Composite Analysis of Multi-Category Behavioral Deficits for Increasing the Translational Relevance of the Mouse Monofilament Stroke Model

Yamileck Olivas-Garcia[1] MD (yolivasgarcia@health.ucsd.edu), Jose Villa[1] MD, Yingxin Chen[1] MD, Kaley Sauer[1] BS, Esmeralda Sajche Sapon[1] BS; Rachana Balakrishnan[1] BS, Chunli Liu[1] MD, Yujung Park[1] MD, Emily Osterli[1] PharmD, and Bingren Hu[1,2] MD, PhD

[1]Departments of Emergency Medicine and Neurosciences, University of California San Diego, La Jolla, CA; [2]Veterans Affairs San Diego Healthcare System, 3350 La Jolla Village Dr, San Diego, CA.

UC San Diego

NIH National Institutes of Health

VA U.S. Department of Veterans Affairs

INTRODUCTION

Most post-stroke behavioral deficits are short-lived in rodent stroke models. This issue poses a significant challenge when using a rodent stroke model to test therapeutic interventions.

OBJECTIVES

This study aims to explore a composite analysis of behavioral outcomes for increasing the drug testing utility of the mouse Middle Cerebral Artery Occlusion (MCAO) model.

METHODS

Mice were subjected to 0 (sham), 20, 40, or 60 min MCAO, followed by 21 days of recovery. Regional cerebral blood flow (rCBF) was kept below 10% and body temperature was maintained at 37C during MCAO. Behavioral outcomes (bodyweight, nesting activity, and pole test) were documented on days -3, -7, -10, -14, and -21. Dead mice were excluded (non-imputed) or given the worst behavioral score (imputed). For the composite analysis, the sham group was assigned a functional performance score of 100%. Each category (motor, nesting, and body weight) contributed 33% to this total or 16.6% when combined with survival, which contributed 50%.Each subject's performance was compared to the mean of the sham group, and a proportion of the corresponding category score was assigned based on their performance. The total composite score was calculated by summing the proportions of all categories.

RESULTS

Quantitative Analysis of Histopathology

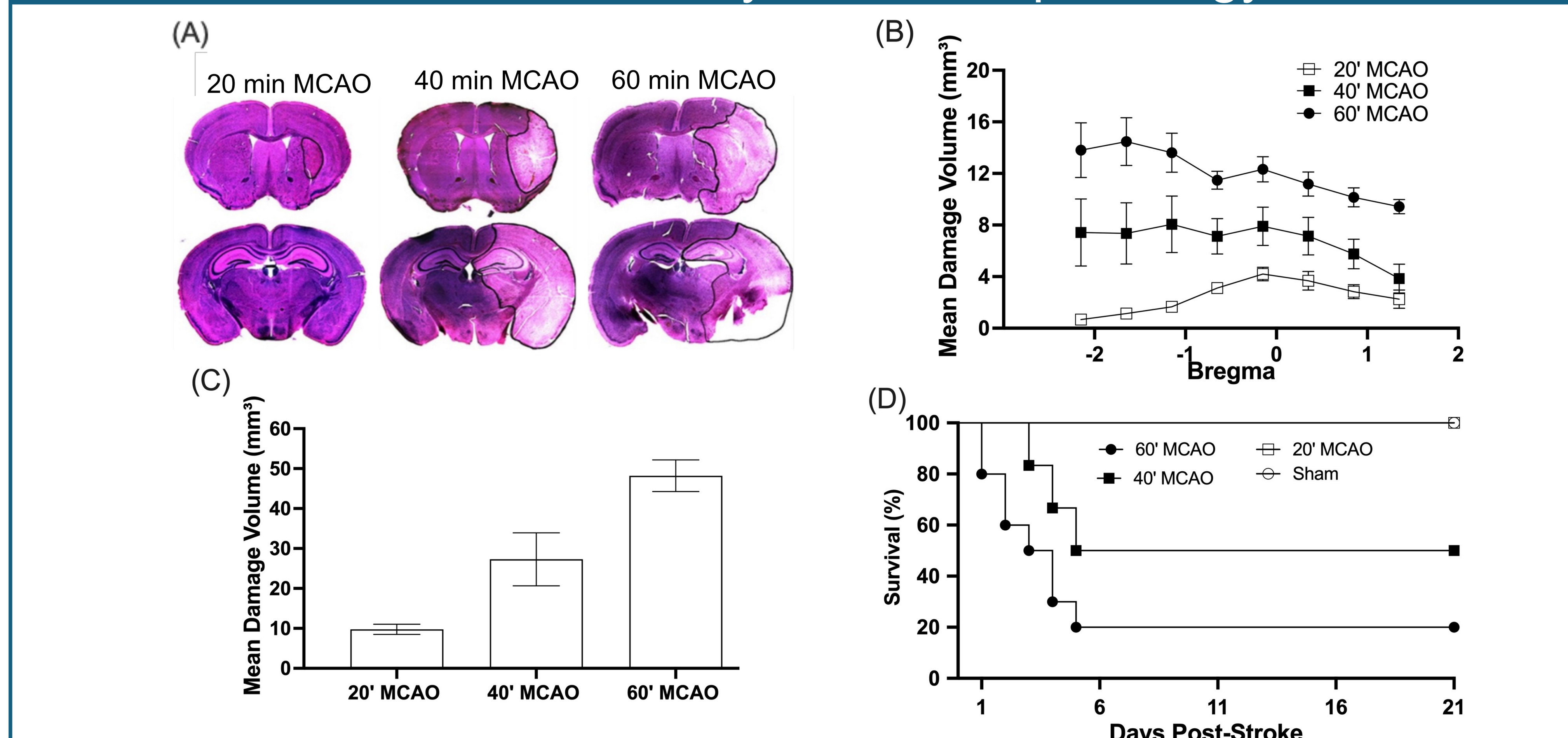

Figure 1. (A) Cresyl violet-stained coronal sections show increasing infarct size with longer MCAO durations (20, 40, and 60 min). (B) Infarct volume increases across Bregma levels for each MCAO duration.(C) Total mean infarct volume increases with longer occlusion times.(D) Kaplan-Meier survival curves for mice, longer occlusion times result in lower survival rates over 21 days.

Post-Stroke Functional Performance

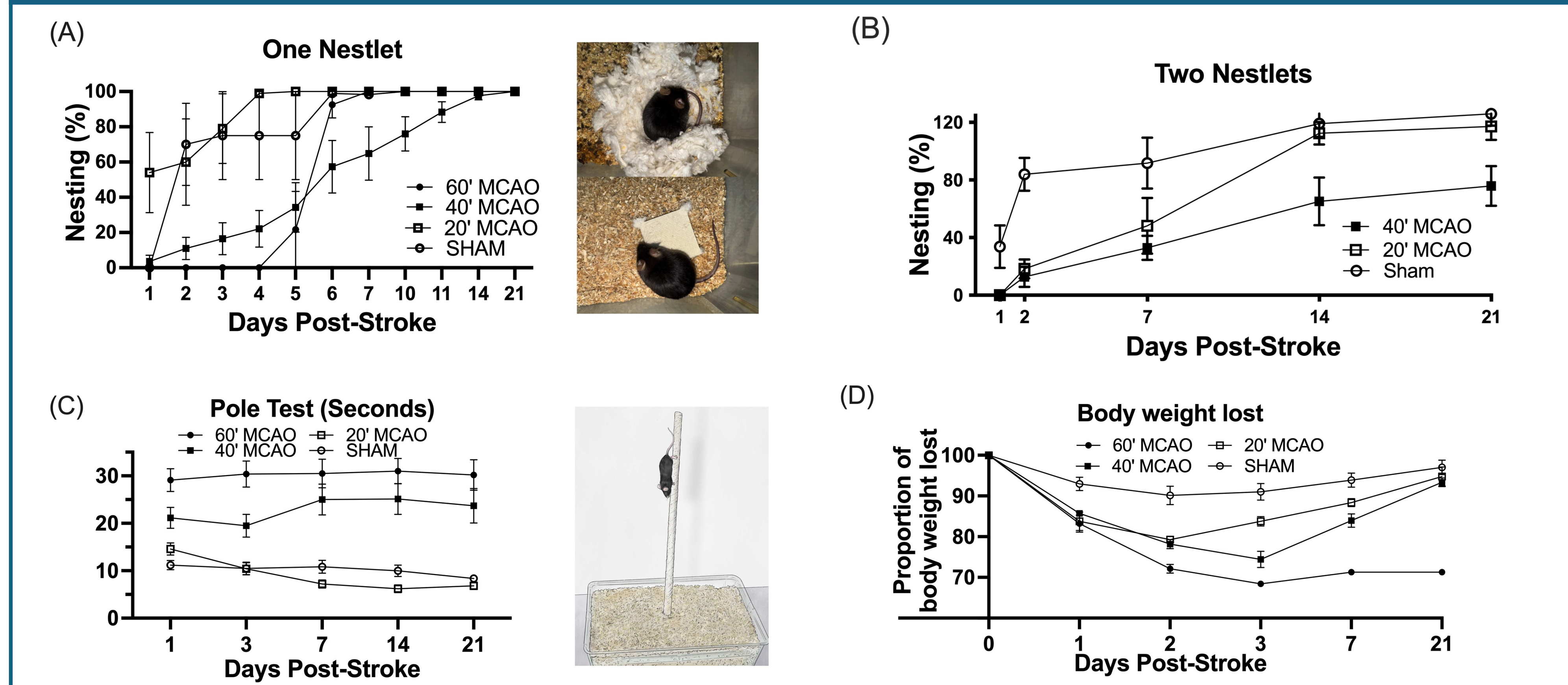

Figure 2. A-B (A)Nesting Activity with one Nestlet; (B)Nesting Activity with two nestlets in mice subjected to sham-operation, 20' MCAO or 40' MCAO Followed by different periods of reperfusion. (C)Pole Test: Time to descend the pole was recorded, showing no significant differences between the 40- and 60-minute MCAO groups. (D)Body Weight Loss: Weight loss from baseline was calculated. All groups, except the 60-minute MCAO group, recovered by day 7.

RESULTS

Composite Analysis of Functional Deficits and Survival

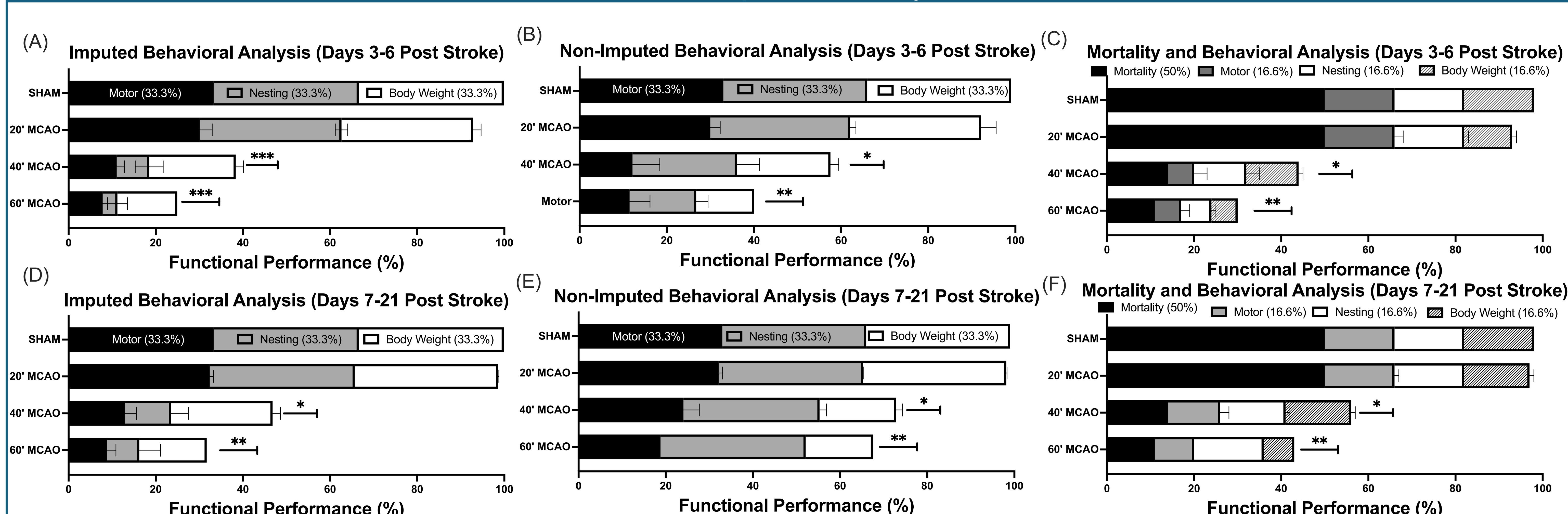

Figure 3. Composite Behavioral Analysis of MCAO Stroke Severity. This approach integrates motor performance (pole test), well-being (nesting behavior), and physical integrity (body weight) for a comprehensive assessment of stroke severity. Imputed Analysis: (A) Days 3-6: Longer MCAO duration worsens deficits, with 40' and 60' groups significantly impaired ($p < 0.001$). (D) Days 7-21: Partial recovery occurs, but severe deficits persist ($p < 0.05$, $p < 0.01$).Non-Imputed Analysis: (B) Days 3-6: Similar trends, but with more variability, confirming the value of imputation. (E) Days 7-21: Persistent deficits in 40' and 60' groups ($p < 0.05$, $p < 0.01$).Mortality-Adjusted Analysis: (C) Days 3-6: Higher mortality Higher mortality correlates with worse outcomes ($p < 0.05$, $p < 0.01$). (F) Days 7-21: Survivors still show lasting deficits, emphasizing long-term sensibility of the analysis.

CONCLUSIONS

This multi-category composite analysis integrates various aspects of behavioral deficits (physical, sensorimotor, and nest-building daily activity), enhancing statistical power by reducing variability, and improving reliability. This approach strengthens the translational relevance of the mouse MCAO model, making it a more effective tool for evaluating stroke drug interventions.