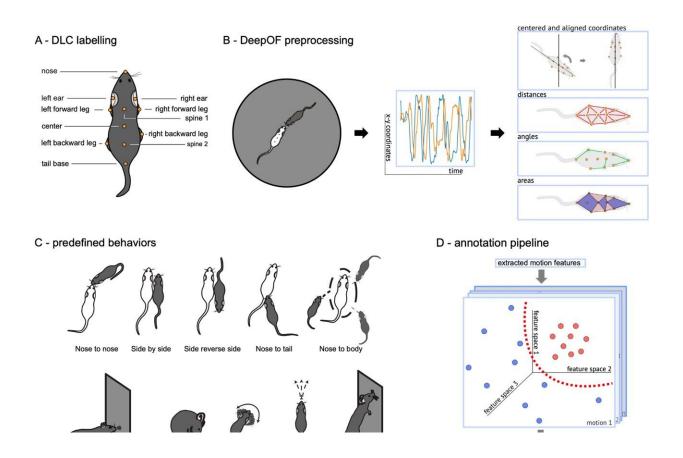


Machine learning meets behavioral neuroscience: Allowing for a more precise phenotyping

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DeepOF workflow. A 11 labels were tagged on each annotated mouse using DeepLabCut. **B** DeepOF preprocessing pipeline. One or two mice (a C57Bl/6N experimental subject and a CD1 social companion depending on the dataset) were tagged using the provided DeepLabCut models. After tracking body parts with DeepLabCut, DeepOF was used to smooth the retrieved trajectories, interpolate outliers, and extract features (including coordinates, distances, angles,



areas, speeds and accelerations). C Set of predefined behaviors that the DeepOF supervised pipeline can retrieve. These include dyadic motifs (such as nose-tonose contacts) and individual motifs (such as climbing), which are reported individually for all tracked mice. The stopped-and-huddled classifier²⁸ is abbreviated as "huddle" in DeepOF output (not to be confused with group huddling behavior⁶⁷). **D** Schematic representation of the supervised pipeline in DeepOF. A set of extracted motion features (only three dimensions are shown for visualization purposes) are fed to a set of rule-based annotators and pretrained classifiers, which report the presence of each behavioral trait at each time by learning how the corresponding trait is distributed in the feature space (red dots). The set of classifiers then yields a table indicating the presence of each motif across time, which can be used for further analysis. Note that annotators are not necessarily mutually exclusive, as several predictors can be triggered at the same time. E Graph representation of animal trajectories used by DeepOF in the unsupervised pipeline. All 11 body parts per animal are connected using a pre-designed (but customizable) adjacency matrix. Nodes are annotated with x, y coordinates and speed of each body part at each given time, and edges with the corresponding distances. This representation can also handle multi-animal settings, where the graphs of individual animals are connected with nose-to-nose, nose-to-tail, and tail-to-tail edges. F Schematic representation of the deep neural network architecture used for the unsupervised clustering of behavior. Data is embedded with a sequence-aware spatio-temporal graph encoder, and clustered at the same time by selecting the argmax of the likelihood of the components of a mixture-of-Gaussians latent posterior. Unidirectional black arrows indicate forward propagation, and gray arrows indicate the reconstruction and KL divergence terms of the loss function, the latter of which minimizes the distance to an also mixture-of-Gaussians prior. Credit: Nature Communications (2023). DOI: 10.1038/s41467-023-40040-3

A new computer program allows scientists to observe the behavior of multiple animals simultaneously and over extended periods, while automatically analyzing their motion. What may seem obvious marks a significant milestone, and paves the way for robust and accessible standardization and evaluation of such complex observations.



Imagine a researcher in the 19th century wearing a pith helmet, observing animals in their natural habitat. Or envision Konrad Lorenz, a veteran of the Max Planck Society, in the 1970s closely following his gray geese near Lake Starnberg—the beginning of behavioral research involved observing and recording what one sees.

The next step took place in the laboratory, where standardized environments were created to establish comparability. Researchers gained invaluable insights, but there were always limitations: the environment and test setup, the number of animals, and the duration of the observations did not correspond to the complexity of certain natural behaviors, either individual or social.

Moreover, observing animal behavior aims not only to better understand how specific species react to given stimuli, but also to help researchers better define mental disorders in humans in order to provide improved and individualized treatment.

A few years ago, scientists achieved a breakthrough using the opensource toolbox DeepLabCut. They were not only able to track the center point of individual animals in simple environments but also automatically detect the complex body posture of multiple animals in real-world environments. This paved the way for the development of new tools capable of extracting information from these data, as capturing posture is not the same as analyzing the underlying behaviors.

Linking movement to behavior

Two research groups at the Max Planck Institute of Psychiatry took on this task. The teams led by Mathias V. Schmidt and Bertram Müller-Myhsok developed a Python package called DeepOF, which links the position of individual body markers over time with behavioral patterns. This allows them to analyze the behavior of animals, in their case mice,



in a semi-natural environment in detail over any desired timeframe.

Two different approaches are used. In a supervised analysis pipeline, behaviors are predefined based on body postures over time, and the obtained data can be directly read and analyzed.

"Even more exciting is the unsupervised analysis pipeline," says statistician Müller-Myhsok. "Our program searches for similar behavioral episodes and classifies them," biologist Mathias Schmidt adds, "This approach opens up entirely new dimensions, enabling hypothesis-free automated investigation of complex social behavior and yielding highly interesting results."

This type of tool opens up new possibilities and brings behavioral biology, in terms of complexity, to a level comparable to molecular or functional biological analysis methods.

"In the future we can now better combine our results with other measurement dimensions, such as EEG recordings, neural activity data, or biosensor data," reports biologist Joeri Bordes. Lucas Miranda, the author of the DeepOF program, is enthusiastic about "<u>open science</u>" because "our program is freely available to researchers around the world, our code is of course open, and anyone is welcome to contribute to the project."

Nature Communications has given the program an independent seal of approval by publishing the teams' study. Moreover, thorough code and functionality reviews were assessed by the Journal of Open Source Software (JOSS). The program also represents an improvement for <u>animal welfare</u>, as the <u>animals</u> are subjected to fewer experiments.

Ultimately, the detailed analysis of <u>behavior</u> through this new dimension represents a significant step towards better translating the data regarding



the exploration of human diseases and their treatment.

More information: Joeri Bordes et al, Automatically annotated motion tracking identifies a distinct social behavioral profile following chronic social defeat stress, *Nature Communications* (2023). DOI: 10.1038/s41467-023-40040-3

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