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AI-nalyzing Mouse Behavior to Combat Epilepsy

Hidden Behavioral Fingerprints in Epilepsy

Gschwind T, Zeine A, Raikov I, Markowitz JE, Gillis WF, Felong S, Isom LL, Datta SR, Soltesz I. *Neuron*. 2023;111(9):1440-1452.e5. doi:10.1016/j.neuron.2023.02.003. PMID: 36841241; PMCID: PMC10164063

Epilepsy is a major disorder affecting millions of people. Although modern electrophysiological and imaging approaches provide high-resolution access to the multi-scale brain circuit malfunctions in epilepsy, our understanding of how behavior changes with epilepsy has remained rudimentary. As a result, screening for new therapies for children and adults with devastating epilepsies still relies on the inherently subjective, semi-quantitative assessment of a handful of pre-selected behavioral signs of epilepsy in animal models. Here, we use machine learning-assisted 3D video analysis to reveal hidden behavioral phenotypes in mice with acquired and genetic epilepsies and track their alterations during post-insult epileptogenesis and in response to anti-epileptic drugs. These results show the persistent reconfiguration of behavioral fingerprints in epilepsy and indicate that they can be employed for rapid, automated anti-epileptic drug testing at scale.

Commentary

Epilepsy is characterized by spontaneous recurrent seizures, most of which associate with sudden changes in motor behavior. Many of these changes—twitching or jerking motions, freezing, staring into space, collapsing—are easily observed. But more subtle ones such as a short lapse of consciousness or quick bout of chewing may go unnoticed by the human eye. Moreover, seizures represent just one side of epilepsy. A variety of comorbidities (motor, speech, cognitive, and social deficits, as well as anxiety, depression, etc.) drastically decrease the quality of life of many individuals with epilepsy. These comorbidities manifest in complex and often subtle behaviors that may be more difficult than ictal behaviors to identify and quantify, both in patients and in animal models. To date, the assessment of behavior changes linked to epilepsy remains subjective, labor-intensive, and time-consuming even when done by trained professionals. Furthermore, these assessments concentrate on known and pre-selected behaviors. The ability to carry out more comprehensive and unbiased assessments of behavior in patients or animal models of epilepsy would greatly improve our ability to understand epilepsy as a whole, test potential therapeutics, and adapt them to individual patients.

To tackle this challenge, Tilo Gschwind and colleagues turned to computer vision and artificial intelligence (AI).[1] Their study, published in *Neuron*, shows that a faster, more precise, and less costly method to evaluate behavior in mouse models of epilepsy might be close at hand. The authors used movement analysis software called motion sequencing (MoSeq), which does not require human assistance to detect and identify behaviors, making it both unbiased and potentially able to discover new behaviors linked to epilepsy. Developed in 2015 by Sandeep Robert Datta and colleagues,[2,3] MoSeq is one of the most powerful animal behavior analysis methods based on AI to date. MoSeq breaks down complex behaviors into sub-second-long motifs called "syllables" (an analogy to birdsong) linked by transitions called "grammar." This breakdown allows researchers to analyze and quantify behaviors in unprecedented detail. Syllables such as "head down" or "turn right" are very different from classic definitions of complex behavior, such as anxiety-related thigmotaxis in the open field or dark chamber preference in the light/dark box, used in neuroethology. But they can be more easily detected and identified with computer vision than complex behaviors and are starting to show their predictive value. Gschwind and colleagues are the first to investigate AI and computer vision as tools to assess epilepsy-related behaviors in preclinical studies.

The authors used 2 well-known means of inducing epilepsy in mice: intrahippocampal kainic acid injection to model acquired temporal lobe epilepsy (TLE),[4,5] and a heterozygous mutation of the *Scn1b* locus (*Scn1b*$^{+/-}$ mice) to model Dravet syndrome,[6] a type of genetic epilepsy. Mice underwent recordings using depth video cameras for 1 hour in an open field setup in which they moved freely. Recorded videos were then analyzed offline. Remarkably, using only these presumably

inter-ictal recordings, MoSeq distinguished mice with epilepsy from nonepileptic controls faster and more accurately than did trained human observers, demonstrating the value of data- versus human-driven behavior analysis. In the process, MoSeq discovered that female but not male *Scn1b*$^{+/-}$ mice over- or under-performed certain syllables compared to littermate controls, which had escaped classic behavioral assessments. The significance of these sex-specific behaviors remains to be investigated, but they illustrate MoSeq's promise for the discovery of new and subtle behaviors that could be correlates of epilepsy. In the TLE mouse model, MoSeq revealed how behaviors changed between kainate injection and the manifestation of epilepsy, a period known as epileptogenesis. If these findings translate to human behaviors, they could help link certain behaviors to the eventual development of epilepsy, and predict which patients are at risk for acquired epilepsy after brain insults. Importantly, MoSeq proved capable of differentiating mice that had received different doses of 3 common antiseizure medications (ASMs), levetiracetam, phenytoin, and valproic acid. This feature provides the field with a much-needed opportunity to study on- and off-target effects and precisely tailor the dose of ASMs in preclinical studies. Finally, using a third mouse model, where seizures are induced via electrical stimulation of the hippocampus,[7,8] the authors showed that MoSeq could segment seizure behaviors in a manner similar to the traditional Racine scores based on human observation, confirming the credibility of MoSeq's analysis for both ictal and inter-ictal behaviors.

While these findings demonstrate the potential of MoSeq for epilepsy research and drug screening, they also raise new questions and open new avenues of research. First, what do the altered behavioral motifs in between the seizures mean? We know that abnormal brain activity may occur between seizures, in the form of inter-ictal spikes and non-convulsive discharges that are only detected by EEGs. Do any of the behaviors picked up by MoSeq coincide with these inter-ictal spikes or discharges? If so, they might be a direct readout of epileptic activity rather than of a comorbidity. Second, one of the most devastating and stigmatizing aspects of epilepsy for patients and families is the unpredictability of seizures; might MoSeq identify behavioral patterns indicative of an impending seizure hours or minutes before the seizure occurs? At the moment, EEG or intracranial recordings are the most reliable way to anticipate seizures, but continuous monitoring of brain activity is impractical for patients. Moreover, to be predictive, EEGs may need to tap the seizure-initiating focus, which can be challenging to locate in epilepsies that implicate complex networks. Third, and on a related note, might MoSeq discover behavioral patterns that are specific to the epileptic circuit involved? A study by the Datta lab showed that MoSeq syllables correlate with particular activities in the dorsolateral striatum.[9] Might MoSeq reveal behavioral signatures of thalamocortical versus temporal lobe epilepsies and the networks involved? Answering these questions will require combining MoSeq with EEG and intracranial electrical recordings. This type of combination has already proven doable with a platform such as Consistent EmBeddings of high-dimensional Recordings using Auxiliary variables (CEBRA), an AI-driven technology capable of mapping behavioral actions to neural activity across species.[10]

Some computer vision methods to detect animal behavior use black box AI, a type of AI based on deep learning neural networks that are so complex that the decision-making process cannot be explained in a way that humans can understand easily. This complexity makes it difficult for humans to remove biases or troubleshoot logical errors the AI might make, which is a serious concern for applications of AI to self-driving cars or medical diagnoses. In preclinical research, another concern is whether the AI's decision process reflects plausible biology. However, MoSeq uses transparent AI based on Hidden Markov models, a statistical method that can be explained in detail. This means that it is possible to understand how MoSeq works, and to design ways to improve its performance.

The study by Gschwind and colleagues will open novel avenues of epilepsy research. It is also remarkably timely. With the ever-increasing number of animal models of epilepsy and volume of behavioral data, there is a dire need to process data quickly and efficiently. The last 5 years have seen an explosion in technology for detecting and quantifying animal behavior with AI, and similar technologies for EEG-based seizure detection are following closely. These technologies are expected to propel epilepsy research forward by facilitating the rigorous screening of large numbers of animals, biological variables, or drug doses, in a noninvasive way.

The next big question is whether this powerful technology can one day be applied to treat epilepsy in patients. Ideally, AI-assessment of the patient's body language would predict whether they are likely to have a seizure, whether their medications are effective, or how treatment could be tailored to their specific symptoms. Given the current momentum of technologies such as MoSeq, this scenario may not be far-fetched.

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Declaration of Conflicting Interests

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