Impact of Stress on the Progression of Dystonic Attacks in \textit{tg/tg} Mouse

Anant Naik\textsuperscript{1}, Madelyn Gray\textsuperscript{2}, Russell Carter\textsuperscript{2}, Timothy Ebner\textsuperscript{2}

\textsuperscript{1}Department of Biomedical Engineering, \textsuperscript{2}Department of Neuroscience, University of Minnesota, Twin-Cities

\section{Introduction}

The tottering (\textit{tg/tg}) mouse is the leading model organism for studies of the human disease episodic ataxia type 2 (EA2). EA2 is characterized by marked cerebellar symptoms, such as random occurrences of dystonic motor attacks, vertigo, imbalance, and transient episodes of ataxia\textsuperscript{1},\textsuperscript{2} as well as non-cerebellar symptoms, including absence seizures and migraine\textsuperscript{3}. An analogous mutation in the \textit{tg/tg} mouse of the \textit{CaCNA1A} gene results in markedly more severe symptoms in \textit{tg/tg} mice, encompassing mild ataxia, pronounced episodic ascending dystonic attacks\textsuperscript{4}, and absence seizures\textsuperscript{5}. As a fully effective therapy has not yet been discovered for EA2, the \textit{tg/tg} mouse is vital in understanding the underlying mechanism of the disease so as to work toward a better tailored treatment for EA2 patients\textsuperscript{6}.

In order to elucidate the complex mechanism of the progression of EA2 in humans, the implications of the analog mutation in \textit{tg/tg} mice must be understood. The most notable consequence of this mutation in \textit{tg/tg} mice is the occurrence of pronounced episodic ascending dystonic attacks that are characterized at times by full loss of muscular control\textsuperscript{7}. These attacks can be reliably induced in both humans and mice by caffeine, ethanol, and the introduction of stressful situations\textsuperscript{8,9}. While the presence of these attacks has been noted since the inception of the \textit{tg/tg} mouse\textsuperscript{10}, the exact progression and severity of attacks is poorly understood. By performing an in-depth study of \textit{tg/tg} mice’s dystonic attacks with respect to provocation duration, and early termination, we wanted to be able to implicate neural circuitry involved in the attacks as well as better understand the consequences of the mutation of the \textit{CaCNA1A} gene.

\section{Methods}

\subsection{Evolving Motor Attacks}

\textbf{Adult (3-24 month old) wild-type or \textit{tg/tg} mice}

- Removal of mouse from home cage
- Treatments: 75\% Caffeine (25\%L), 75\% Saline, Cage transfer
- Individual transfer to new cage for observation

\subsection{Attack Scoring}

- Observations every 5 minutes starting at ti=0 for duration and latency
- Full Body Score (0-3) and Specific Regional Score (Binary: 0 or 1)

\begin{table}
\begin{tabular}{|c|c|}
\hline
\textbf{Score Characteristics} & \textbf{Definition} \\
\hline
0 & Full mobility of mouse, no visible contractions \\
1 & Partial loss of mobility with minimal contractions, stillness without successive contractions \\
2 & Limited capacity for movement, abnormal posture, Tripod position with prominent contractions \\
3 & Inability to sustain contractions, rolling on belly and curling, prolonged contractions \\
\hline
\end{tabular}
\end{table}

\subsection{Tail Pickup}

- After 3 consecutive full body scores of 3, mouse lifted by the tail for 30 seconds
- Returned to test cage or home cage
- Monitoring every 30 seconds for 2 min pre-pickup and 8 min post-pickup

\subsection{Full Scoring of attack post pickup}

\section{Results}

\subsection{Attack Progression}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{A. Representative full-body scoring during a 2 hour observation period showing the progression of the dystonic attack Series of images showing the progress of the attack (from Green andSolman, 1982). B. Summary data comparing the attack severity and duration between caffeine, saline, or new cage animals (n=10 each). Caffeine injected animals were significantly different than saline and new cage animals (ANOVA F(4)=7.98, p<0.001). D. Comparison of the time to attack onset (top) and attack duration (bottom) between the three different groups.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Correlations of age, gender, and weight on attack duration. A. Data comparing the effects of weight on attack duration in the three different groups. No significant effect was found on attack duration. B. No significant effect was found on the comparison of gender on the attack duration. C. The effect of age on attack progression. Only the saline group had a significant effect on age on attack duration (r=+.48, p<.02). D. No significant effect was found on the latency to attack.}
\end{figure}

\subsection{Tail Pickup}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{A & B. Plots of the full body score from mice injected with either caffeine (A) or saline (B) that were picked up by the tail (t=0) and placed back into the test cage. Each line represents a different observation session. In all animals teste, the attack was briefly halted a secondary attack. C & D. Zoomed in region during the tail pickup period for the caffeine (C) and saline (D) injected mice. E. Comparison of the combined pre- and post-tail pickup, post-tail pickup, and the uninterrupted attack duration (Fig 1E) for each group. Attack duration for the post-tail pickup for caffeine was significantly different than the post-tail pickup duration for saline (p<.01). The latency to attack post tail pickup was not significantly different between caffeine or saline injected mice (right). F & G. During a different observation period, attack onset was placed back into the home cage following tail pickup to remove the potential confound of new cage stress. Preliminary data shows that caffeine injected mice (F) still re-attack the attack following tail pickup. Interestingly, saline injected mice (G) never re-entered the attack, suggesting that residual caffeine still in the system may be responsible for the post-tail pickup attack.}
\end{figure}

\subsection{Optogenetics and Motion Tracking}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{A. Diagram of the viral vector injection into the deep cerebellar nuclear cell. B. Example image taken from ANY-mouse, an open-source tracking software program. The mouse is automatically detected and shaded (blue) with the center of mass indicated by the orange dot. The mouse is connected to a fiber optic guide tube with the stimulation parameters controlled by the software. C. Preliminary tracking data for total distance traveled in a mouse that has been injected with CHIRP. The shaded regions indicate the time of the evaluation (8pm, 2a, 4a, 8a). Data is stored in 30s time bins. Green bars show time period indicated that the animal was showing signs of the motor attack. The stimulation during the attack failed to stop the progression, however the second stimulation may have started a secondary attack.}
\end{figure}

\section{Conclusions}

- The onset of the attack is a complex synthesis of various stressors experienced by the tottering mouse. Thus our objective was to reduce as many stressors as possible to determine the true progression of the attack.
- On average, attacks initiated by saline injections were less severe than attacks from caffeine, despite comparable durations. The severity of saline attacks was comparable to those from new cage transfer.
- Characteristics of the attack are consistent across the sex, age and weight of the \textit{tg/tg} mice studied.
- While in the attack, a dramatic increase of stress (tail pickup) halts the attack.
- The return of the attack, we hypothesize, is a discrete and distinct attack, as latency and duration of the post-pickup attack is statistically comparable and significant to those of uninterrupted attacks.
- When returned to their home cage post pickup, \textit{tg/tg} mice injected with saline do not exhibit any further attacks in the monitoring period, suggesting that the tail pickup completely halts the first attack.
- Further studies can look into other stressors to interrupt the attack, investigate the effects of antagonists to the stress response, as well as using optogenetics to influence the dystonic attacks.

\section{Acknowledgments}

Supported by: Undergraduate Research Opportunities Program (UROP), Undergraduate Research Scholarship (URS), T32 GM008244, T32 GM008471, P30 NS062158, and R01 NS18531

\section{References}

4. Raike RS, Weiszb C, Hoebeek FC Terzid MT, DeZeeuw CE, van den Maagdenberg AN, Jinnahd, HA, Hess, C. Dopamine Norepinephrine. Correlations of age, gender, and weight on attack duration. A. Data comparing the effects of weight on attack duration in the three different groups. No significant effect was found on attack duration. B. No significant effect was found on the comparison of gender on the attack duration. C. The effect of age on attack progression. Only the saline group had a significant effect on age on attack duration (r=+.48, p<.02). D. No significant effect was found on the latency to attack.
5. Figure 5. Proposed mechanism of the impact of stress on the episodic dystonic attack of \textit{tg/tg} mouse, through the inhibition of any with vascular calcium defects, signifying two potential pathways. We hypothesize that strong, rapid stressors can stop the observed dystonic attack, whereas prolonged, mild stressors help to initiate the dystonic attack.