addition, we will explore changes in microRNA and other small molecules as potential biomarkers of mTBI.

2. KEYWORDS:

MTBI, concussion, neurocognitive

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim # 1 – Initiate, Plan and Design Study (Months 1-24)
Task 1. Develop Protocol. (Months 1-12)
1.a. Prepare protocol supporting documents and budget (Month 1-12)
Task 2. Obtain Study Approval. (Months 13-15) (Current PI assumed responsibility)
2.a. Fort Benning Command Approval
2.b Submit protocol and consent documents to the Eisenhower Regional Command IRB and U.S. Army Medical Research and Material Command's IRB for review and approval.
Task 3. Preparation of MRI SOP at Auburn University. Quality assurance of MRI system (Months 12-15)
Task 4. Hire remaining personnel (17).

Specific Aim # 2 – Execute Study (Months 20-42)
Task 1. Recruit Subjects (Months 9-42)
Task 2. Consent Subjects (Months 9-42)
Task 3. Collect Baseline, Post-Training, and Post-Incident Data (Months 9-42)
Specific Aim # 3 – Monitor and Control (Months 42-54)
Task 1. Perform data quality assessment/checks (Months 20-50)
1.a. Ensure that all study personnel are compliant with guidelines set forth in the protocol.
Task 2. Pre-process MRI data at Auburn University (Months 42-50).
2.a. Provide quality assurance of data.
2.b. Preparation for post-processing.
Task 3. Analyze data to evaluate hypotheses as follows (Month 45-50)
3.c. Analyze MRI data to explore injury biomarkers and correlations with K-D and other metrics (Months 50-55).
Task 4. Prepare Interim Research Progress Report to Geneva Foundation (Month 50)
Task 5. Analyze final data (Month 45-54)

Specific Aim # 4 – Conclude Study
Task 1. Prepare Final Research Progress Report to (Fort Benning, MRMC's IRB, and Geneva Foundation (Months 50-54)
Task 2. Prepare scientific manuscripts for submission to peer reviewed journal (Months 50-54)

What was accomplished under these goals?

1) (A-18002) Evaluation of the King-Devick Test to Assess Eye Movements and the Performance of Rapid Number Naming in Concussed and Non-Concussed Service Members. (151 enrolled with baseline data. 99 of which also have post-training assessments. 33 of which also have post-injury assessments.
    a. Aim/Objectives:
        i. The primary objective of this study is to evaluate the use of the K-D to Assess Eye Movement and Rapid Number Naming in Concussed and Non-Concussed Service Members. The purpose of this test is to measure and identify changes in Service Members' cognitive performance due to training or injury resulting in blows to the head and or concussive symptoms. Changes in cognitive performance are the most common impairment among Service Members with a concussion sometimes refered to as mild traumatic brain injury (mTBI). As a secondary aim, a small amount of saliva will be collected (<1mL) to explore potential peripheral mRNA biomarkers of concussion.
    b. Method
        i. The study has two groups of subjects: Soldiers who completed Combatives Training without experiencing a concussion and a group of subjects who experienced a concussion during training. All subjects will have three data collection phases: 1) Screening Phase, 2) Baseline Data Collection Phase, and 3) either a Post-training or post-injury. Participants who enrolled came solely from the advanced Combatives school. Recruitment from the airborne school was unsuccessful.
        ii. In later protocol amendment, we attempted to re-assess individuals who receive a post-injury assessment after 72 hours post-injury, but were unsuccessful. This was partially due to losing the RA who quit to PCS with her spouse stationed OCONUS.
        iii. The study enrolled 152 participants. Three participants did not complete the study for unknown reasons, therefore, data from149 participants were analyzed. Data from one participant were excluded from the analysis after quality inspection revealed both baseline and post-training KD scores for this individual were extreme outliers using Q-Q plots. Out of the remaining 148 participants, 19 (13%) did not receive a follow up assessment (baseline only), 98 (66%) completed the combatives training without sustaining a concussion, and 31 (21%) were screened positive to having sustained a concussion. All but one of the individuals who screened positive for a concussion reported sustaining strikes (i.e., punch or kick) to the head. The one individual who did not get concussed via strikes to the head was injured after being body-slammed to the mat.
        iv. Salivary biomarkers: In addition to behavioral and self-report measures, the protocol was amended in June 2017 to include the collection of participant saliva to explore unique biomarkers that might have potential to be used for screening concussive injuries (http://www.npr.org/sections/health-shots/2017/05/04/526782407/spit-test-may-reveal-the-severity-of-a-child-s-concussion). Micro-RNA and other small, non-coding RNA sequences were tested for concentrations and/or specific patterns of RNA sequences. For the salivary biomarker analysis, samples were collected on 28 of the 155 participants (18 with baseline and post-training samples; 6 with baseline and post-injury samples; 4 with baseline only). Due to this sample size constraint, biomarker values analyzed with those from other study data sets (see, Hicks et al., 2020a attached).

c. Results

i. Headache, balance problems, and dizziness were the most severe concussive symptoms (See table 1 below; Dretsch et al., 2019). KD scores for those who sustained a concussion (n = 31) were significantly worse compared to baseline ($p < .05$), but not for participants who finished the course with no concussion (n = 98).

Table 1. Rank order of symptom severity at post-concussion.

Order	Symptom	M(SD)
1	headache	2.11(2.05)
2	balance problems	1.29(2.17)
3	dizzy	1.05(1.69)
4	visual disturbances	0.85(1.81)
5	concentration	0.74(1.67)
6	irritability	0.61(1.69)
7	nausea/vomiting	0.53(1.24)
8	memory problems	0.42(1.36)
9	ringing in ears	0.42(1.23)

ii. The concussed group was more likely to have reported having a prior lifetime concussion (29%) than those who did not sustain a concussion (13%), $\chi 2$ (1) = 4.14, p = .042. For concussed, 74.2% had scores that were worse from baseline (slower) compared to 39.8% of the post-training group. KD scores were worse 34.4% more in individuals who sustained a concussion compared to those who did not. However, there was poor discriminant ability of the KD test (AUC = .60, sensitivity/specificity) to distinguish between concussed and non-concussed participants.

iii. For the salivary biomarkers, salivary non-coding RNA (ncRNA) were assessed as a diagnostic adjunct for concussion (i.e., mTBI). Analyses included data from 538 individuals, including 251 with concussion, enrolled ≤ 14 days post-injury from 11 sites. There were 28 miRNAs, 21 Refseq RNAs, and 1378 wiRNAs with significant (FDR < 0.05) differences between concussed and control groups. There were 16/28 (57%) miRNAs, 12/21 (57%) Refseq RNAs, and 675/1378 (49%) wiRNAs up-regulated in the concussed group.

iv. A predictive model utilizing seven ratios, involving seven ncRNAs along with participant age and chronic headache status, differentiated concussed and control participants with AUC of 0.857 (95% CI: 0.816-0.903). The model correctly identified 190/251 (76%) concussed participants and 232/287 (81%) control participants (PPV = 81%, NPV = 76%). AUCs varied when combining various measures (see figure 1).

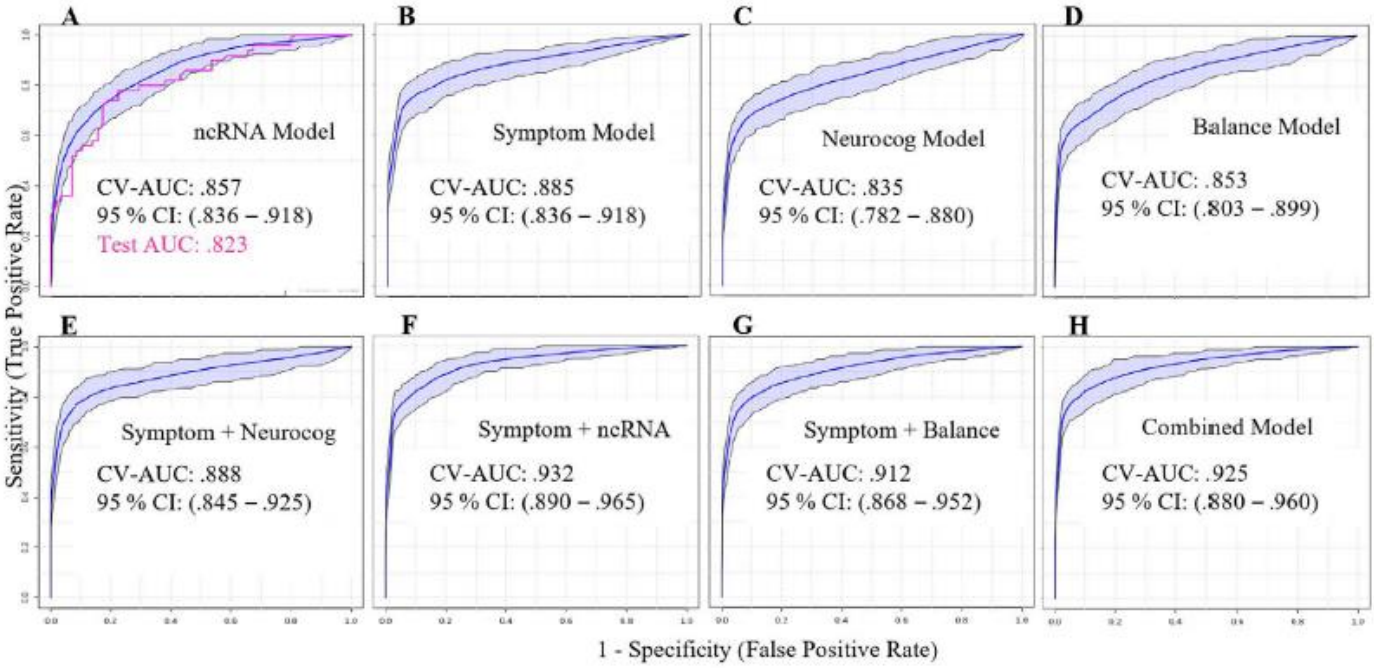

Figure 1. AUCs of varied measures to mTBI (Hicks et al., 2020).

v. In a secondary saliva biomarker study (see Hicks et al., 2020b attached), assessed concussed and non-concussed athletes recruited across 11 sites to include ours. RNA sequencing was used to measure levels of salivary miRNAs within 479 samples from 326 individuals to assess effects of sports-related concussion (SRC), cumulative head impacts, and various exercise regimens on miRNA concentrations. A predictive model employing four of these miRNAs (miR-192-5p, miR-27a-5p, miR-30e-5p, miR-7-1-3p) differentiated 57/75 concussed and 71/97 non-concussed athletes (AUC = 0.84; 95% CI: 0.76-0.92). There were 11/25 SRC-related miRNAs significantly affected by both exercise and season-long participation in contact sport (adj $p<0.05$).
vi. Overall, the validation of salivary markers is ongoing. Continued replication with larger samples is necessary for establishing the reliability of specific miRNA.

2) (A-18002.2) Imaging Assessment of Neurological Changes Associated with Subconcussive and Concussive Events in US Soldiers. [Control group (n=32). mTBI group (n=4)]
   a. Aim/Objective:
      i. The primary objective of this study was explore differences in healthy and concussed military service members participating in Army combatives training. The aim was to explore structural and functional differences in neuroarchitecture. Participants who enrolled had brain imaging conducted at Auburn University's MRI Research Center.
      ii. Due to logistical and personnel issues, it was difficult to recruit the numbers of participants needed to run all of the analyses that were initially proposed. As such, only differences in functionality (i.e., connectivity) was assessed, and not structural differences (i.e., diffusion tensor imaging for assessing white matter tract integrity).
   b. Method:
      i. A total of 36 active duty Soldiers participating in an Army combatives training course at Fort Benning, GA, who were enrolled in study protocol A-18002, and voluntarily enrolled in this secondary study (A-18002.2) where they were scanned with a Siemens Magnetom 7T MRI at the MRI Facility in Auburn University. Of the 36 participants, 32 were healthy controls in that their data were collected at the start of the combatives course, prior to any sparring or potential head injury. Of the 32 controls, only imaging

data on 23 were included due to missing data, head movement and other artifacts, and technical problems during data collection. During the course, 4 of the 36 Soldiers (n = 4), independent from those in the control group, sustained a concussion while sparring, were immediately enrolled into the study, and received an MRI scan. The mean age of the concussed group was 27 years (50% female) and 28 years (4% female) for the controls.

i. Participants traveled approximately 40 miles to Auburn University for a functional MRI (fMRI) scan. Participants were instructed to relax, keep their eyes open, and keep their head as still as possible throughout the duration of the scan. Participants underwent resting state scans, where they were instructed to remain awake and alert, but are given nothing specific to focus on, recall, or do for the duration of the scan. These scans examined the activity of the brain in its natural state. The method of classification (controls vs. concussed) used in this study was normative modeling through Scalable Multi-Task Gaussian Process Regression (S-MTGPR).

2. Results

i. Using the normative model, a Predicted Yhat Layout was used to estimate which subjects contained outliers, and identified where the outliers were located. Each pane of the image showed a slice of the brain in axial view, cutting through the middle of the brain. Each slice corresponds to a different participant (Figure 2), providing a summary image of each participant. Most participants look relatively similar, with three exceptions, concussed participants 3, 6, and 7 (but not concussed participant 16). These three exceptions appear to have had greater activity in their AMGY, HIPP, and Frontal Lobe. When compared to the mask of the normal functional neuronal activity of the sample, it is clear that these three subjects varied significantly from the created model.

ii. The results revealed promising findings of a resting state imaging, analytic approach that has modest sensitivity (75%), but high specificity (95.7%) to acute concussion. Based on the normative model, 3 of the 4 participants (3, 6, and 7, but not 16) fell outside of a standard deviation of 1.5. These same individuals performed poorly on the KD test.

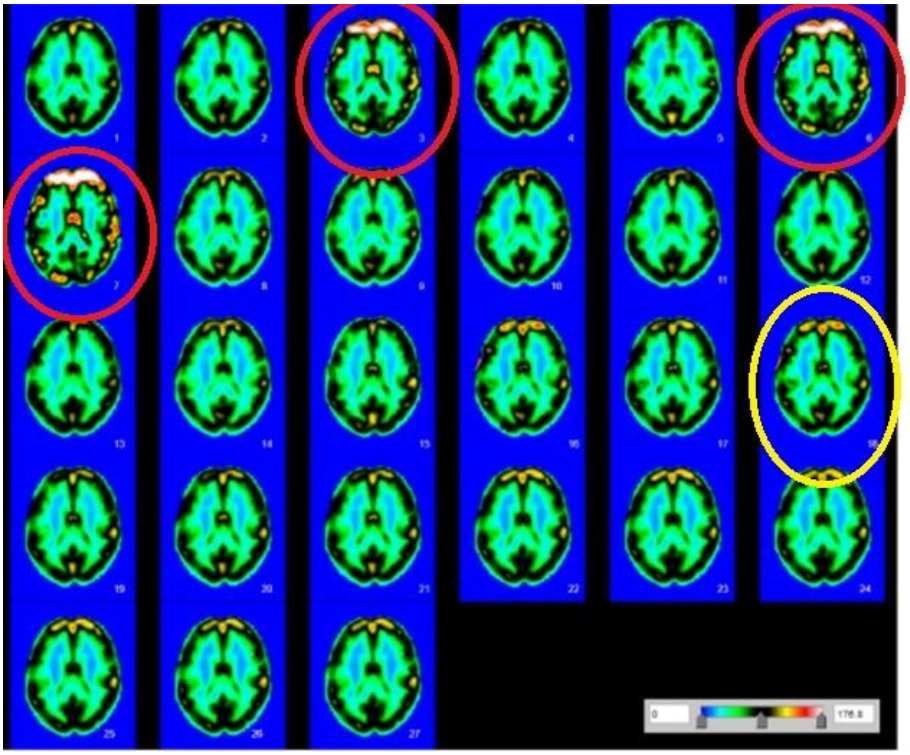

Figure 2. S-MTGPR model. Axial view by participant. Concussed participants 3, 6, and 7 with abnormal activation of specific ROIs highlighted by red circle and concussed participant 16 highlighted in yellow.

iii. For a comprehensive report, see Dretsch, Oldag, and Deshpande, 2020 attached.
iv. The use of larger samples of concussed subjects is likely to improve sensitivity, and is essential for validating this finding.

What opportunities for training and professional development has the project provided?

The project provided opportunities for professional development for RAs and the site PI. The project exposed them to neuroscience techniques (i.e., salivary biomarkers and neuroimaging) of which they were not familiar with. It also provided an opportunity for a junior researcher to have the experience being a site PI.

How were the results disseminated to communities of interest?

With referral to published article and two pending manuscripts under review with journals (see #6 below)

What do you plan to do during the next reporting period to accomplish the goals?

N/A

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The findings from this project have the potential to impact policy for screening concussion during training in CONUS. The secondary and tertiary effects may result in continued research within other military populations and operational environments. Policy change would come through dissemination of findings to USAMRDC, TRADOC, DHA, and DVBIC. Overall, the results will inform the community of practice of the utility and properties of existing and emerging concussion screening methods.
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Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications. *Yes*

Dretsch, M.N., Fauth, J., Moya, M., Connaboy, C., T., Kontos, A. (2019). Modest utility of brief oculomotor test for concussion screening in military mixed-martial arts training. Brain Injury, 33(13-14):1646-1651.

Hicks, S., Onks, C., Kim, R.Y., Zhen, K.J., Loeffert, J., Loeffert, A.C…Dretsch, M., Middleton, F. (2020a). Diagnosing mild traumatic brain injury using saliva RNA compared to cognitive and balance testing. Clinical and Translational Medicine, 10(6): e197.

Hicks, S. D., Olympia, R. P., Onks, C., Kim, R. Y., Zhen, K. J., Fedorchak, G., DeVita, S., Rangnekar, A., Heller, M., Zwibel, H., Monteith, C., Gagnon, Z., McLoughlin, C. D., Randall, J., Madeira, M., Campbell, T. R., Fengler, E., Dretsch, M. N., Neville, C., & Middleton, F. A. (2020b). Saliva microRNA Biomarkers of Cumulative Concussion. International journal of molecular sciences, 21(20), 7758. https://doi.org/10.3390/ijms21207758

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

"King-Devick Study Assesses Head Injury in the Army" July 28th 2016. Available at: http://www.bayonetandsaber.com/2016/07/27/1031335/king-devick-study-assesses-head.html / https://www.army.mil/article/172262/

-
- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: MAJ Michael Dretsch
Project Role: PI
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.6
Contribution to Project: Dr. Dretsch served as the overall study PI on this research project
Funding Support: N/A

Name: Jenifer Fauth
Project Role: Project Director
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Jenifer Fauth served as the Project Director and on-site lead for this research project.
Funding Support: The Geneva Foundation

Name: Rian Ory
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: Intermittent
Contribution to Project: Rian Ory trained for data collection, administration, and analysis.
Funding Support: The Geneva Foundation

Name: CPT Marcelo Moya
Project Role: Site PI
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Recruitment and data collection. Administrative actions
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Provide the following information for each partnership:
Organization Name: Auburn University
Location of Organization: Auburn AL
Partner's contribution to the project:
- Collaboration (e.g., partner's staff work with project staff on the project);

Organization Name: SUNY Molecular Analysis Core facility (SUNYMAC)
Location of Organization: Syracuse, NY
Partner's contribution to the project:
- Collaboration (e.g., partner's staff work with project staff on the project);

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to Report

QUAD CHARTS:
The Quad Chart: Attached.

9. APPENDICES:
None