Trajectory Data Mining

in Mouse Models of Stroke

Justin Shenk

Author
Justin Shenk

Cover illustration
Hannah Payette Peterson

Printer
Ipskamp, Enschede, The Netherlands

ISBN
9789462848887

This document was typeset with LaTeX using classicthesis developed by André Miede and Ivo Pletikosić, based on Robert Bringhurst's *"The Elements of Typographic Style"* and prepared for print by Theo Hafmans.

Georg Duffner's *EB Garamond* type-faces are used. Monospaced text uses Roboto Mono.

The publication of this thesis was financially supported by the Department of Medical Imaging, Anatomy, Donders Institute for Brain Cognition & Behaviour, Radboud university medical center.

Trajectory Data Mining

in Mouse Models of Stroke

Proefschrift ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van promoties
in het openbaar te verdedigen op

dinsdag 4 oktober 2022
om 14:30 uur precies

door

Justin Christopher Shenk
geboren op 30 juni 1985
te Texas (Verenigde Staten)

Promotor
prof. dr. A.J. Kiliaan

Copromotor
dr. M. Wiesmann

Manuscriptcommissie
prof. dr. J.A.W.M. van der Laak
prof. dr. J.R. Homberg
prof. dr. K.-U. Kühnberger (University of Osnabrück, Duitsland)

[The universe] cannot be understood unless one first learns to comprehend the language and to read the alphabet in which it is composed. It is written in the language of mathematics, and its characters are triangles, circles and other geometrical figures, without which it is humanly impossible to understand a single word of it.

Galileo Galilei, *The Assayer*

What more can I say? I wouldn't be here today if the old school didn't pave the way.

Maxwell Dixon, *"Dedication"*

In memory of Rhonda and Ellis Shenk.

CONTENTS

1

INTRODUCTION

Neurological diseases are among the leading causes of death and disability today [1]. Research on these diseases–including stroke, Parkinson's disease, Alzheimer's disease (AD), and many others–is thus crucially important, at both the basic and clinical level for the development of preventatives and/or interventions. Technological development is changing how research in this field is carried out, as new tools make both collection and analysis of large amounts of behavioral phenotype data easier. For preclinical research involving behavior and locomotion in rodents, automated home cage monitoring systems enable the collection of data 24/7 with minimal interaction with animals. In addition to reducing the amount of stress on animals and eliminating many other confounding factors, automated home cage monitoring expands the scope of the parameters that can be measured and the total amount of data collected, which also creates a need for new software to process these data.

Traja, the novel software developed and presented in this thesis, is one such tool designed for the processing and analysis of large amounts of movement tracking data. Indeed, many fields of scientific research are moving towards a more data-driven approach, and tools like Traja will enable researchers to open up new avenues of scientific discovery.

1.1 AIMS OF THE THESIS

Main contribution

This thesis introduces Traja, a new, openly available software built for analysis of behavioral data collected with home cage monitoring. With the creation of this software, we have developed a method for data collection and analysis that opens up a new, previously inaccessible avenue of research in neuroscience. Increased application of advanced and state-of-the-art modeling techniques allow for the identification, description and visualization of previously undetectable patterns in movement data. This software framework employs methods such as automated pattern and anomaly detection, dimensionality reduction, and deep neural networks (Figure 1.3), which have never before been applied to mouse home cage locomotion data.

The primary use case for Traja is with automated home cage monitoring of animals, which provides many advantages over traditional locomotion and behavioral testing. To this end, the thesis includes two studies (Chapters 3 and 4) examining the effect of diets on mice following ischemic stroke. In this research, Traja was used to measure recovery of the animals by the analysis of locomotion data collected from mouse home cages.

Scope of research

The intended audience of the research in this thesis includes researchers looking to improve work in laboratory animal sciences (see §1.2.4 below and §5.1 for an extended discussion), as well as neuroscientists interested in finding new patterns in animal behavior, especially those handling large amounts of data, and extending the analysis of movement data to advanced computational and statistical techniques. Further, this thesis is also relevant to biologists and neuroscientists interested in computational and data science tools (Figure 1.2) and how these can revolutionize their research strategies.

Description of methods is given at a level sufficient to encourage exploration of data and prototyping of approaches, and is thus application- rather than theoretical-focused. For this reason, some technical concepts are treated lightly, while others assume comfort with reading mathematical expressions at the level of an introductory course in machine learning, in order to provide the reader with sufficient context. For the reader interested in an extensive treatment of machine learning, please see Christopher Bishop's excellent introduction [2].

1.2 LOCOMOTION ANALYSIS IN NEUROSCIENCE RESEARCH

Abnormalities in movement can be an indicator of cognitive impairment associated with neurodegenerative disorders due to aging and disease [3]. Examples of such diseases marked by changes in movement include Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Huntington's disease, and stroke, among others. Since many of these neurological conditions can be studied using animal models, non-invasive methods of diagnosing and following the progression of disease and recovery are crucial for research in this area. Thus, locomotion analysis (also referred to as gait analysis) — a subset of behavioral tests that examine information about the motion of animal models or human subjects — has historically been an effective and invaluable tool [4, 5]. Similar forms of gait analysis can be useful in clinical settings [6]. Locomotion analysis may also enable earlier diagnosis of diseases than other methods, with both animal models and human patients, due to early manifestation of symptoms [7–9]. For many neurological conditions, including Parkinson's and stroke, early diagnosis can drastically improve the chances of successful therapeutic intervention. As technology improves, research has begun to shift from using traditional gait analysis tools towards using systems of automated home cage monitoring (HCM) [10, 11]. These methods allow for continuous

tracking of locomotor activity, circadian rhythms, and other behaviors in rodents [12–14].

Tools traditionally used for locomotion analysis generally fall within two categories: sensor platforms (including computerized visual systems) or wearable sensors [3]. Sensor platforms usually measure movement parameters when test subjects are walking on them. They can be useful for studies carried out in a lab environment and do not require subjects to wear potentially uncomfortable sensors, but are not always easily portable or useful for measuring daily activity. Wearable sensors can collect data while a subject performs daily activities, but can be uncomfortable to wear and are more often used with human subjects rather than in animal research. These methods of locomotion analysis have traditionally been coupled with other behavioral tests in the evaluation and tracking of sickness and recovery in rodent models of neurological diseases.

New systems of automated HCM improve upon these more traditional techniques. The ability of researchers to record the movement or position of rodents 24 hours a day provides new advantages: rodents are recorded constantly including at night when they are naturally most active, a wide range of behaviors is captured without the need to disturb the rodents, and the reduction of animal handling can reduce experimenter influence [12–14] and stress for the animal, because it can stay in the home cage instead a new experimental environment [15, 16]. These new methods of HCM result in the collection of large amounts of data; thus, new tools for analysing this data are needed, which elevates the importance of software designed for this purpose. Traja is a new software developed in this PhD study for the purpose of analyzing HCM data.

1.2.1. *Neurological diseases characterized by movement abnormalities*

Locomotion analysis and now automated HCM have broad research and clinical applications due to the fact that many neurological diseases are characterized by irregularities in movement and behavior. These tools can be used for diagnosis as well as for monitoring the progression of and recovery from various diseases in rodents used for research purposes.

One such disease in which locomotion analysis serves as a relevant tool is Parkinson's. Indeed, neurologists have long observed that patients with Parkinson's disease demonstrate gradual changes in gait which are visible to the naked eye [17]. Gait patterns can also be an indicator of cognitive decline associated with Parkinson's [18]. Gait analysis can additionally be used to study Parkinson's in animal models [19, 20], which has pertinent applications for research on, for example, effect of interventions. Studies using automated HCM of Parkinson's mice models have helped to measure reduction of home cage locomotor activity [21, 22].

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that primarily affects motor neurons. Early symptoms can include muscle twitching, cramping, fatigue and difficulty swallowing and speaking, and then progress to paralysis [23, 24]. Over the progression of the disease, gait is also affected, and thus locomotion analysis can be used to aid in its diagnosis and observing the impact of treatments [25–27]. ALS can be difficult to diagnose early on in its progression, but locomotion analysis can improve the probability of early detection; indeed, it can be used to detect the development of ALS even before the onset of more noticeable symptoms [8]. One recent study using automated HCM also found that ALS mouse models exhibited irregular activity potentially indicating sleep disturbances [28].

In Alzheimer's disease, like many other forms of dementia, cognitive decline is often accompanied by gait disturbances [29, 30] as well as behavioral changes such as increased anxiety and hyperactivity [31, 32]. Because gait abnormalities vary according to the stage of the disease, gait can serve as an indicator of the severity of dementia [29]. Locomotion analysis also has the potential to be used as a tool for early diagnosis of Alzheimer's disease in human patients [33]. Automated HCM can also be used to characterize behavioral changes in mouse models of Alzheimer's disease [34].

Huntington's disease, an autosomal-dominant neurodegenerative disorder, is characterized by both cognitive and motor decline and is also marked by changes in gait [35, 36]. Mouse models of Huntington's have traditionally been studied using behavioral tests such as the rotarod and open field test [37]. HCM can also be used to examine the behavior of animal models, and studies using these tools have found early behavioral disturbances such as repetitive behaviors, increased drinking and sleep disturbances followed by an overall decrease in motor activity as the disease progresses [38–42].

Finally, stroke is often characterized by both motor and cognitive impairment [43–45]. Motor impairment due to stroke can be studied using locomotion analysis or HCM in the context of rodent models [46–48]. The mouse stroke model is the primary focus of the studies included within this thesis, and it will be further discussed in §1.2.2.

Movement and behavioral disturbances are a valuable indicator of neurological disease or injury (Figure 1.1). Movement analysis can also be used to track recovery after injury and assess the effectiveness of preventative measures or treatments. It is useful to understand the broad applications for locomotion analysis and HCM across many diseases in both clinical and preclinical research settings, and therefore the potential for the usage of HCM data analysis software such as Traja in other contexts in addition to those detailed in this thesis.

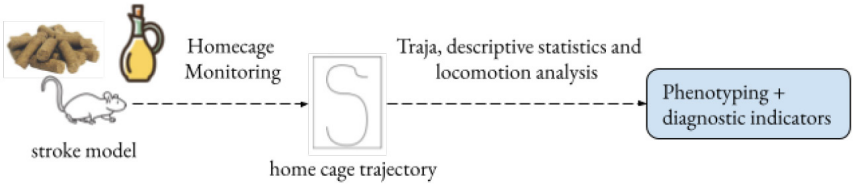


Figure 1.1: Homecage monitoring can be used to observe phenotype in stroke mouse models. The studies performed in this PhD presented in Chapters 3 and 4 of this thesis are interested in analysis of behavior as an indicator of dietary treatment efficacy in single-housed mouse stroke models.

1.2.2. *Vascular dementia and stroke*

Vascular dementia is the second most common form of dementia after Alzheimer's disease [49–52]. It is caused by reduced blood flow to the brain (hypoperfusion) due to stroke, and there are multiple subtypes of vascular dementia, including single-infarct dementia, multi-infarct dementia, hereditary vascular dementia (CADASIL) and others [49, 50, 52]. Risk factors for both AD and vascular dementia include age, diabetes, hypertension, and metabolic syndrome (including obesity) [49, 50, 52, 53]. Hypoperfusion is suggested to play an important role in cognitive impairment and dementia, and can also lead to motor impairment [45, 46]. Further, several studies suggest that stroke and AD are comorbid [54]. Indeed, it is one of the most common causes of death and disability worldwide [55–57]. As the average age of the general population increases, the prevalence of stroke also increases [56]. Research in this field, both in clinical and laboratory settings, is therefore increasingly important.

Research into stroke can lead to more effective methods of diagnosis and the ability to monitor patient recovery, as well as new methods of prevention and treatments. In a clinical context, gait analysis can be used to identify movement deficits and measure patient performance during recovery; findings from this research can then lead to improved diagnosis and treatment of post-stroke patients [46, 58, 59].

In a laboratory setting, rodent models of stroke are essential for studying potential new treatments and tools for stroke prevention. There are many different rodent models commonly used in research, including middle cerebral artery occlusion (MCAo) in rats or mice, photothrombosis, embolic MCAo, and endothelin-1 application, among others [60, 61]. Mice subjected to MCAo, for example, perform less successfully than control mice on tests to assess behavioral aspects after stroke, including gait analysis [62, 63], Rotarod [64], Pole test [65, 66], and Morris water maze [67].

A common model for studying ischemic stroke in mice, transient MCAo (tMCAo) has several advantages including relatively non-invasive surgery and ability to parameterize the severity by modifying the occlusion time [61]. In tMCAo, surgery can be performed in a short time period, thus limiting confounders such as effects from extensive anesthesia, and it produces highly reproducible lesions. In Chapters 3 and 4 of this thesis, the stroke model used is a 30-min tMCAo induced in mice [47, 48, 68, 69]. This model involves the proximal occlusion of the middle cerebral artery for 30 minutes with a filament, and mimics one of the most common forms of ischemic stroke in humans [70,71].

1.2.3. *Traditional methods of locomotion and behavioral analysis*

Previously, diagnosis of neurological diseases and monitoring of recovery in both rodents in laboratory studies and patients in clinical settings was carried out using a combination of locomotion analysis and behavioral tests [3].

One of the simplest traditional methods of locomotion analysis is to apply ink to the paws of rodent models or feet of human subjects and have them walk across paper to record the prints [3, 72]. Many researchers today use electronic systems of locomotion analysis, such as pressure-sensitive platforms [73, 74] and transparent platforms through which gait can be recorded on video [75]. There are also commercially available tools for locomotion analysis, such as DigiGait (Mouse Specifics, Inc.), CatWalk (Noldus IT) and GAITRite (CIR Systems, Inc.) [3]. DigiGait records high-speed video of the underside of mice as they walk on top of a transparent treadmill, which can then be analyzed to examine gait [76–78]. CatWalk is a camera-based gait analysis system that records the unforced movement of mice or rats over a transparent platform in an enclosed walkway [79–81]. GAITRite is a portable walkway with embedded pressure sensors that can measure spatial and temporal gait characteristics and is designed for use with human patients [82]. In addition to sensor platforms as described above, there are many wearable sensors which are more commonly used with human patients. These include the SmartShoe (developed by Sazonov et al. [83]) [84–86], GaitShoe (developed by Bamberg et al. [87]), ForceShoe (Xsens) [88], and CODA motion analysis system (Charnwood Dynamic Ltd.) [3, 89, 90], among others.

In laboratory research settings, locomotion analysis is typically paired with other behavioral tests used to diagnose and monitor neurological injury. Among rodents, some of the most commonly used tests include the Morris water maze and radial arm maze to test spatial learning and memory, the corner test to assess sensorimotor and postural asymmetries, and the open field test to assess exploratory behavior and generalized locomotor activity. These tests are explained in further detail in §4.2. While these tests benefit from broad

usage among animal researchers, there are several limitations to manual testing: i) mice are nocturnal, but mostly tested during the day period, ii) they only capture a snapshot of behavioral phenotype, and iii) they risk researcher bias and interference [14]. As a result, animal welfare (based on the principle of the 3RS - reduce, refine and replace) and reproducibility have been called into question. HCM has the potential to address these concerns by reducing animal usage and refining experiments to improve animal welfare and complement the description of the animal model to offer better translation to human studies. A discussion of animal welfare and the potential of HCM to increase efficiency is provided in §5.1.

1.2.4. *Movement towards automated home cage monitoring*

In laboratory research, new technologies have resulted in a shift from gait analysis techniques and behavioral tests towards automated HCM. This allows for constant recording of the movements of rodents and, as previously mentioned, provides many new advantages over traditional methods. There are multiple tools available for automated HCM, including PhenoTyper (Noldus IT), PhenoMaster (TSE Systems), Intellicage (TSE Systems), Home Cage Analyser (Actual Analytics), and Digital Ventilated Cage (Tecniplast SpA) [12, 91–94]. These automated HCM systems use a variety of different methods for tracking animal movement, such as video, infrared sensors, or microchips and antennae. The Digital Ventilated Cage (DVC), which is the HCM system used in this thesis, continuously tracks the position of animals via electrical capacitance [92, 93] and has been used to identify behavioral phenotypes related to change or shift in circadian rhythm [95] as well as recovery from stroke [47, 48, 96].

1.2.5. *Challenges and limitations of HCM*

HCM can greatly increase the variety and volume of data by tracking movement 24/7, and can reduce animals' stress by decreasing unnecessary interactions with researchers and testing in a new environment. It is thus promising for the future of research into neurological, movement and behavioral disorders.

While HCM systems have several advantages for identifying behavioral phenotypes (see below and §5.3.1 for a complete comparison), there are new challenges posed by this shift towards automated movement analysis, such as greater reliance on individual housing, lack of ability to measure behaviors aside from movement tracking, and technical limitations of data capturing and analysis.

HCM like DVC is currently only capable of tracking individual mice, necessitating single housng. Social isolation of home cage environment can lead to drifts in behavior [97] and possibly diminished validity. For example, in one study involving socially-isolated mice in PhenoTyper cages during an 8-day recording period, total daily distances gradually declined with similar increases in total daily sleep, as well as feeding and licking times [98]. Controlling for the effects of social isolation may be achieved by enriching the environment with objects such as running wheels, which have been shown to ameliorate several aspects of neuropsychiatric symptomology in rodent models [99]. In addition, several studies have demonstrated the ability to track animals in pairs or group housing using RFID-based individual identification [100–102] or fluorescent markers [103]. These approaches involving enriched environments and complex tracking favor end-to-end HCM systems that can more easily integrate various types of sensors, such as from running wheels, or that can track multiple animals simultaneously, a task not possible with the resolution provided by the DVC data.

HCM systems like DVC only track the movement of rodents. While this is useful, it doesn't capture the whole range of behaviors, for example, whether the animal is sitting and grooming or sleeping. Other systems exist which can infer more high-level behaviors for individually-housed mice like grooming, rearing, sniffing, digging etc. [104, 105], however they generally require more space for housing and larger equipment than the DVC, making them less practical for large-scale studies.

Video HCM systems, which may capture a wider range of behaviors, carry other limitations. Camera-based monitoring systems have a greater reliance on environmental factors such as lighting and spatial constraints. For example, infrared-based systems require consistent infrared lighting to provide tracking in dark phases, however are somewhat limited in their ability to distinguish between individual subjects within a group [98]. Some classical movement behavior tests like spatial memory tasks be difficult to implement with HCM when the task requires spatial separation from the home cage [106]. Thus, extending HCM to testing in locations separate from the home cage may require additional equipment or software [97]. Additionally, some home cage environments may not be monitored with a single camera or sensor but may require multiple cameras or sensors, increasing the complexity of the data collection. The ability of DVC to track mice without additional cage space is a particular advantage of the system [92], which enabled the large-scale studies present with minimal intervention in animal handling.

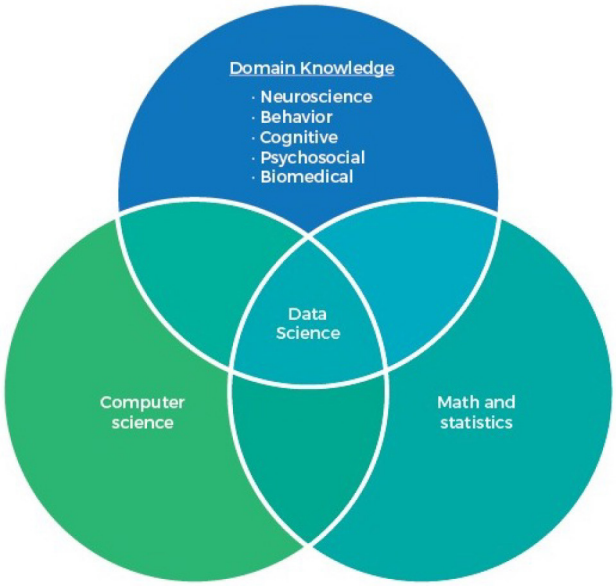


Figure 1.2: Data Science in neuroscientific research (2013) [107].

A further significant challenge for implementing HCM systems is the extensive data collection and management required. Detection of movement in several days of HCM data requires optimal processing and interpretation of high-throughput data, such as video or sensor data. Such data is often only available offline, thus limiting the observer from intervening until the event as passed. Further, the large volume of data involved carries an increased risk of tracking errors, which can increase the probability of outliers or anomalies in the data.

Indeed, the shift towards automated HCM creates a need for user-friendly software to screen and analyze large amounts of data collected from the home cage over extended periods of time to ensure data and measurement quality. The combination of software with domain expertise and mathematical and statistical techniques is commonly referred to as "data science," owing to the unique tools and skills needed (Figure 1.2) for working with data and the hypothesis-driven approach [107]. Existing software such as the R package Trajr [108] are not designed for HCM data: they are either not flexible enough to use raw centroids, are not extensible to advanced methods of modeling, such as deep learning (see §1.3), or are not available in the common data science language Python (see Table 5.2 for a complete comparison of existing package features). This thesis addresses this need with the development of Traja, a software toolkit designed for trajectory analysis of animal models.

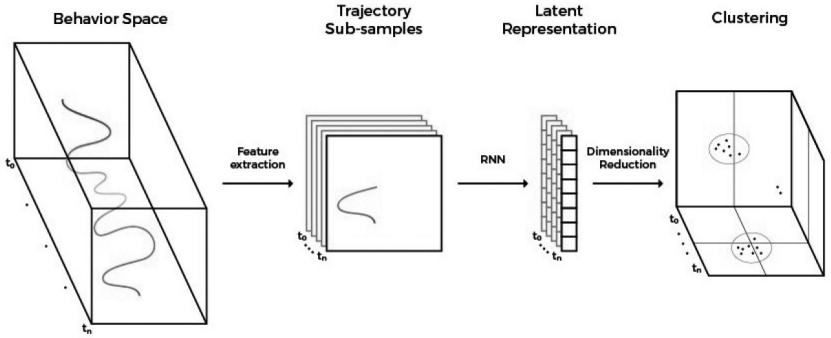


Figure 1.3: Increased amounts of data combined with statistical learning methods allow data-driven modeling of animal behavior for neuroscientific and medical research. Advanced methods such as recurrent neural networks (RNNs) are implemented in Traja (§2.1.10). An overview of methods implemented in Traja can be found in Chapter 2.

1.3 TOWARDS DEEP LEARNING FOR TRAJECTORY DATA MINING

Animal and human motility researchers traditionally relied on painstaking data collection methods (e.g. observational studies, mark-recapture, travel diaries) and/or aggregate data (e.g. seasonal distribution maps, origin-destination flows, intercept counting) [109]. Technological breakthroughs have led to not only increased usage of automated HCM in laboratory settings, but also an increase in the use of Global Positioning System (GPS) for analyzing motion of animals in ecological research [110].

Use of GPS data and other tracking techniques has provided insights into animal behavior in the wild, such as foraging and mating. Availability of tracking data has allowed large-scale, high resolution analysis of animal movement and trajectories [111]. With the rapid development of information and communication technology, the amount of data is growing at a rapid rate. For example, the healthcare system in the United States alone produced more than 150 exabytes (10^{18}) in 2011 [112] and the worldwide health records were expected to reach 40 yottabytes (10^{24}) in 2020 [113]. In the biomedical informatics domain, the volume of data is growing exponentially [114–117]. This big data is generally defined by Dash et al. [118] as data which is "large and unmanageable using traditional software or internet-based platforms." Such a growth in data implies considerable opportunity for identifying patterns in clinical and biomedical data, leading to more efficient treatments.

Extracting useful information from large datasets is often referred to as data mining, or "knowledge discovery" [119, 120]. Data-driven modeling of movement allows identifying patterns in an automated fashion, thus leading to improved methods for diagnosis and prognosis and thus improved

strategies for treatment [121] (Figure 1.3). Broadly speaking, virtually any pattern which is apparent to a trained researcher is theoretically perceptible to a machine learning system trained on sufficient data. Automated analysis of locomotion opens the possibility of identifying patterns which are *not* apparent to a trained researcher, due to effects being manifested below a researcher's perceptive threshold. Cognitive-behavioral effects which are manifested over not only seconds but hours or weeks are thus within the potential scope of an automated pattern detector. Identifying inexpensive, precise, and real-time effects of interventions for stroke from non-invasive biomarkers is the holy grail of clinical research model development.

Previous examples of modeling animal movement in ecological contexts with deep learning include interpolating paths for bird [122] as well as bears [123], seals [124], mice [125], worms [123], and insects [126]. Modeling behavior with deep learning involves extracting features from the movement data and identifying latent numerical representations (or variables, in statistical terms) which predict the underlying dynamics which in turn lead to expression of certain bhaviors. Similar techniques are used in application to pedestrian path modeling [127] as well as particle motion analysis [128]. Crucially, Traja enables mining patterns in animal trajectories, owing to its implementation in Python, the *de facto* programming language for machine learning, and integration with deep learning libraries (see §2.1). Further discussion of modeling behavior with advanced techniques is provided in §2.1.10.

1.3.1. *Machine learning*

Machine learning is the study of computer algorithms that automatically improve performance through experience. The field combines approaches from computer science, statistics, probability theory, optimization theory, decision theory and mathematics. It is generally different from mathematics or statistics, where mathematical rules are applied primarily for inferring the relationship between variables, whereas machine learning seeks to identify generalizable predictive patterns [129]. Many types of tasks can be solved with machine learning, for example:

- *forecasting* disease trajectories [130]
- image-based *classification* of tumor type or growth rate [131]
- *regression analysis* to predict life expectancy [132]
- *clustering* clinical data into profiles [133]
- *recommendation systems* for clinical decision support [134]
- *generation of data* such as realistic medical images [135]

Distinctive of machine learning algorithms as opposed to traditional modeling techniques is the relevance of the amount of data to train the models [2]. Generally speaking, the more clean data available, the better the models.

A remarkable feature of machine learning is that it allows one to make sense of data about which one has no prior understanding nor domain specific knowledge. Machine learning has been used to model animal trajectories [122, 123].

Machine learning has become increasingly relevant to biological research in recent years due to the following factors:

- increase in accessibility and number of sensors which collect data
- decrease in size, cost, and energy requirements of sensors
- increase in computational power and development of algorithms and tools for handling large amounts of data.

1.3.2. *Deep learning*

While prior research in modeling human and animal locomotion mainly relied on hidden Markov models[1] and rule-based approaches, significant advances have been made in recent years by learning representations of trajectories via neural networks. Deep learning has emerged as a powerful tool enabling solving challenges in computer vision [136] such as medical imaging [137], as well as neuroscientific applications such as analyzing brain activations [138].

[1] *see §2.1.10*

Deep learning is a subset of machine learning where models are structured into sequential "layers" in an artificial neural network (Figure 1.4). A neural network is a biologically-inspired computational graph organized into layers of computational units (*neurons*) which fire sequentially based on their inputs. Neural networks are organized into an input layer which receives features of the data, one or more hidden layers, and an output layer. Deep neural networks are distinguished by the plurality of hidden layers which make a model "deep" and allow complex computational routines for learning efficient representations of the data; this is particularly useful for high-dimensional, unstructured data. Deep learning has led to breakthroughs in biomedical and neuroscientific research such as with medical imaging [139] and neural decoding [138]. They have distinct advantages over traditional methods, such as being non-parametric, self-learning, noise-tolerant and having minimal assumptions [140].

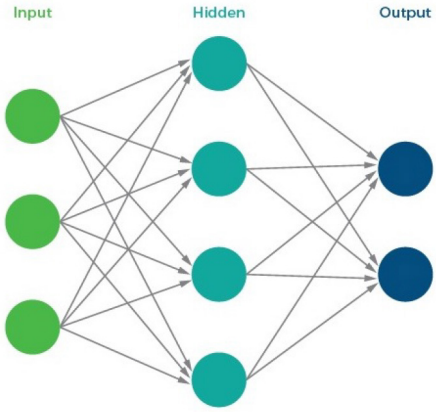


Figure 1.4: Neural network.

1.3.3. *Deep learning for trajectory analysis*

Deep learning has several applications for time series data ranging from stock price prediction to solving ordinary differential equations [141]. With sufficient volume and quality of animal trajectory data, one can build probabilistic models that describe present and/or future states, including:

- trajectory forecasting
- phenotyping
- diagnosis
- prognosis
- effectiveness of treatment.

Learning methods are further divided into classification, regression and clustering. In classification, a machine learning algorithm is trained on input data which classifies the unknown instances either into two classes (binary classification) or multiple classes (multiclass classification). However, in the case of regression, the output is continuous, for instance, prediction of house prices given that area of a house, age, and rooms. Both categories (classification and regression) are supervised techniques; clustering, however, is generally an *unsupervised* method where inputs are divided into groups and the identity of these groups are not previously known.

Various machine learning methods have been proposed for the above tasks such as logistic regression, linear discriminant analysis (LDA), decision trees, linear regression, K-means clustering, and recently deep artificial neural networks; these include convolutional neural networks, which learn translationally invariant features, typically of image inputs, and recurrent neural networks (RNNs), which learn time-dependent features of sequence inputs. Trajectory data mining is often performed with Long-Short-Term Memory (LSTM) networks, a type of RNN.

Dynamical processes such as locomotion can be modeled with continuous-time recurrent neural networks. An implementation of an LSTM with Traja is provided in §2.1.10. As opposed to classical sequence modeling methods such as Markov models, deep learning focuses on learning representations of the data in an end-to-end fashion that allows for solving a task without handcrafted features extracted from the data. A typical example of this is a face detector which learns to recognize faces based on seeing thousands of faces without any special underlying knowledge of the problem domain, simply by identifying edges and other abstract patterns of the images.

An introduction to the analytical and learning methods available in Traja is provided in Chapter 2.

1.4 DIETARY INTERVENTION DURING STROKE RECOVERY

We want to investigate the impact of dietary intervention on behaviors affected by stroke, and therefore we will use Traja software developed during this thesis to analyze mouse locomotion and activity during stroke recovery.

Generally, much emphasis is placed on pharmaceutical treatments after stroke [142, 143], and often too little attention is given to diet and exercise. However, research suggests that diet can play a significant role in the brain's recovery from injury. For example, the Fortasyn diet has been shown to improve cerebral blood flow (CBF) and neurogenesis in mice [69, 144] as well as motor function in male stroke mice [69]. In Chapter 3, we further examine the effects of this diet in mice after the induction of stroke. In order to do this, we measure the recovery of animals in both control and experimental groups. We use automated HCM to track mice in their home cages, and apply Traja to analyze movement and behavioral data. We demonstrate use of Traja with HCM for providing descriptive statistics of mouse behavior in the home cage, including comparing distance moved, velocity, and turning direction, and laterality.

A diet rich in hydroxytyrosol (HT) has also been shown to increase CBF and brain derived neurotrophic factor (Bdnf) in mice, indicating its potential as a beneficial neurogenic therapeutic approach. Consequently, in Chapter 4, we test the influence of HT treatment after induced stroke in mice. In this case, in order to measure recovery from stroke, we use Traja to analyze automated HCM data in conjunction with standard behavioral tests and found a left (ipsilateral) turn preference for stroke mice in home cage during nighttime.

1.5. THESIS OVERVIEW

In this thesis, I present Traja, a software framework for data analysis that opens up a new avenue of research in neuroscience that was not previously accessible through the standardization and application of data science methods for detecting patterns in behavioral data.

To assess its utility, we apply it to home cage mouse locomotion data collected during several experiments of dietary interventions for ischemic stroke. We use it to support analysis of circadian rhythms as well as various proxies for health and prognosis. Though Traja has multiple applications[1] (see Chapter 2), and has been recognized in the spatiotemporal analysis literature for animal trajectory analysis [145, 146], it is particularly useful for analyzing data from automated home cage monitoring, which records rodent activity and locomotion patterns 24/7 in the home cage environment without stressing the animals by bringing them into new test environments.

In §1.2, I introduce the biological context of the thesis, which sets up the importance of locomotion analysis in neurological research. In §1.3, I introduce the technical aspects which are relevant to the development of Traja. In §1.4, I introduce the application of Traja to HCM data in stroke mice.

The HCM data used in these experiments consists of noisy, high-volume raw centroid coordinates corresponding to the mouse position [92], collected over a period of 8 months. This data requires extensive cleaning and preprocessing in order to generate the reproducible, accurate statistical analysis. In Chapter 2, published in *Journal of Open Source Software*, I present and explain the computational and analytical methods available in Traja. There I describe various methods useful for preprocessing, analyzing, and modeling spatio-temporal data, using data derived from the study on multinutrient intervention after ischemic stroke in mice [48]. These methods include essential preprocessing techniques common to working with spatio-temporal data, including trip grid computation (Figure 2.2), feature scaling (§2.1.5), missing data handling (§2.1.5), dimensionality reduction (Figure 2.8), and a wide range of techniques used for exploratory analysis of movement data. Further, to support scientific reproducibility and transparency, I provide a link to the complete documentation of the API with complete descriptions of methods, data structures and examples of usage.

Then, I apply Traja to mouse home cage data collected with DVC in investigation of mouse models of stroke next to standard behavioral tests like open field, grip strength, and pole test. Chapter 3 is based on the paper "Automated analysis of stroke mouse trajectory data with Traja," which was published in *Frontiers in Neuroscience* [48]. In this chapter we explore the effects of receiving a multicomponent dietary treatment on stroke recovery. Using Traja, we analyze activity (§3.3.1), distance travelled (§3.3.2), velocity (§3.3.3), and turns and laterality (§3.3.4). I identify increased walked distance and velocity in both control and Fortasyn groups over time with Traja and DVC, which was not able to be seen in Open Field tests [147], and distinguish between changes in nighttime and daytime behavior (Figure §3.3.4).

1 Traja has reportedly been used by researchers in fields ranging from geoinformatic systems to moth flight analysis.

Chapter 4 is based on "Hydroxytyrosol, the major phenolic compound of olive oil as acute therapeutic strategy after ischemic stroke," published in *Nutrients* [47]. There, I apply Traja to the extensive study of stroke treatment in mice with hydroxytyrosol (HT), the foremost phenolic component in olive oil. In this study, we analyze the effects of light phase and HT treatment on activity, distance travelled, walking velocity, total turnings, and laterality index 24/7 (§4.5). We compare the laterality following surgery and identify a left turn preference in the home cage following stroke, whereas this was undetectable with traditional behavioral tests. These results confirm previous results with corner test where stroke mice exhibited preference towards the nonimpaired (ipsilateral) side in distal middle cerebral artery occlusion (MCAo) [148, 149] as well as combined distal and proximal MCAo [150], in contrast to sham mice which showed no directional bias. In these studies, I demonstrate that Traja combined with DVC confirms several results from traditional behavioral tests without experimental intervention, and is additionally able to identify distinct behavioral phenotypes not possible with traditional tests.

Chapter 5 provides a unifying discussion for this thesis. I address recent advances in technology used for behavioral analysis, particularly automated home cage monitoring, and how these create a need for software like Traja. I also further discuss the capabilities and advantages of Traja, and the applications of the software presented in this thesis. Finally, I consider other possible applications of Traja and reflect on how the software fits into the changing landscape of scientific research today.

2

TRAJA: A PYTHON TOOLBOX FOR ANIMAL TRAJECTORY ANALYSIS

Several tools exist for the analysis of animal locomotion. However, none are designed for working with high-throughput homecage data coordinates provided by systems employing movement sensors like DVC. Traja provides a way to automatically analyze such data, opening up a new, previously inaccessible avenue of research in neuroscience.

This chapter is published in Journal of Open Source Software as:
Shenk, J., Byttner, W., Nambusubramaniyan S., Zoeller, A. (2021). Traja: A Python Toolbox for Animal Trajectory Analysis. *Journal of Open Source Software* 6:3202. doi: 10.2110S/joss.03202.

2.1 SUMMARY

There are generally four categories of trajectory data: mobility of people, mobility of transportation vehicles, mobility of animals, and mobility of natural phenomena [151]. Animal tracking is important for fields as diverse as ethology, optimal foraging theory, and neuroscience. Mouse behavior, for example, is a widely studied in biomedical and brain research in models of neurological disease such as stroke.[1]

Several tools exist which allow analyzing mouse locomotion. Tools such as Ethovision [152] and DeepLabCut [153] allow converting video data to pose coordinates, which can further be analyzed by other open source tools. DLCAnalyzer[2] provides a collection of R scripts for analyzing positional data, in particular visualizing, classifying and plotting movement. B-SOiD [154] allows unsupervised clustering of behaviors, extracted from the pose coordinate outputs of DeepLabCut. SimBA [155] provides several classifiers and tools for behavioral analysis in video streams in a Windows-based graphical user interface (GUI) application.

These tools are primarily useful for video data, which is not available for the majority of animal studies. For example, video monitoring of home cage mouse data is impractical today due to housing space constraints. Researchers using Python working with non-visual animal tracking data sources are not able to fully leverage these tools. Thus, a tool that supports modeling in the language of state-of-the-art predictive models [127, 156, 157], and which provides animal researchers with a high-level API for multivariate time series feature extraction, modeling and visualization is needed.

Traja is a Python package for statistical analysis and computational modelling of trajectories. Traja extends the familiar pandas [158, 159] methods by providing a pandas accessor to the `df.traja` namespace upon import. The API for Traja was designed to provide an object-oriented and user-friendly interface to common methods in analysis and visualization of animal trajectories. Traja also interfaces well with relevant spatial analysis packages in R (e.g., trajr [108] and adehabitat [160]), Shapely [161], and MovingPandas [162] allowing rapid prototyping and comparison of relevant methods in Python. A comprehensive source of documentation is provided on the home page (`http://traja.readthedocs.io`).

1 The examples in this paper focus on animal motion, however it is useful for other domains.

2 `https://github.com/ETHZ-INS/DLCAnalyzer`

2.1.1 *Statement of Need*

The data used in this project includes animal trajectory data provided by Tecniplast S.p.A., manufacturer of laboratory animal equipment based in Varese, Italy, and Radboud University, Nijmegen, Netherlands. Tecniplast provided the mouse locomotion data collected with their Digital Ventilated Cages (DVC). The extracted coordinates of the mice requires further analysis with external tools. Due to lack of access to equipment, mouse home cage data is rather difficult to collect and analyze, thus few studies have been done on home cage data. Furthermore, researchers who are interested in developing novel algorithms must implement from scratch much of the computational and algorithmic infrastructure for analysis and visualization. By packaging a library that is particularly useful for animal locomotion analysis, future researchers can benefit from access to a high-level interface and clearly documented methods for their work.

Other toolkits for animal behavioral analysis either rely on visual data [153, 163] to estimate the pose of animals or are limited to the R programming language [108]. Prototyping analytical approaches and exploratory data analysis is furthered by access to a wide range of methods which existing libraries do not provide. Python is the *de facto* language for machine learning and data science programming, thus a toolkit in Python which provides methods for prototyping multivariate time series data analysis and deep neural network modeling is needed.

2.1.2 *Overview of the Library*

Traja targets Python because of its popularity with data scientists. The library leverages the powerful pandas library [158], while adding methods specifically for trajectory analysis. When importing Traja, the Traja namespace registers itself within the pandas dataframe namespace via `df.traja`.

The software is structured into three parts. These provide functionality to transform, analyse and visualize trajectories. Full details are available at https://traja.readthedocs.io/. The `trajectory` module provides analytical and preprocessing functionalities. The `models` subpackage provides both traditional and neural network-based tools to determine trajectory properties. The `plotting` module allows visualizing trajectories in various ways.

Data, e.g., x and y coordinates, are stored as one-dimensional labelled arrays as instances of the pandas native `Series` class. Further, subclassing the pandas `DataFrame` allows providing an API that mirrors the pandas API which is familiar to most data scientists, thus reducing the barrier for entry while providing methods and properties specific to trajectories for rapid prototyping. Traja depends on Matplotlib [164] and Seaborn [167] for plotting and NumPy [166] for computation.

Trajectory Data Sources
Trajectory data as time series can be extracted from a wide range of sources, including video processing tools as described above, GPS sensors for large animals or via home cage floor sensors, as described in the section below. The methods presented here are implemented for orthogonal coordinates *(x, y)* primarily to track animal centroids, however with some modification they could be extended to work in 3-dimensions and with body part locations as inputs. Traja is thus positioned at the end of the data scientist's chain of tools with the hope of supporting prototyping novel data processing approaches. A sample dataset of jaguar movement [167] is provided in the `traja.dataset` subpackage.

2.1.3 *Mouse Locomotion Data*

The data samples presented here[3] are in 2-dimensional location coordinates, reflecting the mouse home cage (25x12.5 cm) dimensions. Analytical methods relevant to 2D rectilinear analysis of highly constrained spatial coordinates are thus primarily considered.

High volume data like animal trajectories has an increased tendency to have missing data due to data collection issues or noise. Filling in the missing data values, referred to as *data imputation*, is achieved with a wide variety of statistical or learning-based methods. As previously observed, data science projects typically require at least *95%* of the time to be spent on cleaning, preprocessing and managing the data [168]. Therefore, several methods relevant to preprocessing animal data are demonstrated throughout the following sections.

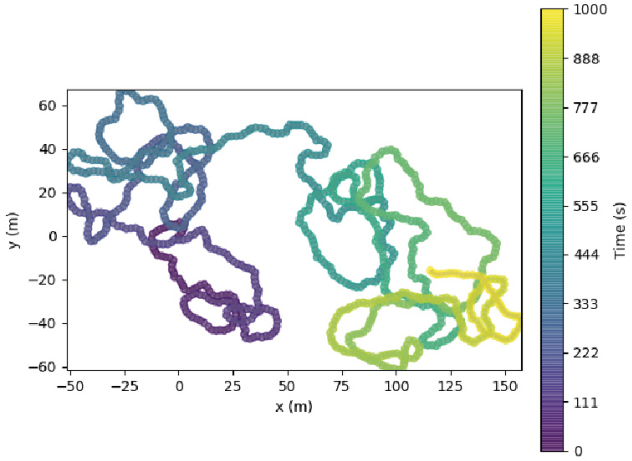


Figure 2.1: Generation of a random walk

3 This dataset has been collected for other studies of our laboratory [48].

2.1.4 *Spatial Trajectory*

A *spatial trajectory* is a trace generated by a moving object in geographical space. Trajectories are traditionally modelled as a sequence of spatial points like:

$$T_k = \{P_{k1}, P_{k2}, ...\}$$

where $P_{ki} (i \geq 1)$ is a point in the trajectory.

Generating spatial trajectory data via a random walk is possible by sampling from a distribution of angles and step sizes [108, 169]. A correlated random walk (Figure 2.1) is generated with `traja.generate`.

2.1.5 *Spatial Transformations*

Transformation of trajectories can be useful for comparing trajectories from various geospatial coordinates, data compression, or simply for visualization purposes.

Feature Scaling
Feature scaling is common practice for preprocessing data for machine learning [170] and is essential for even application of methods to attributes. For example, a high dimensional feature vector $\mathbf{x} \in R^n$ where some attributes are in (0,100) and others are in (−1, 1) would lead to biases in the treatment of certain attributes. To limit the dynamic range for multiple data instances simultaneously, scaling is applied to a feature matrix $X = \{\mathbf{x}_1, \mathbf{x}_2, ..., \mathbf{x}_N\} \in R^{n \times N}$, where n is the number of instances.

Min-Max Scaling To guarantee that the algorithm applies equally to all attributes, the normalized feature matrix $\hat{X}$ is rescaled into range (0, 1) such that $\hat{X} = \frac{X - X_{min}}{X_{max} - X_{min}}$.

Standardization The result of standardization is that the features will be rescaled to have the property of a standard normal distribution with $\mu = 0$ and $\sigma = 1$ where μ is the mean (average) of the data and σ is the standard deviation from the mean. Standard scores (also known as **z**-scores) are calculated such that $z = \frac{x - \mu}{\sigma}$.

Scaling Scaling a trajectory is implemented for factor f in scale where $f \in R : f \in (-\infty, +\infty)$.

Rotation
Rotation of a 2D rectilinear trajectory is a coordinate transformation of orthonormal bases x and y at angle θ (in radians) around the origin defined by

$$\begin{bmatrix} x' \\ y' \end{bmatrix} = \begin{bmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} x \\ y \end{bmatrix}$$

with angle θ where $\theta \in R : \theta \in [-180, 180]$.

Trip Grid
One strategy for compressing the representation of trajectories is binning the coordinates to produce an image as shown in Figure 2.2.

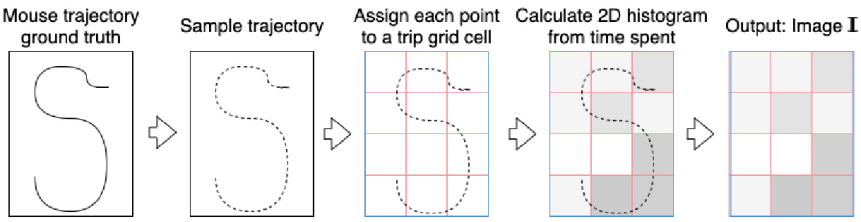


Figure 2.2: Trip grid image generation from mouse trajectory.

Allowing computation on discrete variables rather than continuous ones has several advantages stemming from the ability to store trajectories in a more memory efficient form.[4] The advantage is that computation is generally faster, more data can fit in memory in the case of complex models, and item noise can be reduced.

Creation of an M ∗ N grid allows mapping trajectory T_k onto uniform grid cells. Generalizing the nomenclature of [171] to rectangular grids, $C_{mn}(1 \le m \le M; 1 \le n \le N)$ denotes the cell in row m and column n of the grid. Each point P_{ki} is assigned to a cell C(m, n). The result is a two-dimensional image M ∗ N image I_k, where the value of pixel $I_k(m, n)(1 \le m, n \le M)$ indicates the relative number of points assigned to cell C_{mn}. Partionining of spatial position into separate grid cells is often followed by generation of hidden Markov models [172] (see below) or visualization of heat maps (Figure 2.3).

Smoothing
Smoothing a trajectory can also be achieved with Traja using Savitzky-Golay filtering with `smooth_sg` [173].

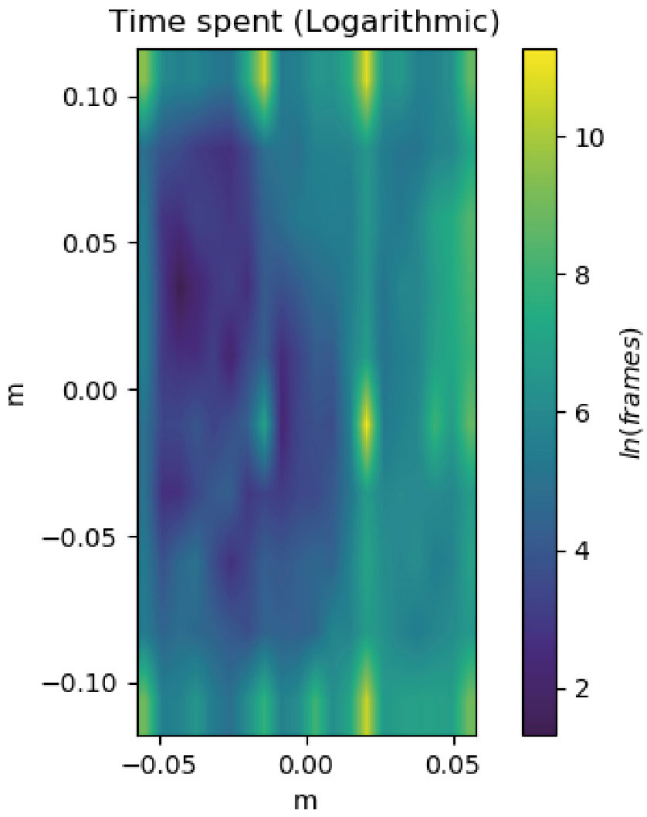


Figure 2.3: Visualization of heat map from bins generated with `trip_grid`. Note regularly spaced artifacts (bright yellow) in this sample due to a bias in the sensor data interpolation. This type of noise can be minimized by thresholding or using a logarithmic scale, as shown above.

2.1.6 *Resampling and Rediscretizing*

Trajectories can be resampled by time or rediscretized by an arbitrary step length. This can be useful for aligning trajectories from various data sources and sampling rates or reducing the number of data points to improve computational efficiency. Care must be taken to select a time interval which maintains information on the significant behavior. If the minimal time interval observed is selected for the points, calculations will be computationally intractable for some systems. If too large of an interval is selected, we will fail to capture changes relevant to the target behavior in the data.

4 In this experiment, for example, data can be reduced from single-precision floating point (32 bits) to 8-bit unsigned integer (*uint8*) format.

Resampling by time is performed with `resample_time` (Figure 2.4). Rediscretizing by step length is performed with `rediscretize`.

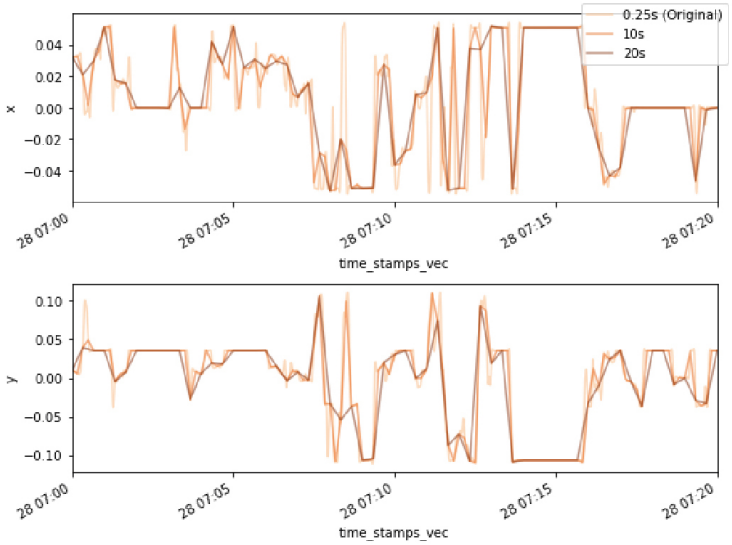


Figure 2.4: Resampling trajectories by different time scales is performed with `resample_time`.

For example, the Fortasyn dataset [48] demonstrated in this paper was sampled at 4 Hz and converted to single-precision floating point data. Pandas dataframes store this data in 4 bytes, thus there are approximately 4.15 MB[5] bytes required to store data for x and y dimensions plus an index reference for a single day. In the case of [48], 24 mice were observed over 35 days. This translates to 3.4 GB (10^9) of storage capacity for the uncompressed datasets prior to feature engineering. Thus resampling can be a useful way to reduce the memory footprint for memory constrained processes that have to fit into a standard laptop with 8 GB memory space. A demonstration of how reduction in precision for trajectory data analysis is provided in Figure Figure 2.4, as applied to a sample from the Fortasyn experiment [48]. Broad effects such as cage crossings, for example, can still be identified while downsampling data to a lower frequency, such as 0.1 Hz, reducing the memory footprint by a factor of 40 (4 Hz/0.1 Hz) and providing significant speedups for processing.

2.1.7 *Movement Analysis*

Traja includes traditional as well as advanced methods for trajectory analysis.

5 4 x 4 Hz x 60 seconds x 60 minutes x 24 hours x 3 features (x, y, and time)

Distance traveled

Distance traveled is a common metric in animal studies - it accounts for the total distance covered by the animal within a given time interval. The distance traveled is typically quantified by summing the square straight-line displacement between discretely sampled trajectories [174, 175]. Alternative distance metrics for the case of animal tracking are discussed in [176].

Let $p(t) = [p_x(t), p_y(t)]$ be a 2×1 vector of coordinates on the ground representing the position of the animal at time t. Then, the distance traveled within the time interval t_1 and t_2 can be computed as a sum of step-wise Euclidean distances

$$s(t_1, t_2) = \Sigma_{t=t_1+1}^{t_2} d(t),$$

where

$$d(t) = \sqrt{(p_x(t) - p_x(t-1))^2 + (p_y(t) - p_y(t-1))^2}$$

is the Euclidean distance between two positions in adjacent time samples.

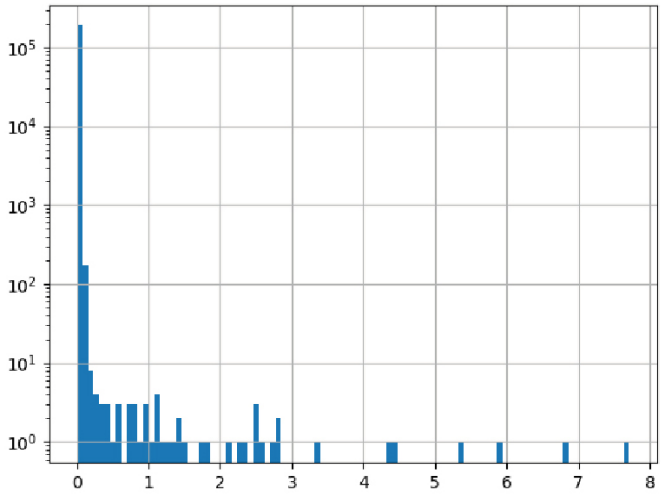


Figure 2.5: Velocity histogram from one day of mouse activity.

Speed

Speed or velocity is the first derivative of centroids with respect to time. Peak velocity in a home cage environment is perhaps less interesting than a distribution of velocity observations, as in Figure 2.5. Additionally, noise can be eliminated from velocity calculations by using a minimal distance moved threshold, as demonstrated in [48]. This allows identifying broad-scale behaviors such as cage crossings.

Turn Angles
Turn angles are the angle between the movement vectors of two consecutive samples. They can be calculated with `calc_turn_angles`.

Laterality
Laterality is the preference for left or right turning and a *laterality index* is defined as $\mathrm{LI} = \frac{\mathrm{RT}}{\mathrm{LT}+\mathrm{RT}}$ where RT is the number of right turns observed and LT is the number of left turns observed. Turns are counted within a left turn angle $\in (\theta, 90)$ and right turn angle $\in (-\theta, -90)$. A turn is considered to have a minimal step length.

2.1.8 *Periodicity*

Periodic behaviors are a consequence of the circadian rhythm as well as observing expression of underlying cognitive traits. Some basic implementations of periodic analysis of mouse cage data are presented.

Autocorrelation
Autocorrelation is the correlation of a signal with a delayed copy of itself as a function of the decay. Basically, it is similarity of observations as a function of the time lag between them. An example is shown in Figure 2.6.

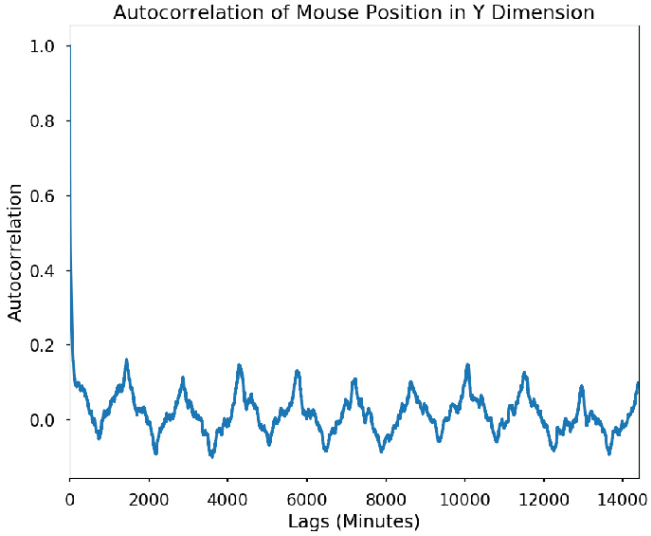


Figure 2.6: Autocorrelation of the y-dimension reveals daily (1440 minutes) periodic behavior

Power Spectrum
Power spectrum of a time series signal can be estimated (Figure 2.7). This is useful for analyzing signals, for example, the influence of neuromotor noise on delays in hand movement [177].

2.1.9 *Algorithms and Statistical Models*

Machine Learning for Time Series Data

Machine learning methods enable researchers to solve tasks computationally without explicit instructions by detecting patterns or relying on inference. Thus they are particularly relevant for data exploration of high volume datasets such as spatial trajectories and other multivariate time series.

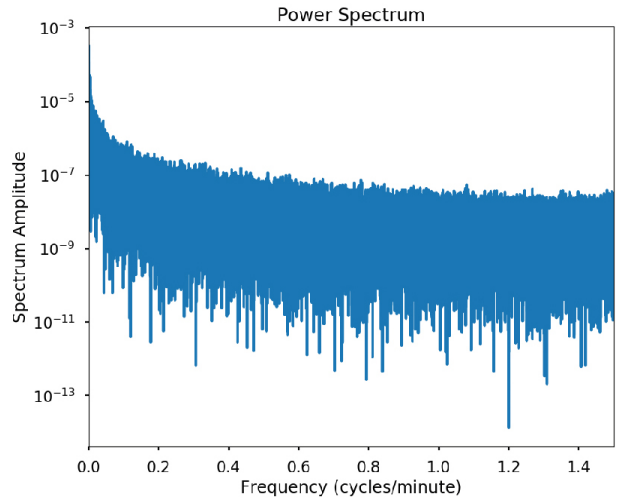


Figure 2.7 : Power Spectral Density. One day of activity reveals fairly smooth power spectral density.

Principal Component Analysis

Principal Component Analysis projects the data into a linear subspace with a minimum loss of information by multiplying the data by the eigenvectors of the covariance matrix.

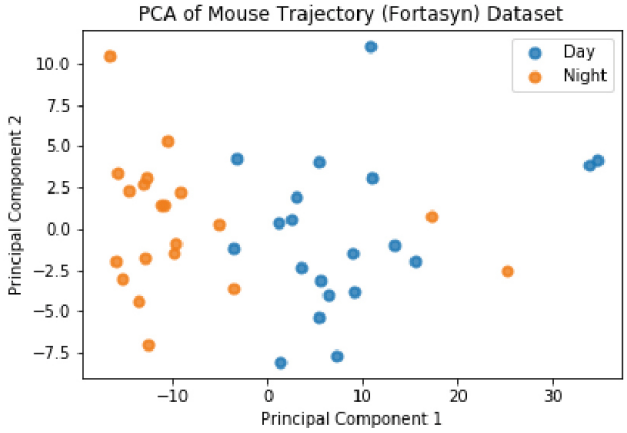


Figure 2.8: PCA of Fortasyn trajectory data. Daily trajectories (day and night) were binned into 8x8 grids before applying PCA.

This requires converting the trajectory to a trip grid (see Figure 2.2 and performing PCA on the grid in 2D (Figure 2.8) or 3D (Figure 2.9). Structure in the data is visible if light and dark time periods are compared.

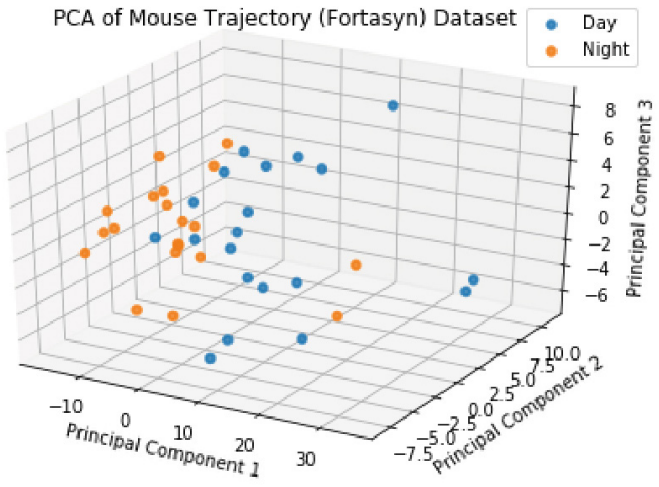


Figure 2.9: 3D PCA of Fortasyn trajectory data. Daily trajectories (day and night) were binned into 8x8 grids before applying PCA.

Clustering

Clustering of trajectories is an extensive topic with applications in geospatial data, vehicle and pedestrian classification, as well as molecular identification. K-means clustering is an iterative unsupervised learning method that assigns a label to data points based on a distance function [2] (Figure 2.10).

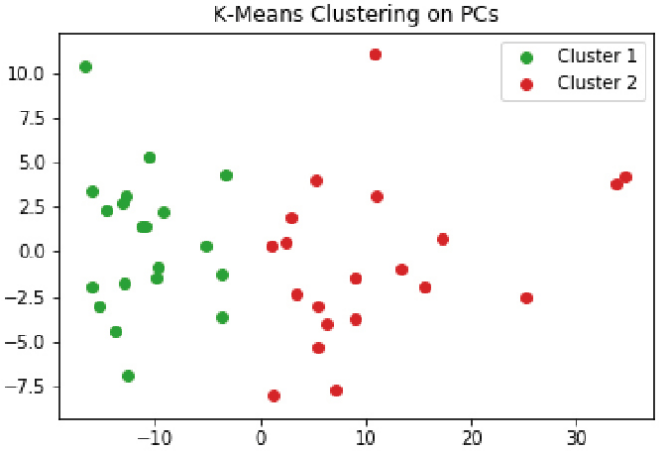


Figure 2.10: K-means clustering on the results of the PCA shown above reveals a high accuracy of classification, with a few errors. Cluster labels are generated by the model.

Hierarchical Agglomerative Clustering
Clustering spatial trajectories has broad applications for behavioral research, including unsupervised phenotyping [178]. For mice, hierarchical agglomerative clustering can also be used to identify similarities between groups, for example periodic activity and location visit frequency [179].

Gaussian Processes
Gaussian Processes is a non-parametric method which can be used to model spatial trajectories. This method is not currently implemented in Traja and is thus outside the scope of the current paper, however the interested reader is directed to the excellent text on Gaussian processes by Rasmussen and Williams [180] for a complete reference and [181] for an application to spatial trajectories.

2.1.10 *Other Methods Fractal Methods*

Fractal (i.e. multiscale) methods are useful for analyzing transitions and clustering in trajectories. For example, search trajectories such as eye movement, hand-eye coordination, and foraging can be analyzed by quantifying the spatial distribution or nesting of temporal point processes using spatial Allen Factor analysis [182, 183].

Recurrence plots and derivative recurrence factor analysis can be applied to trajectories to identify multiscale temporal processes to study transition or nonlinear parameters in a system, such as postural fluctuation [184] and synchrony [185] in humans and to movement of animals such as ants [186] and bees [187]. These methods are not yet implemented in Traja, but are planned for a future release.

Graph Models
A graph is a pair G = (V, E) comprising a set of vertices and a set of connecting edges. A probabilistic graphical model of a spatial occupancy grid can be used to identify probabilities of state transitions between nodes. A basic example is given with hidden Markov models below.

Hidden Markov Models
Transition probabilities are most commonly modelled with Hidden Markov Models (HMM) because of their ability to capture spatial and temporal dependencies. A recent introduction to these methods is available provided by [188]. HMMs have successfully been used to analyze movement of caribou [189], fruit flies [190], and tuna [191], among others. Trajectories are typically modelled as bivariate time series consisting of step length and turn angle, regularly spaced in time.

Traja implements the rectangular spatial grid version of HMM with transitions.

The probability of transition from each cell to another cell is stored as a probability within the transition matrix. This can be visualized as a heatmap and plotted with `plot_transition_matrix` (Figure 2.11).

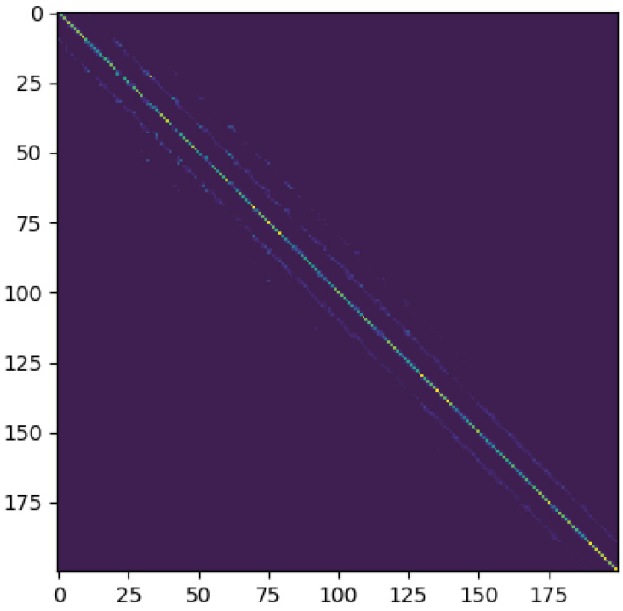


Figure 2.11: Transition matrix. Rows and columns are flattened histogram of a grid 20 cells high and 10 cells wide. Spatially adjacent grid cells are visible at a spacing of -11, -10, -9, 1, 10, and 11 cells from the diagonal. The intensity of pixels in the diagonal represents relative likelihood to stay in the same position.

Convex Hull

The convex hull of a subtrajectory is the set X of points in the Euclidean plane that is the smallest convex set to include X. For computational efficiency, a geometric k-simplex can be plotted covering the convex hull by converting to a Shapely object and using Shapely's `convex_hull` method.

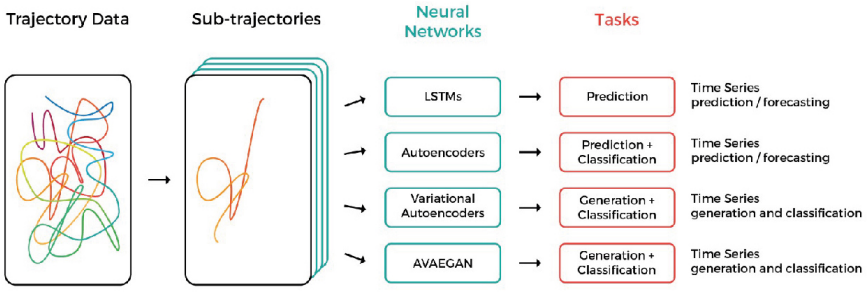


Figure 2.12: Neural network architectures available in Traja

Recurrent Neural Networks

In recent years, deep learning has transformed the field of machine learning. For example, the current state of the art models for a wide range of tasks, including computer vision, speech to text, and pedestrian trajectory prediction, are achieved with deep neural networks. Neural networks are essentially sequences of matrix operations and elementwise function application based on a collection of computing units known as nodes or neurons. These units perform operations, such as matrix multiplication on input features of a dataset, followed by backpropagation of errors, to identify parameters useful for approximating a function.

Recurrent Neural Networks (RNNs) are a special type of Neural Networks that use a state $S(t_{i-1})$ from the previous timestep t_{i-1} alongside $X(t_i)$ as input. They output a prediction $Y(t_i)$ and a new state $S(t_i)$ at every step. Utilising previous states makes RNNs particularly good at analyzing time series like trajectories, since they can process arbitrarily long inputs. They remember information from previous time steps $X(t_{i-k})$, ..., $X(t_{i-1})$ when processing the current time step $X(t_i)$.

Trajectory prediction lets researchers forecast the location and trajectory of animals [126]. Where this technique works well, it is also a sign that the trajectory is highly regular and, fundamentally, follows certain rules and patterns. When tracking an animal live, it would also let researchers predict when it will arrive at a particular location, or where it will go, letting them rig cameras and other equipment ahead of time.

A particularly interesting type of RNN is the Long Short Term Memory (LSTM) architecture. Their layers use stacks of units, each with two hidden variables - one that quickly discards old states and one that slowly does so - to consider relevant information from previous time steps. They can thus look at a trajectory and determine a property of the animal – whether it is sick or injured, say – something that is time-consuming and difficult to do by hand. They can also predict future time steps based on past ones, letting researchers estimate where the animal will go next. LSTMs can also classify trajectories, determining whether a trajectory comes from an animal belonging in a specific category. This lets researchers determine how a controlled or semi-controlled variable (e.g., pregnancy) changes the movement pattern of an animal.

Traja implements neural networks (§2.1.10) by extending the widely used open source machine learning library PyTorch [192], primarily developed by Facebook AI Research Group. Traja allows framework-agnostic modeling through data loaders designed for time series. In addition, the Traja package comes with several predefined model architectures which can be configured according to the user's requirements.

Because RNNs work with time series, the trajectories require special handling. The `traja.dataset.MultiModalDataLoader` efficiently groups subsequent samples and into series and splits these series into training and test data. It represents a Python iterable over the dataset and extends the PyTorch `DataLoader` class, with support for

- random, weighted sampling,
- data scaling,
- data shuffling,
- train/validation/test split.

`MultiModalDataLoader` accepts several important configuration parameters and allows batched sampling of the data. The two constructor arguments `n_past` and `n_future` specify the number of samples that the network will be shown and the number that the network will have to guess, respectively. `batch_size` is generally in the dozens and is used to regularise the network.

The RNNs also need to be trained this is done by the high-level `Trainer` class below. It performs nonlinear semicolor optimisation with a Stochastic Gradient Descent-like algorithm. The `Trainer` class by default implements the Huber loss function [193], also known as smooth L_1 loss, which is a loss function commonly used in robust regression:

$$L_\delta(a) = \begin{cases} \frac{1}{2}a^2 & \text{for } |a| \leqslant \delta, \\ \delta(|a| - \frac{1}{2}\delta), & \text{otherwise.} \end{cases}$$

In comparison to mean-squared error loss, Huber loss is less sensitive to outliers in data: it is quadratic for small values of a, and linear for large values. It extends the PyTorch `SmoothL1Loss` class, where the d parameter is set to 1.[6] A common optimization algorithm is ADAM and is Traja's default, but several others are provided as well. Although training with only a CPU is possible, a GPU can provide a 40 – 100x speedup [194].

Recurrent Autoencoder Networks

Traja can also train autoencoders to either predict the future position of a track or classify the track into a number of categories. Autoencoders embed the time series into a time-invariant latent space, allowing representation of each trajectory or sub-trajectory as a vector. A class of well-separated trajectories would then be restricted to a region of the latent space. The technique is similar to Word2vec [195], where words are converted to a 100+ dimensional vector. In this approach, forecasting and classification are both preceded by training the data in an autoencoder, which learns an efficient representation of the data for further computation of the target function.

6 https://pytorch.org/docs/stable/generated/torch.nn.SmoothL1Loss.html

Traja allows training a classifier that works directly on the latent space output - since each class of trajectories converges to a distinct region in the latent space, this technique is often superior to classifying the trajectory itself. Traja trains classifiers for both Autoencoder-style and Variational Autoencoder-style RNNs. When investigating whether animal behavior has changed, or whether two experimental categories of animals behave differently, this unstructured data mining can suggest fruitful avenues for investigation.

3

APPLICATION OF TRAJA TO MOUSE STROKE MODEL ON ENRICHED DIET

Quantitative characterization of mouse activity, locomotion and walking patterns requires the monitoring of position and activity over long periods of time. Manual behavioral phenotyping, however, is time and skill-intensive, vulnerable to researcher bias and often stressful for the animals. We present examples for using a platform-independent open source trajectory analysis software, Traja, for semi-automated analysis of high throughput mouse homecage data for neurobehavioral research. Our software quantifies numerous parameters of movement including travelled distance, velocity, turnings, and laterality which are demonstrated for application to neurobehavioral analysis. In this study, the open source software for trajectory analysis Traja is applied to movement and walking pattern observations of transient stroke induced female C5 BL/6 mice (30 min middle cerebral artery occlusion) on an acute multinutrient diet intervention (Fortasyn). Mice were housed individually in Digital Ventilated Cages (DVC, GMS00, Tecniplast S.p.A., Buguggiate (VA), Italy) and activity was recorded 24/7 every 250 ms using a DVC board. Significant changes in activity, velocity, and distance walked are computed with Traja. Traja identified increased walked distance and velocity in Control and Fortasyn animals over time. No diet effect was found in preference of turning direction (laterality) and distance travelled. As open source software for trajectory analysis, Traja supports independent development and validation of numerical methods and provides a useful tool for computational analysis of 24/7 mouse locomotion in home-cage environment for application in behavioral research or movement disorders.

This chapter is published in Frontiers in Neuroscience as:
Shenk, J., Lohkamp, K. J, Wiesmann M., Kiliaan, A. J. (2020). Automated Analysis of Stroke Mouse Trajectory Data With Traja. *Frontiers in Neuroscience* 14:518. doi: 10.3389/fnins.2020.00518.

3.1 INTRODUCTION

Rodent locomotion has been studied in the context of various disease models such as spinal cord injury [196–198] neurodegenerative diseases such as Parkinson's [199–201] and Down syndrome [202, 203], assessment of pharmacological agents [204], genetic mutations [205, 206], and stroke [207, 208]. Locomotion monitoring has been used both as a proxy for measuring illness and fatigue as well as overall development and recovery. Automated quantitative analysis of mouse phenotype allows researchers to objectively assess cognitive and motor abilities and disturbances brought about by genetics, disease processes, and interventions. The ability of these effects to be observed, measured and communicated, is constrained by the availability of assays which reflect physiological and cognitive changes occurring over indeterminate time intervals. Further, the time resolution of observations and analysis are limited by the availability of behavioral data and analytical methods. Sharing data and analytical methods allows for increasing the validity of experimental modalities.

Phenotyping mouse models typically involves screening mice through various behavioral tests to measure anxiety, learning, memory, or locomotion in experimental setups outside the home-cages. Several mouse tracking tools exist for phenotyping; a recent review can be found at [209]. These tools however either are not designed to be used in home-cage environments or require expensive commercial software which limits the reproducibility of data collection and analysis. Monitoring in artificial environments introduces an additional stress and discomfort to the animals, and the short-term nature of the experiments risk missing important behavioral patterns which can only be discovered during long-term observation.

The Open Field (OF) test is a widely used experimental paradigm for quantifying mouse locomotion and monitoring behavior. It allows providing descriptive statistics of mobility and stress-related behaviors such as tendency to remain near walls or corners [210]. OF is limited, however, because it is subject to experimenter bias, requires trained animal handler's to be present, management of equipment, and specialized space for experimentation. Multiple exposure to the OF environment leads to habituation, which decreases exploratory behavior and can mask recovery after an ischemic insult. In addition, the novelty of the environment causes stress to the animal which may affect the observed response. Similarly, the Corner Test [211] is widely used to identify sensory-motor functional deficits like laterality preference after an experimental stroke, but suffers the same drawbacks. Reproducibility of movement data analysis depends on widespread access to the data and analytical methods for verification within the scientific community [210]. The vast majority of researchers use commercial software for recording and digitally analyzing the OF test results. A number of applications capture locomotion with video [212,

213] or photo-beams [214]. Most tracking systems require specialized cages, illumination [215, 216] or the presence of intrusive devices which do not fit within standard mouse home-cage and interfere with mouse behavior, or are based upon proprietary methods or software specific to the data sources. Such experimental setups rely on a novel environment during recording, or are otherwise difficult to scale to large studies without considerable investment in equipment. Ability to customize parameters used for analysis is a priority in defining quantitative statistics, since the validity of the construct in relation to the features of interest in many cases cannot be determined empirically [217]. For example, laterality is defined in various ways in the literature, e.g., with various thresholds for distance travelled or angular movement. Software which allows customizing the parameters for analysis supports independent verification of the results and fine-tuning of analytical methods for increased internal validity [217].

Open source software has been developed in the past years for tracking mice in OF (M-Track) [216] and other specialized cage environments (Live Mouse Tracker) [209]. Singh et al. 2019 developed a camera-based system for mouse tracking for mouse home-cage locomotion tracking and analysis. Their setup requires the user to be experienced in configuring hardware and does not provide tools for analyzing the distance travelled.

Trajr is an R package developed for analysis of animal movement in two-dimensional space [219]. It provides several methods for trajectory analysis and data preprocessing. The source code however is not optimized for analysis of the millions of data points needed to track the lifetime position of mice, the most common used experimental animal model. Further, there are many advanced data modelling tools for machine learning, such as TensorFlow and PyTorch, which are not accessible in R, thus unnecessarily restricting the user to traditional analytical techniques.

The Python programming language, on the other hand, is a general-purpose programming language and is the primary language used for implementation of current state-of-the-art trajectory prediction models (eg, Social Ways [127], Next [156], and TraPHic [220]). A library which bridges the most widely used machine learning packages with mouse home-cage trajectory is thus needed.

We present a Python package, Traja, for automated analysis of activity and position extracted via the 12 capacitive home-cage sensors in the DVC (Tecniplast S.p.A., Buguggiate (VA), Italy) [221, 222] to quantify several behavioral modalities in a stroke mouse model. Stroke is a motor impairment disease; therefore, Traja is a suitable application for identifying changes in behavior and motor function relevant to surgical intervention and treatments like diet and exercise [223]. We analyze mouse activity, distance, velocity, and turning bias/laterality from data collected in a comparison of a group on

Control diet and a group treated with the multinutrient intervention, Fortasyn [223]. Fortasyn consists of fatty acids, phospholipids, and vitamins stimulating neuronal membrane formation, and improving vascular health by increasing cerebral blood flow (CBF) which suggest that this diet may also improve damages caused by cerebrovascular diseases (CVD) such as stroke. This study on the impact of Fortasyn on behavior (Open Field, Pole test), neuroimaging and post-mortem brain measures was previously published [147]. In short, the study provides evidence that Fortasyn leads to improved brain integrity, sensorimotor integration and neurogenesis, while motor skills did not recover in female stroke mice on Fortasyn diet [147]. The present results computed by Traja are a valuable addition to the previously performed study, since DVC derived data might pick up more effects than classical behavioral tests (e.g. Open Field). We demonstrate the capability of metrics derived from home-cage activity and position tracking to study differences in the neurobehavioral phenotypes of mice over extensive lengths of time, within a method that is accessible to researchers possessing moderate programming background. Thus, by making use of the DVC dataset of this female stroke mouse model, we show that the Traja software package is a valid method for semi-automated trajectory analysis.

3.2 MATERIALS AND METHODS

3.2.1 *Description of the Traja Python package*

The user of the Python package Traja can carry out data selection by using filter settings like minimal distance moved to eliminate slight "apparent" movements. Several quantitative measures of behavior are available and can be described with descriptive statistics relevant to neurobehavioral research, including activity, distance, velocity, turn angle, and laterality. A list of functionalities and specifications of Traja are listed in the Supplementary materials (Table 1 and 2).

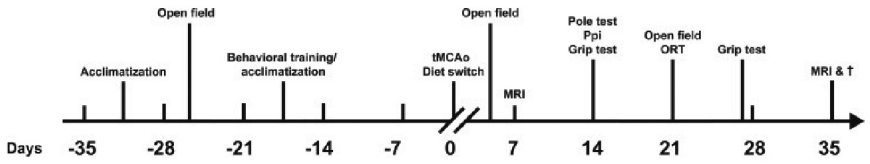


Figure 3.1: Study design. Acclimatization, behavioral training and baseline behavioral experiments started 35 days before tMCAo. Immediately after stroke induction (day 0), diets were switched to either Fortasyn multinutrient diet (n=11) or to an isocaloric diet (n=12). Additionally, all animals were individually housed in DVC to monitor their locomotive behavior, including activity, distance moved, velocity, turns and laterality. Until 35 days post-surgery, behavioral tests [pole test, prepulse inhibition (PPI), grip test, Open Field, novel object recognition test (ORT)] were performed and animals underwent two MRI scanning sessions.

3.2.2. *Automated locomotion and trajectory analysis*

Activity and centroids

Activity and centroids were measured by sensing boards, equipped with 12 capacitive-based electrodes, underneath each Digital Ventilated Cage (Tecniplast S.p.A., Buguggiate (VA), Italy) as previously described in detail [221, 222]. In short, proximity sensors are able to measure electrical capacitance of each electrode in 250ms time intervals 24/7. As soon as an animal is moving in the cage, the electrical capacitance of the proximity sensors are influenced by the dielectric properties of matter in close proximity to the electrode. Consequently, animals moving across the electrodes are detected and recorded as change in capacity over a limited time interval. An activity event describes the absolute value of the difference between two consecutive measurements for each electrode that is compared with a set threshold to control for noise. Centroids include the x, y coordinates of the mouse position inside the cage. For the x, y-value calculation, the mouse position is estimated in the average position between the centers of the active electrodes weighted by using change in capacity, as described elsewhere in more detail [222].

Distance and velocity

Distance and velocity were calculated using the first and second derivative, respectively, of the centroid coordinates with respect to time [222]. The distance computed with Traja was matching the distance obtained with Ethovision XT 14, indicating that Traja accurately computes trajectory parameters (Supplementary Figure 1) [147]). In Traja this is accomplished with `traja.calc_displacement()` and `traja.calc_derivatives()`, respectively. Velocity was measured with a minimum velocity threshold of 0.02 m/s.

Turn angle and laterality

Angular velocity has been used in several animal models including mice and fish [224] for observing reflexes and locomotion. Extending the nomenclature in [215] with heading at time step n as HE_n and time coordinate t, relative turn angle $RTA_n = HE_n - HE_{n-1}$ and relative angular velocity $RAV_n = \frac{RTA_n}{t_n - t_{n-1}}$
We calculate laterality index $LI = \frac{R}{R+L}$ where R is the number of right turn angles RTA ∈ [30, 90], L is the number of left turn angles RTA ∈ [−90, −30], and LI ∈ [0, 1] and the minimum velocity is 1 cm/s. Turn bias of trajectories can be visualized using `traja.polar_bar()`.

3.2.3. *Stroke disease model*

All results regarding behavioral tests, neuroimaging, and post-mortem brain analysis of the dietary intervention Fortasyn in a female stroke mouse model were published earlier by Wiesmann et al. [147]. The focus of the present study is the trajectory analysis of the female stroke model in DVC with the software Traja.

Transient middle cerebral artery occlusion
Ischemic stroke was induced in female C57BL/ 6JRj mice by a transient middle cerebral artery occlusion (tMCAo), which is mimicking one of the most common types of ischemic stroke in patients [225]. The intraluminal occlusion model was performed as described elsewhere with minor modifications [147, 225]. In short, a 7-0 monofilament (tip diameter 190–200lm, coating length 2–3 mm, 70SPRePK5, Doccol Corp., Sharon, MA, USA) was inserted in the right common carotid artery and placed to block blood supply via the middle cerebral artery. The filament was held in place for 30 min followed by retraction of the filament leading to reperfusion. A Laser Doppler probe (moorVMS-LDF2, Moor Instruments, UK) was placed on the skull of the mice to monitor cerebral blood flow, considering a drop of ≥ 80% CBF as a successful stroke induction. Animals were anesthetized during the whole time of surgery, using 1.5% isoflurane (Abbott Animal Health, AbbottPark, IL, USA) in a 2:1 air and oxygen mixture.

Animals, diet, housing and study design
At 3-4 months of age, 24 female C57BL/ 6JRj mice (Harlan Laboratories Inc., Horst, the Netherlands) arrived at the preclinical imaging center of the Radboud university medical center (Radboudumc) (Nijmegen, the Netherlands) where all experiments were performed (see Figure 1 for study design). Animals were group housed (four animals per cage) in DVC (Tecniplast S.p.A., Buguggiate (VA), Italy) which contained corn based bedding material (Bio Services, Uden, The Netherlands), wood wool nesting material (Bio Services, Uden, The Netherlands), and a mouse igloo (Plexx, Elst, The Netherlands). Standard food pellets (Ssniff rm/h V1534-000, Bio Services, Uden, The Netherlands) and autoclaved water were available ad libitum. The room had constant temperature (21 ± 1°C), humidity (55% ± 10%), background music and an artificial 12h light-dark cycle (light on at a.m.). After letting the animals acclimatize, baseline behavioral measurements were performed pre-stroke. All parameters measured in the Open Field (walked distance, velocity, manual scored behaviors) and grip strength test did not differ between the Control and Fortasyn group prior surgery [147]. Immediately after tMCAo, mice were randomly divided into two experimental groups using a random sequence generator switching from normal chow (Ssniff rm/h V1534-000, Bio Services, Uden, The Netherlands) to either a multinutrient intervention Fortasyn diet (n = 12) or an isocaloric Control diet (n = 12) [147]. Both the Fortasyn and the Control diet were based on AIN-93M (Reeves et al., 1993) with 5% fat, but differed with respect to their fatty acid composition and some additional nutrients. The Fortasyn diet contained 0.1% coconut oil, 1.9% corn oil, and 3.0% fish oil, while the Control diet contained 1.9% soy oil, 0.9% coconut oil, and 2.2% corn oil. Furthermore, the Fortasyn diet contained a specific multinutrient composition comprising uridine, omega-3 polyunsaturated fatty acids (PUFAs), choline, B vitamins,

phospholipids, and antioxidants (the specific composition is specified in [226]. Both diets were manufactured and pelleted by Ssniff (Soest, Germany) and stored at −20°C until use. Group sizes were calculated based on the effect sizes (Type I error: 0.05, statistical power: 0.80), exclusion and mortality rates determined in our previous study [226]. Before and 1 day after stroke surgery, all mice were injected Carprofen (Rimadyl, Pfizer Animal Health, Cappele aan de IJssel, the Netherlands) subcutaneously adjusted to their weight (0.1 ml Carprofen l per 10 gram) to prevent discomfort. After surgical intervention, the animals were housed separately in clean DVC to optimize healing of surgical wounds. During the post-surgery period (35 days) it was not necessary to clean the cages due to single-housing. Furthermore, physiological parameters as well as stroke related disturbances in motor function during the recovery period were monitored for each individual mouse. Body weight did not differ between experimental groups, however, during the first week post-stroke, Fortasyn fed mice ate significantly more than Control animals although the diets were isocaloric. No further diet effects on both body weight and food intake were found during the poststroke weeks 2-5. Body weight increased in weeks 2 and 3 and stabilized in week 3 and 5 to baseline levels [147].

Ethics statement

Our study was in concurrence with the European regulations on ethics and responsible conduct regarding scientific communication as previously described [147]. Experiments were performed according to Dutch federal regulations for animal protection and the European Union Directive of 22 September 2010 (2010/63/EU). They were approved and pre-registered by the Animal Ethics Committee (called the Dierexperimentencommissie; DEC, RU-DEC 2014-1 1) of the Radboudumc. Furthermore, our experiments were performed according to the (updated) recommendations made by the Stroke Therapy Academic Industry Roundtable (STAIR) for the preclinical development of therapies for ischemic stroke [227] and ARRIVE guidelines [228]. All applicable international, national, and institutional guidelines for the care and use of animals were followed. Our study was also in concurrence with the European regulations on ethics and responsible conduct regarding scientific communication.

3.2.4. *Data analysis*

Mouse tracking was performed using DVC collecting data 24/7 via capacitance sensors placed underneath the home-cage. The raw data of mouse position centroids and activity were provided by Tecniplast. The data was analyzed with Traja.

Coding
Traja was written using Python 3.6 and several Python libraries for data management and analysis, with links and descriptions provided in Supplementary Table 2 [147].

Software
The software and documentation for Traja are freely available for download at `http://traja.readthedocs.io` or from the repository `https://github.com/justinshenk/traja` [223]. It is compatible with Microsoft Windows, Mac OSX and Linux.

Statistical analysis
Before analysis, data was aggregated for Fortasyn and Control groups. Unless otherwise stated, data were pooled within experimental groups by days from surgery and split into nighttime (7pm - 7am, light off) and daytime (7am -7pm, light on). Furthermore, effects found across 24 hours are considered as overall effects. All data are presented as mean ± SEM, unless otherwise stated. Statistical significance was set at $p<0.05$. Effects and interactions of longitudinal data were calculated by generalized linear models treating time and diet as fixed effects with the Python statsmodel software package (program items are in Supplementary 1). Moreover, for our GEE regression additional statistical measures are provided in the Supplementary material (e.g. number of observations/clusters, min./max./ mean cluster size, skew and kurtosis, standard errors, z-values) clarifying our statistical approach.

3.3. RESULTS

3.3.1 *Activity*

Recovery following stroke induction was monitored by analyzing mouse activity during day- and nighttime. In the first three days after surgery, Fortasyn fed animals were overall significantly more active than the Control group ($p<0.050$) (Figure 2A). In the same day range, activity increased in all animals during daytime ($p<0.001$) (Figure 2A). Both groups showed increased activity over time between day ranges 1- (daytime ($p<0.001$); nighttime ($p<0.001$); overall ($p<0.001$)) and 1-33 (nighttime ($p<0.017$)) (Figure 2B). Contrary to nighttime activity, daytime activity decreased between day 1-33 in both groups ($p<0.020$) (Figure 2B).

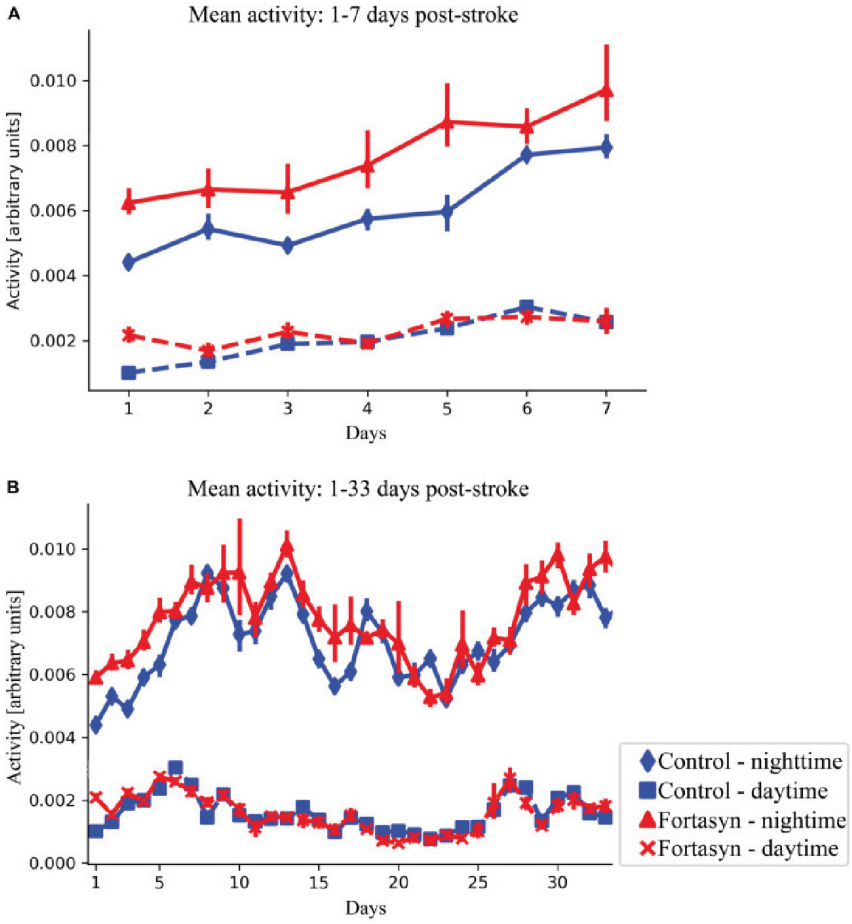


Figure 3.2: Mean activity measured in Control and Fortasyn fed animals during day- and nighttime between day ranges 1–7(A) and 1–33 (B). (A) Fortasyn fed animals were overall significantly more active between day 1–3 ($p<0.050$). (A,B) In both groups, activity increased over time during day- time [days: 1–3 ($p<0.001$), days: 1–7($p<0.001$)], nighttime [days: 1–7($p<0.001$), days: 1–33 ($p<0.01$)], and overall (days: 1–7 $p<0.001$). During daytime activity decreased in Control and Fortasyn animals between day 1–33 ($p<0.020$).

3.3.2. *Distance*

In all animals the average distance travelled was observed to increase during daytime between day ranges 1-3 ($p<0.038$) and 1-7 ($p<0.012$) (Figure 3.3A). Between day 1-33 an increase in travelled distance was observed during nighttime ($p<0.001$) and overall ($p<0.001$) (Figure 3.3B). No diet effects were found over time.

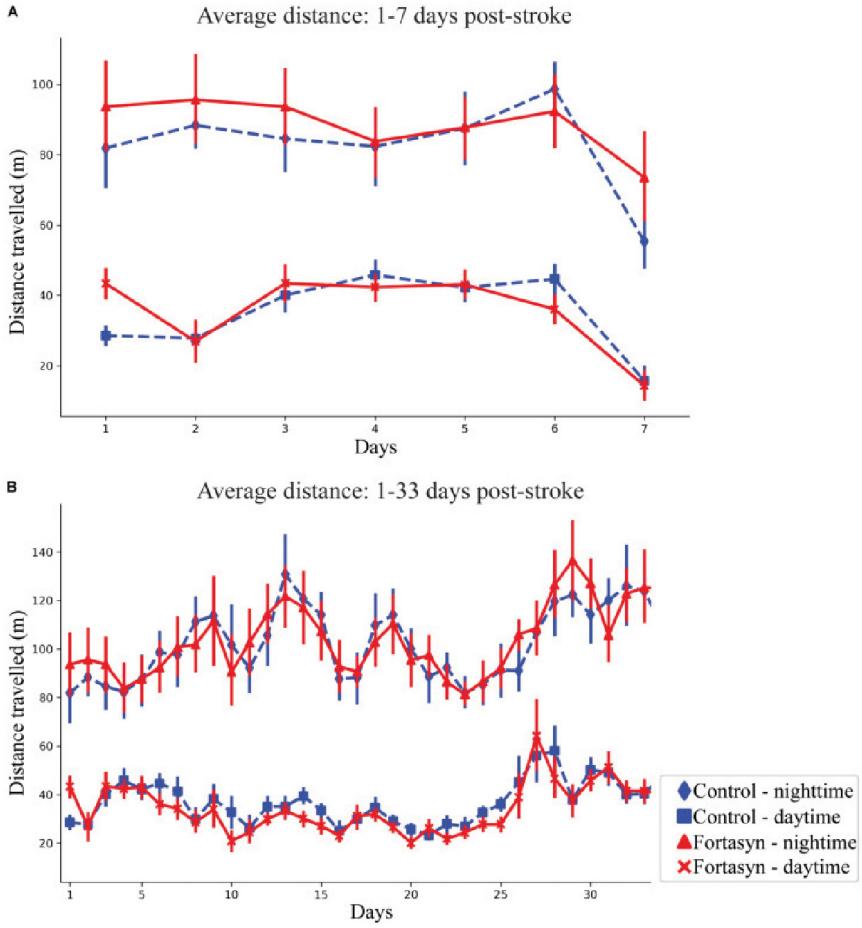


Figure 3.3: Average distance traveled by Control and Fortasyn fed animals during day- and nighttime between day ranges 1–7 (A) and 1–33 (B). (A,B) In all animals the distance traveled increased over time during daytime [days: 1–3 ($p<0.038$), days: 1–7($p<0.012$)], nighttime (days: 1–33 $p<0.001$), and overall (days: 1–33 $p<0.001$).

3.3.3. *Velocity*

All animals displayed increased mean velocity over time during daytime between day ranges 1-3 ($p<0.004$), 1-7 ($p<0.001$), 1-33 ($p<0.001$), and also overall between day range 1-33 ($p<0.008$) (Figure 3.4B). A diet effect was detected between day 1-3 during daytime (Figure 3.4A). In detail, Fortasyn fed animals were significantly faster in comparison to the Control group ($p<0.008$) (Figure 4A).

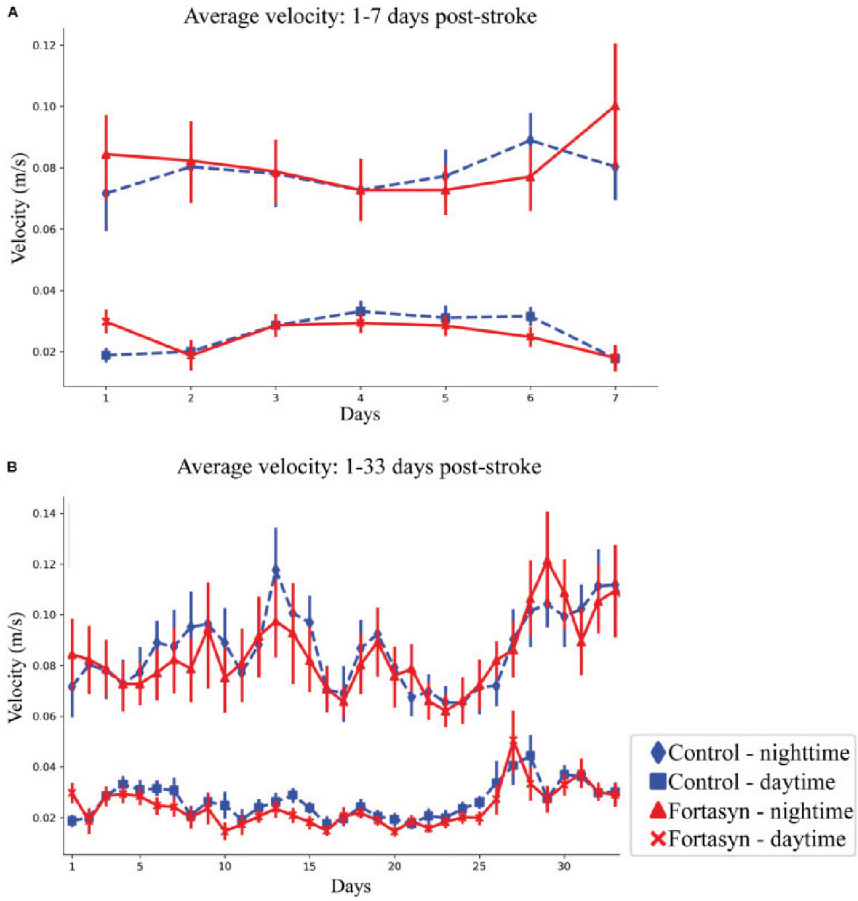


Figure 3.4: Average velocity measured in Control and Fortasyn fed animals during day- and nighttime between day ranges 1–7 (A) and 1–33 (B). (A) Average velocity was significantly higher in the Fortasyn group compared to Control during daytime between day ranges 1–3 ($p < 0.008$). (A,B) In all animals walking velocity increased over time during daytime [days: 1–3 ($p<0.004$), days: 1–7 ($p<0.001$)], nighttime [days: 1–33 ($p<0.001$)], and overall [days: 1–7 ($p<0.059$), days: 1–33 ($p<0.008$)].

3.3.4. *Turns & Laterality*

No significant diet or time differences were found regarding the number of right and left turns during day- and nighttime (Figure 3.5). Laterality is defined as proportion of right turns over all turns, thus laterality <0.5 indicates left turn preference (Figure 3.6). No significant effects due to diet or time were found between groups. In both experimental groups Traja was able to detect left and right turns. Laterality index was observed to be consistently around 0.5, indicating similar number of left and right turns during recovery.

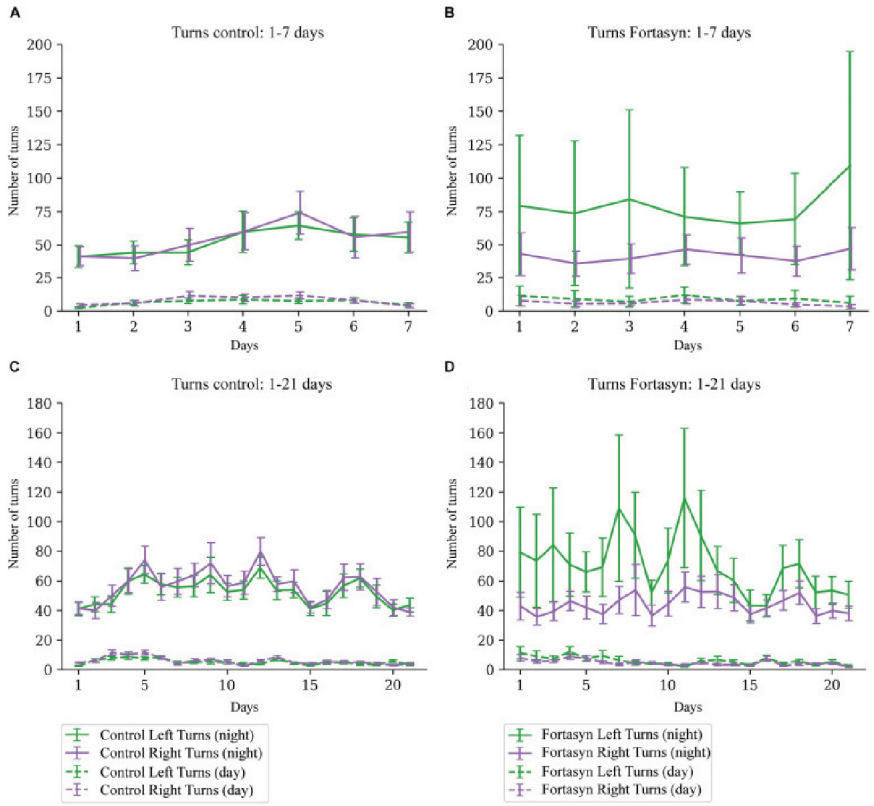


Figure 3.5: Number of turns measured in Control [(A) 1–7 days, (C) 1–21 days] and Fortasyn [(B) 1–7 days, (D) 1–21 days] group during day- and nighttime between different day ranges. No effect of time or diet was detected.

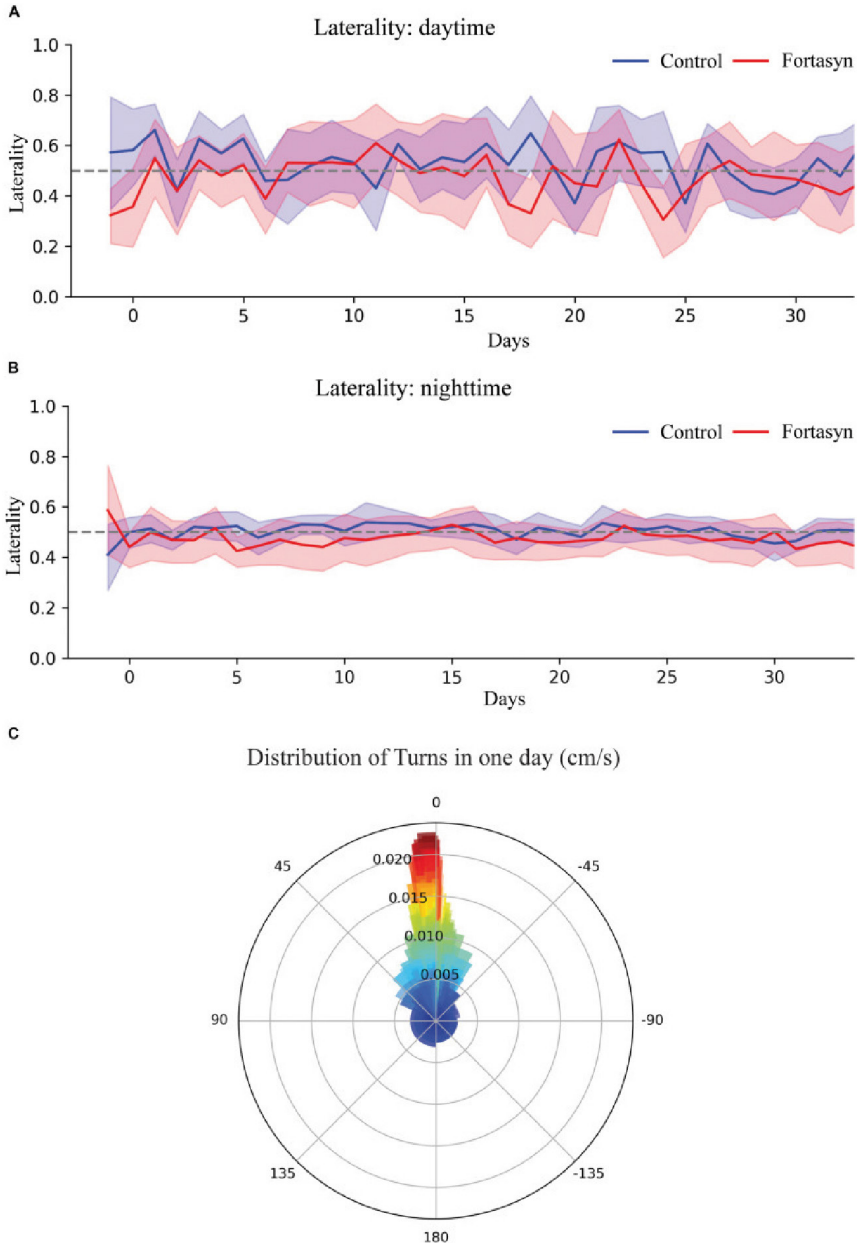


Figure 3.6: Visualization and analysis of turns. (A) Laterality in Fortasyn and Control group during daytime (A) and nighttime (B) following stroke. (C) Polar bar chart of angular movement of one mouse over the period of one day produced by Traja, visualizing laterality.

3.4 DISCUSSION

We have demonstrated several capabilities of Traja relevant to behavioral analysis of a stroke mouse model. An increasing number of sensors for animal tracking in recent years has led to a plethora of possibilities to analyze activity, each having advantages and disadvantages (reviewed in [209]). Monitoring single housed mice in their home-cages allows to increase the validity of observations for ongoing experiments. The disruption of mouse activity caused by cage changes has been previously documented [222]. It is clear that home-cage analysis is useful to monitor interference effects of novel environments. Testing animals in an environment consistent with daily living is thus crucial to maximizing the validity of behavioral assays, and Traja supports analysis of such data.

In the present experimental setting, we investigated the effect of a dietary intervention with Fortasyn acute after stroke induction in female, wildtype C57BL/ 6JRj mice [147]. Parameters, including activity, distance, velocity, and laterality were analyzed based on DVC metric measures with Traja to detect differences in recovery between intervention and Control group. Aforementioned parameters were analyzed between different day ranges to explore stroke or diet effects on short term (day range 1-3, 1-7) and long term post-surgery (day range 1-33). Traja calculated a subtle increase in overall activity and daytime velocity in the Fortasyn group compared to the Control group between day 1-3. Previous studies have clearly shown that Fortasyn has neuroprotective effects after an ischemic stroke, however short-term effects have not been shown before and need to be further investigated [147, 229]. Further diet effects were negligible regarding distance, turns and laterality. Both, the Control and Fortasyn group showed progressive recovery from stroke over time. More precisely, in both diet groups Traja calculated an increase of activity, distance, and velocity on short-term and/or long-term after stroke induction. In contrast, activity was overall found to be decreased during daytime (day range 1-33). This lower daytime activity is likely to be the consequence of the many behavioral tests which were performed during daytime on several days during the experiment (detailed overview in Supplementary Figure 2) [147].

In comparison to standard behavioral tests as the OF, automated analysis of DVC trajectory data with Traja was able to detect differences in walked distance and velocity in the present female stroke animal model during the post-surgery period [147]. Previously, neither time nor diet differences on locomotion (distance, velocity) have been found in the OF [147]. In future, further exploration of hyperparameters for laterality (i.e, distance threshold and turn angle range) could improve observation of stroke-induced turn preference, thus potentially providing a quantitative measure of impairment and recovery. In conclusion, the generated data provide a proof-of-concept of Traja as novel automated analysis method of activity measured via home-cage sensors in DVC to quantify several locomotive parameters in a stroke mouse model.

Automated home-cage 24/7 monitoring is an active area of research and a step forward for reproducible behavioral analysis, accompanied by rapid advances in technology and advanced methods such as machine learning (ML). While researchers seek to control many factors in experimental settings to understand biological and pathological processes, there is still much opportunity to extract and analyze large datasets in an experiment. As the amount of data available to researchers grows beyond the capacity of researchers to process and analyze it, tools which support automated pattern recognition are becoming increasingly relevant to neuropsychiatric research and disease treatment. As an open source, Python-based software, Traja supports collaboration between behavioral researchers for both classical hypothesis testing as well as complementary advanced analytical techniques for pattern detection such as unsupervised ML. Based on these findings, we suggest that Traja can be used to gain insight into mouse locomotion in movement disorders and stroke research.

In future, by combining sensor data provided by devices like DVC with open source tools such as Traja, researchers will be able to gain a deeper insight into underlying cognitive processes relevant to neurological conditions like stroke as well as ordinary behavior. Further potential extensions of this software include development of a graphical user interface to increase the usability to researchers with minimal computer skills. Researchers can use tools like Traja to generate highly reproducible and transparent analysis and visualizations of spatial trajectory data collected through virtually any tracking data source.

3.5 CONFLICT OF INTEREST

The authors thank Bianca Lemmers-van de Weem, Kitty Lemmens-Hermans and Iris Lamers-Elemans for their excellent technical support. No actual or potential competing interests apply for any of the authors. *The authors have no conflict of interest to declare.*

3.6 AUTHOR CONTRIBUTIONS

A.K. and M.W. provided conceptual guidance and were together with J.S. and K.L. involved in the study design. M.W. collected all the experimental data. J.S. performed data analysis with Traja as well as statistical analysis. The manuscript was written by J.S. and K.L. and commented on by all authors.

3.7 SUPPLEMENTARY RESULTS

Supplementary material can be found in the original article.

4

APPLICATION OF TRAJA TO MOUSE STROKE MODEL ON A MEDITERRANEAN DIET

Stroke is one of the leading causes of adult disability worldwide. After ischemic stroke, damaged tissue surrounding the irreversibly damaged core of the infarct, the penumbra, is still salvageable and therefore a target for acute therapeutic strategies. Mediterranean diet (MD) has been shown to lower stroke risk. MD is characterized by increased intake of extra-virgin olive oil, of which hydroxytyrosol (HT) is the foremost phenolic component. This study investigates the effect of a HT-enriched diet directly after stroke on regain of motor and cognitive functioning, MRI parameters, neuroinflammation, and neurogenesis. Stroke mice on HT diet showed increased strength in the forepaws, as well as improved short-term recognition memory probably due to improvement in functional connectivity (FC). Moreover, mice on HT diet showed increased CBF and also heightened expression of brain derived neurotrophic factor (Bdnf) indicating a novel neurogenic potential of HT. This result was additionally accompanied by an enhanced transcription of the postsynaptic marker Psd-95 and by a decreased IBA-1 level indicative of lower neuroinflammation. These results suggest that a HT-enriched diet could serve as a beneficial therapeutic approach to attenuate ischemic stroke-associated damage.

This chapter is published in Nutrients as:
Shenk, J.[1], Calahorra, J.[1], Wielenga, V. H., Verweij, V., Geenen, B., Dederen, P. J., Peinado, Herreros, M. A. P., Siles, E., Wiesmann, M., Kiliaan, A. J.. (2019). Hydroxytyrosol, the major phenolic compound of olive oil as acute therapeutic strategy after ischemic stroke. *Nutrients* 11:2430. doi: 10.3390/nu11102430.

[1] The authors contributed equally to the present work.

4.1 INTRODUCTION

Stroke is a leading cause of death and long-term disability worldwide. Whereas two-third of stroke deaths occur in less developed countries, it is the second most common cause of death in Europe [230]. Ischemic stroke, caused by obstruction of a blood vessel by for example a thrombus, is the most common type of stroke (80-85%). The neuronal injury after ischemic stroke is caused by the absence of oxygen and glucose during the ischemic period and, more importantly, by oxidative stress and increase in inflammation along the reperfusion period [231]. Consequently, neurodegeneration especially in the core of the infarct takes place, giving rise to a gradual and continuous deterioration of behavioral and cognitive functions [232, 233]. The penumbra, the ischemic boundary zone around the irreversibly injured core, is a potentially salvageable tissue and may be the objective of restorative interventions [234].

Endovascular interventions and intravenous thrombolysis restore brain perfusion and limit the acute effects of stroke. However, no further stroke treatments are available, except for some rehabilitative therapies such as training, progressive task-related practice of skills, and neurostimulation [235]. The decrease of the oxidative stress level, the reduction of the inflammatory processes or the stimulation of neuro- and synaptogenesis are some of the strategies that, particularly when combined, could help to reduce the impact of stroke in the potentially salvageable tissue. A dietary approach could play an essential role in this field. In fact, several studies prove its significance [236]. Our group has already demonstrated that a multinutrient intervention (Fortasyn) containing long chain poly unsaturated fatty acids (LCPUFAS), extra vitamins and antioxidants improves sensorimotor integration, brain integrity and neurogenesis after ischemic stroke induction [237, 238]. The PREDIMED trial, which studies the effect of Mediterranean diets in health, has highlighted the positive association between extra virgin olive oil (EVOO) consumption and the risk of stroke in humans [239, 240]. This beneficial effect of EVOO has also been shown to be protective in terms of redox homeostatic balance, lipid and protein damage, activation of NO synthase (NOS) in penumbra and reduction of apoptosis level in chronic ischemic models [241–243].

EVOO, obtained by mechanical processes under cold temperatures, is constituted by two fractions: the major saponificable fraction (98%) composed of fatty acids such as oleic acid, and the minor unsaponificable fraction (2%) existing of more than 230 components, amongst which are the phenolic alcohols [244]. Hydroxytyrosol (HT), together with tyrosol and oleuropein, are the most abundant phenolic alcohols in EVOO [245]. A number of studies have demonstrated that many of the beneficial properties of EVOO are strongly related with HT. This polyphenol has shown numerous biological effects, among others such as antioxidant and anti-inflammatory capacity, antitumour properties and neuroprotective effects [246]. Until now, all studies concerning

the neuroprotective effect of HT under ischemic conditions have been carried out ex vivo, using thick brain sections [247–250]. These sections were incubated with different concentrations of HT or extracted from previously treated animals. The results obtained from these experiments, indicate that HT exerts a neuroprotective effect associated with lower release of lactic dehydrogenase, decreased levels of nitrosative and oxidative stress and decrease in inflammation. The neuroprotective effect of this compound in ischemic processes has also been studied in diabetic rats, and indicated that its neuroprotective action is not exclusively linked to its antioxidant action [251].

With this background, the objective of the present study is to longitudinally evaluate the effect of a HT-enriched diet both on motor and cognitive skills as well as structural and functional MRI outcomes like cerebral blood flow (CBF) and grey and white matter integrity at day and day 35 post-stroke in a well-known and broadly used mouse transient middle cerebral artery occlusion (tMCAo) model. In addition, metabolic, neurogenic and inflammatory markers will be evaluated as well as oxidative levels in serum, in order to investigate the potential of HT as acute therapeutic strategy after stroke.

4.2 MATERIALS AND METHODS

4.2.1 *Animals*

The present study was double-blinded randomized and performed at the Preclinical Imaging Center (PRIME) of the Radboud university medical center (Radboudumc, Nijmegen, The Netherlands) using 28 male 2-3-month-old CS BL/6JRj mice (Harlan Laboratories Inc., Horst, the Netherlands). Before tMCAO, the mice had *ad libitum* access to standard food pellets (Ssniff rm/h V1534-000, Bio Services, Uden, the Netherlands) and autoclaved water and were individually housed in DVC cages (Digital Ventilated Cage, Tecniplast S.P.A., Buguggiate (VA) Italy) during the experiments, to study individual locomotion via calculation of DVC metric measures (activity, walked distance, walked velocity, total turnings, laterality index) during day- and night time, before and after surgery [252, 253]. The animals were kept at artificial 12h light-dark cycle (lights on at a.m.) in rooms controlled for humidity and temperature at 21±1 °C and background music playing during the light cycle. All experiments were performed in accordance with the Dutch federal law for animal experimentation ("Wet op de Dierproeven", 1996) and the regulations of the European Union Directive of 22 September 2010 (2010/63/EU). All experiments were approved by the Animal Ethics Committee of the RadboudUMC (protocol number: RU-DEC 2017-0021) and performed according to the ARRIVE guidelines.

4.2.2 *Transient middle cerebral artery occlusion (tMCAo)*

At ~3 months of age, mice underwent transient (30min) occlusion of the right middle cerebral artery (tMCAo), as previously described [238]. Mice were anesthetized with 1.5% isoflurane in a 2:1 (air:oxygen) mixture and were kept under anaesthesia for the duration of the surgery. Just prior to the occlusion procedure, a Laser Doppler probe (moorVMS-LDF2, Moor Instruments, UK) was placed on the skull of the mice to monitor cerebral blood flow (CBF) as an assessment of the efficacy of the occlusion (≥80% loss of CBF). A 7-0 monofilament (190-200μm, coating length 2-3mm, 70SPRePK5, Doccol Corp., Sharon, MA, USA) was inserted in the right carotid cerebral artery (CCA) and pushed upwards to the proximal part of the middle cerebral artery (MCA). The filament occluded the MCA for 30min, after which it was retracted to allow reperfusion. As a control, part of the mice underwent sham surgery instead of tMCAo. In these mice, the filament was immediately retracted after touching the Willis' circle. After surgery, all mice were carefully assessed for pain and other discomfort, weighed every day at 12 a.m. for days, and food intake was monitored. Exclusion criteria were decreased motor activity (<50% of the baseline measurements combined from the baseline values of each behavioural test) or extreme weight loss (>20% within three consecutive days). Using a T2-weighted RARE sequence to measure lesion size and ratio between stroke (right) and unaffected (left) hemispheres, all stroke animals showed a comparable lesion size at days post-stroke and no dietary effect on lesion size (data not shown). Notably, both dietary groups demonstrated atrophy over time visible in a decrease in left-to-right ratio (D : 0.9 ±0.03; D35: 0.85±0.08; $F(1,12)=31.3$, $p<0.006$). The time line of the experimental design is illustrated in Figure 4.1.

4.2.3 *Group allocation and diet*

After stroke, mice were randomly allocated, using a random sequence generator, to one of two diets: an HT-enriched diet (n=13; stroke (n=6), sham (n=7)) or an isocaloric control diet (n=15; stroke (n=8), sham (n=7)). Group sizes were calculated according to effect sizes (p=0.05, statistical power: 0.80), exclusion and mortality rates previously determined by a similar study of our group [238]. Both diets contained 24.0% kcal protein, 15.0% kcal fat, and 61.0% kcal carbohydrates (Research Diet Services B.V., Wijk bij Duurstede, The Netherlands), based on a previous study from another group [254–256]. The HT-enriched diet was supplied with 0.03g% HT (Seprox Biotech, Spain). Food intake and body weight was measured before and during the weeks after surgery. Excluded mice per test are shown in Table 4.1.

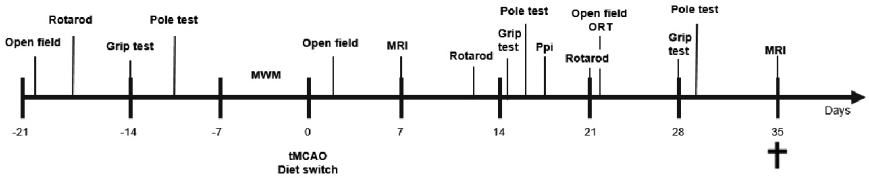


Figure 4.1: Study design. After a transient occlusion of the middle cerebral artery (tMCAo) for 30 min, mice were divided into two dietary groups (Control or HT-enriched). At and 35 days post tMCAo all mice underwent MRI. In between, all mice were tested on motor and cognitive impairments via several behavioral tests, like the Open field, Rotarod, Pole test, Prepulse inhibition (Ppi), grip strength test, and novel object recognition test (ORT). After MRI at day 35, animals were sacrificed, serum samples were recollected and all brains were processed for immunohistochemical stainings and qPCR analysis.

4.2.4 *Open field*

Mice were placed in a square open field (45x45x30cm) for 10 min to assess locomotion and explorative behaviour. The open field test was performed three times: once prior to surgery, and at 3 and 21 days postsurgery. Locomotion was automatically recorded, using EthoVision XT 10.1 (Noldus, Wageningen, The Netherlands). In EthoVision, the floor of the open field arena was divided in zones to distinguish the periphery, corners and center. The frequency of entering these zones was automatically recorded. Additionally, manual scoring of exploratory behaviours (sitting, walking, grooming, wall-leaning, rearing) was performed, as previously described [257].

4.2.5 *Grip test*

Grip strength of the mice was measured with a grip strength meter (Grip Strength Meter, 47200, Ugo Basile, Italy) at three time points: pre-stroke, and day 15 and day 29 (post-stroke). Mice were held by their tail so they could grab a trapeze or grid (connected to the grip strength meter) to measure, respectively, muscle strength in the fore limbs (trapeze) or strength in all four limbs collectively (grid). Trials in which mice grabbed the trapeze with only one forepaw or the grid with less than four paws, were excluded. Additionally, trials in which the mouse grabbed the side of the trapeze were also excluded. The maximum value of peak force (in gram per force (gf)) was averaged per experimental group for both trapeze and grid.

4.2.6 *Pole test*

The pole test is used to monitor motor function. It was performed pre-stroke, and post-stroke at day 14 and day 28. The mouse was placed on a vertical pole with its head pointed upwards and had to turn 180 degrees to walk down the pole. The time needed to fully turn 180 degrees (turning time) and the turning direction were manually recorded. Additionally, video recordings of each trial were automatically analysed with EthoVision XT 10.1 (Noldus, Wageningen, The Netherlands) to calculate the velocity (cm/s) with which the mouse walked down the pole. Trials that were excluded from statistical analysis consisted of: every first trial of a mouse (acclimatization), trials in which the mouse was already turning when placed on the pole, and trials in which the mouse showed no motivation and was assisted by the researcher to go down.

4.2.7 *Rotarod*

As a measure of balance, coordination, physical condition and motor planning the Rotarod (ITC LifeScience INC., Woodland Hills, CA, USA) was performed presurgery, and at day 10 and day 21 postsurgery. The mice were placed on the Rotarod and left to acclimatize for 10-30 sec. Then the Rotarod was turned on to accelerate for 300 sec from 4 to 40 rpm. The latency time to fall was recorded. No significant effects (data not shown) were found.

4.2.8 *Prepulse inhibition (Ppi)*

The prepulse inhibition (Ppi) test was performed 16 days post-stroke to examine the sensorimotor gating integration of the mice, as previously described [258]. To measure the startle reactivity, the mouse was placed in a restrainer in the chamber of the SR-LAB startle response system (San Diego Instruments, San Diego, CA, USA) and exposed to blocks of startle pulses. Prepulse inhibition was calculated during the second block of startle pulses as 100 - response to startle pulse after prepulse/response to startle pulse x 100%. Additionally, habituation to startle pulses was investigated by comparing the startle response to the first startle pulse block to the startle response in the third (last) startle pulse block.

4.2.9 *Morris water maze (MWM)*

The Morris water maze (MWM) was used to test spatial learning and memory in rodents. In short, before surgery all mice were placed in a circular pool, filled with opaque water, and were trained to find a submerged platform in the northeast (NE) quadrant of the pool by using distant visual cues. At the end of the fourth day, mice performed additionally a single probe trial, in which the

platform was removed from the swimming pool. Mice were allowed to swim for 120 s and the time spent swimming and searching in the NE quadrant (where the platform had been located) was recorded. The MWM is used to analyse spatial learning and memory before surgery (data not shown). All mice learned to find the hidden platform revealed by a decrease in latency time from acquisition day 1 to day 4 (-16.33s±5.68s; $F(3,81)=3.8$, $p<0.013$).

4.2.10 *Novel object recognition test (ORT)*

Short-term memory of the mice was measured with the novel object recognition test (ORT), as previously described [238]. This test spanned over 3 days. On the first day, mice were acclimatized to the open field box by letting them explore freely for 10min. On the second and third day, mice underwent object recognition trials. First, mice performed a familiarization trial. In this trial two identical objects (eggs, tea light holders, yellow plastic ice cream cones, or bottles filled with sand) (F1 and F2) were placed in the open field, equidistant from the center, and the mouse was then placed in the open field to freely explore the objects for 4 minutes. After a certain delay (30 min on day 2 and 60 min on day 3) the trial was repeated, with one of the familiar objects (F3) and one object replaced for a novel object (N1). Exploratory behaviour of the mice was measured using EthoVision XT 10.1 (Noldus, Wageningen, The Netherlands) as direct contact with the object, or movement within a 2cm diameter around the object.

To measure object recognition, several indexes were calculated in both the familiarization and the test phase. In the test phase, discrimination between objects was calculated with the discrimination index (DI), as the time spend around N1 minus F3, divided by the time spend around both objects (DI = (N1-F3)/(N1+F3)). From this index, a number between +1 and -1 is obtained, where closer to +1 shows more time spend around N1, closer to -1 more time spend around F3, and 0 shows no difference in time spend at either object. To measure recognition memory, the recognition index (RI) was calculated as the time spend around N1 as a fraction of the time spend around both objects (RI = N1/(N1+F3)). The preference for either object was calculated with the preference index (PI), as time the mouse spend around N1 (or F3) as a percentage of the time spend around both objects (PI = 100 x ([N1 or F3]/(N1+F3)). If N1 is the numerator, closer to 100% indicates preference for N1, 50% indicates no preference, and below 50% preference for F3 (vice versa if F3 is the numerator) [259].

4.2.11 *Digital ventilated cages (DVC)*

Like previously detailed described [252, 253], animals were single housed in DVC cages during the experiment to study individual locomotion via calculation of DVC metric measures (activity, walked distance, walked velocity, total turnings, laterality index) during day- and nighttime before and after surgery. A detailed explanation of the calculations on the aforementioned DVC metric measures can be found in the supplementary material (Figure 4.19). For analysis of the processed results we compared week 1-5 individually to presurgery values.

4.2.12 *In vivo magnetic resonance imaging (MRI)*

MRI measurements were performed and 35 days after surgery with an 11.7T BioSpec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) operating on Paravision 6.0.1. software (Bruker, Karlsruhe, Germany) under full isoflurane anaesthesia (3.5 % for induction and 1.8% for maintenance; in a 2:1 (medical air:oxygen) mixture). Body temperature was monitored with a rectal probe, and maintained at 37 ^{0}C using hot air flow. A pneumatic cushion respiratory monitoring system (Small Animal Instruments Inc, NY, USA) was used to measure the respiration rate of the mouse. Mice with scans that showed motion and/or echo planar imaging artifacts were excluded from MRI analysis.

4.2.13 *Arterial spin labelling (ASL)*

To assess cerebral blood flow (CBF), perfusion imaging was performed using a flow sensitive alternating inversion recovery arterial (FAIR) technique as previously described [234, 238]. Hippocampus, cerebral cortex and thalamus, according to the Paxinos and Franklin atlas [260], were analysed as regions of interest (ROI) by a researcher blinded to the surgery and treatment groups. For each ROI, CBF was analysed in the affected (ipsilateral/right) and unaffected (contralateral/left) hemisphere separately for each group.

4.2.14 *Diffusion tensor imaging (DTI)*

Diffusion of water was imaged as described previously [261–263]. In short, 22 axial slices covering the whole brain were acquired with a four-shot SE-EPI protocol. B0 shift compensation, navigator echoes and an automatic correction algorithm to limit the occurrence of ghosts and artefacts were implemented. Encoding b-factors of 0 s/mm^2 (b0 images; 5×) and 1000 s/mm^2 were used and diffusion-sensitizing gradients were applied along 30 non-collinear directions

in three-dimensional space. The diffusion tensor was estimated for every voxel using the PATCH algorithm [264]; mean water diffusivity (MD) and fractional anisotropy (FA) were derived from the tensor estimation following a protocol as described elsewhere [263]. MD and FA values were measured in several white matter (WM) and grey matter (GM) areas, manually selected based on an anatomical atlas [265].

4.2.15 *Resting state functional MRI (rs-fMRI)*

Subsequently after the acquisition of the anatomical reference images, resting state fMRI (rsfMRI) datasets were acquired using a single-shot spin-echo sequence with echo-planar readout (SE-EPI) sequence. Six hundred repetitions with a repetition time (TR) of 1.8 s and echo time of 16.9 ms were recorded for a total acquisition time of 18 min. The rsfMRI datasets were first realigned using a least-squares method and rigid-body transformation with Statistical Parametric Mapping (SPM) mouse toolbox (SPMS, University College London; http://www.fil.ion.ucl.ac.uk/spm/; [266]. Mean and maximum displacement across the six degrees of freedom (along the x-, y-, and z-axes and on three rotation parameters pitch, roll, and yaw) were measured in each mouse. The mean SE-EPI images for each mouse were then used to generate a study-specific template through linear affine and nonlinear diffeomorphic transformation (ANTs. v1.9; http://picsl.upenn.edu/ANTS/). Visual inspection of the normalised dataset was performed to screen for possible normalization biases. On the template, 12 areas were selected in left and right hemisphere. The selected regions were based on previous work concerning functional connectivity in mice [267], and included: left and right dorsal hippocampus, left and right ventral hippocampus, left and right auditory cortex, left and right motor cortex, left and right somatosensory cortex, and left and right visual cortex. All cortical ROIs were selected 1–2 voxels away from the edge of the cortex, to minimise the impact of susceptibility artefacts, which are more prominent in areas close to tissue interfaces (e.g., near the skull or near the ear canals). In-plane spatial smoothing (0.4 × 0.4 mm), linear detrending, and temporal high-pass filtering (cut-off at 0.01 Hz) were applied to compensate for small across-mouse misregistration and temporal lowfrequency noise. Functional connectivity (FC) group comparison between ROIs were calculated from the BOLD time series using total correlation analyses implemented in FSLNets (FSLNets v0.3; www.fmrib.ox.ac.uk/fsl). Pearson's correlation values were Fisher transformed to Z-scores for group comparisons and statistical analysis.

4.2.16 *qPCR*

RNA was isolated from frontal parts (divided in to left and right) of the brain (Bregma: −0.10 to 4.28 using TRIzol method (Thermo Scientific, Waltham, USA). The samples were treated with RNase-free DNase I (RQ1, Promega, Fitchburg, USA) to eliminate any genomic DNA. cDNA was synthesized using the iScript kit (Bio-Rad, Hercules, USA). qPCR was done in 96-well plates (Thermo Scientific) using a StepOnePlus system (Thermo Scientific). Gene expression of postsynaptic density protein 95 (Psd-95), brain derived neurotrophic factor (Bdnf) and glucose transporter 1 (GLUT-1) were quantitatively assessed by using hypoxanthine guanine phosphoribosyl transferase (Hprt) and beta-2 microglobulin (B2m) as the normalizing genes. The sequences of primers are shown in Table 4.2.

4.2.17 *(Immuno)histochemistry*

After the last scanning session, the mice were sacrificed by transcardial perfusion using 0.1M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS. The brains were harvested and stored separately. The brains were postfixed overnight in 4% paraformaldehyde at 4^0C and transferred to 0.1M PBS containing 0.01% sodium azide the next day. One part of the brain (Bregma: -0.1-4.36) was cut in 30 μm frontal sections using a sliding microtome (Microm HC 440, Walldorf, Germany) equipped with an object table for freeze-sectioning at -60^0C. 24 Hours before cutting, the brains were transferred in 30% sucrose in 0.1M phosphate buffer. 8 Series were cut and stored in 0.1M PBS with 0.01% sodium azide so multiple immunohistochemical stainings could be performed.

All sections were stained in one session to minimize differences in staining intensity. In total three stainings were performed for vascular integrity measured via glucose transporter-1 (GLUT-1), for activated microglia via ionized calcium-binding adapter molecule 1 (IBA-1) as indicator for neuroinflammation, and for immature neurons (measure for neurogenesis) with antibodies against doublecortin (DCX) on free-floating brain sections on shaker tables at room temperature. Immunohistochemistry was performed using standard free-floating labelling procedures, using previously described protocols [268]. The GLUT-1 amount was visualized using polyclonal rabbit anti-GLUT1 antibody (1:40.000, Chemicon AB 1340, Chemicon International, Inc., Temecula, CA, USA) and as secondary antibody donkey anti-rabbit biotin (1:1500 Jackson ImmunoResearch, West Grove,PA, USA). For IBA-1, as primary antibody against IBA-1 polyclonal goat anti-IBA-1 (1:3000; Abcam) and for DCX, polyclonal goat anti-DCX (1:8000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as a primary antibody to

assess neurogenesis. For both as secondary antibody donkey anti-goat biotin (1:1500; Jackson ImmunoResearch, West Grove, PA, USA) was used. From a more frontal part of the brain tissue (Bregma: -0.10 to 0.98) was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH .4) and embedded in paraffin according to a standard protocol.

4.2.18 *Quantification (GLUT-I, IBA-I, and DCX)*

Brain sections (Bregma: -1.46 to -2.30) were preselected for quantification accordingly to the atlas of Franklin and Paxinos [260]. Quantification was done on images at a 5x objective using an Axio Imager A2 (Zeiss Germany). ImageJ (National Institute of Health, Bethesda, MD, USA) was used to analyze the regions of interest (GLUT-1 + IBA-1: Cortex (Bregma 0.62 & -1.94), hippocampus, thalamus, caudate putamen, corpus callosum (only IBA-1); DCX: Hippocampus).

4.2.19 *Determination of Serum NO Level*

Nitric oxide production was indirectly quantified by determining nitrate/nitrite and S-nitroso compounds (NOx), using an ozone chemiluminescence-based assay adapted to serum samples [269, 270]. In brief, serum samples were deproteinized with 0.8 N NaOH and 16% $ZnSO_4$ solutions (1/0.5/0.5). After centrifugation at 10,000g for 5 minutes, the resulting supernatants were removed for chemiluminescence analysis [271] in an NO analyzer (NOA 280i; Sievers Instruments, Boulder, CO). NOx concentration was calculated by comparison with standard solutions of sodium nitrate. Final NOx values were expressed as μM.

4.2.20 *Determination of serum oxidative stress level*

Thiobarbituric acid reactive substances (TBARS), a major indicator of oxidative stress, was determined using an adaptation of the method described by Buege and Aust [272]. Specifically, 8% sodium dodecyl sulfate was added (1:1) to each serum sample. Samples were vortexed and mixed (1:6) with a reagent containing 15% trichloroacetic acid, 0.38% thiobarbituric acid, and 2% HCl and then heated for 30 minutes at 96^0C, cooled, and centrifuged (3000g for 5 minutes). The supernatants were collected, and the absorbance was measured at 532 nm. The concentration of TBARS was determined by extrapolation from a malondialdehyde standard curve. Results were expressed as μM.

4.2.21 *Statistical analyses*

A random and blinded selection procedure was maintained throughout the study. Group means were compared using univariate analysis of variance (ANOVA) with Bonferroni correction for multiple testing with a statistical program, SPSS 24 (IBM SPSS Statistics 24, IBM Corporation, Armonk, NY, USA). Nonparametric tests were used when assumptions of normality and homogeneity of variance were not met. p-values lower than 0.05 were considered significant. Data are presented as mean ± SEM.

4.3 RESULTS

4.3.1 *Food intake and body weight*

Body weight (Figure 4.2A) of sham mice did not decrease over time comparing presurgery with the first week after surgery ($F(1,12)=3.3$, $p<.095$), while body weight of both dietary stroke groups decreased post-stroke versus pre-stroke ($F(1,12)=18.2$, $p<.002$). Food intake (Figure 4.2B) of both stroke and sham mice decreased over time comparing presurgery with the first week after surgery (Stroke: $F(1,12)=19.8$, $p<.001$; Sham: $F(1,12)=40.5$, $p<.001$).

Investigating the development over time of body weight and food intake, these parameters were analyzed weekly after surgery. Sham mice showed a significant time interaction in body weight ($F(1,48)=14.5$, $p<.001$) and also in food intake ($F(1,48)=16.5$, $p<.001$). Specifically, sham mice lost body weight comparing week 1 with week 2 ($p<.001$), while this effect was not present in the following weeks comparing week 2 with week 3, week 3 with week 4, and week 4 with week 5. Sham mice had a lowered food intake comparing week 1 to week 2 ($p<.006$) and also week 3 to week 4 ($p<.009$). Notably, sham mice on HT diet had a higher food intake during all post surgery weeks than sham mice on control diet ($F(1,12)=5.3$, $p<.041$). Stroke mice demonstrated a time interaction in food intake ($F(1,48)=10.2$, $p<.001$). After further statistical analysis, stroke mice ate less comparing week 1 with week 2 ($p<.025$) and started to eat more comparing week 2 with 3 ($p<.008$).

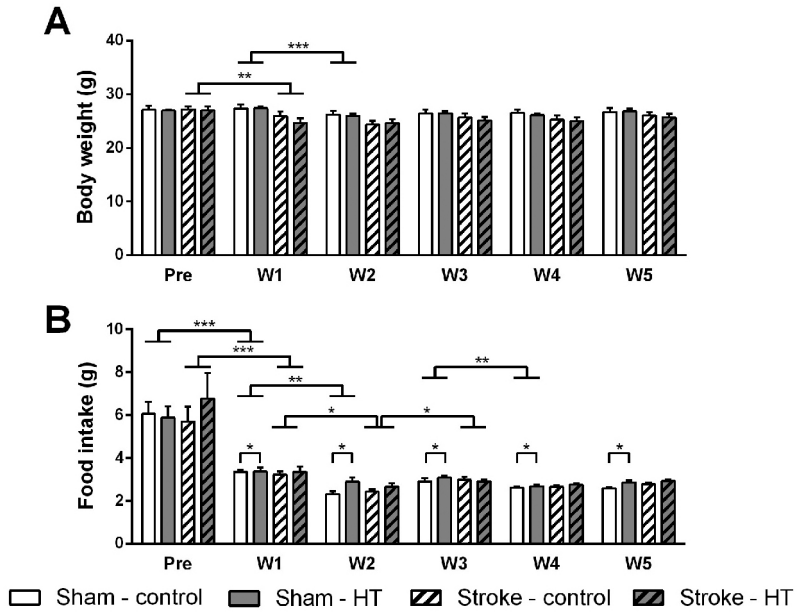


Figure 4.2: Effect of HT-diet on body weight and food intake. (A) Body weight of both dietary stroke groups decreased post-stroke versus pre-stroke ($p<.002$). Sham mice lost body weight comparing week 1 with week 2 ($p<.001$), with no effect in the upcoming weeks. (B) Food intake of both stroke and sham mice decreased over time comparing presurgery and the first week after surgery (Sham: $p<.001$; Stroke: $p<.001$). Sham mice had a lower food intake comparing week 1 to week 2 ($p<.006$) and also week 3 to week 4 ($p<.009$). Sham mice on HT diet had a higher food intake during all postsurgery weeks than sham mice on control diet ($p<.041$). Stroke mice ate less comparing week 1 with week 2 ($p<.025$) and started to eat more comparing week 2 with 3 ($p<.008$). Only sham mice showed a significant time interaction in body weight ($p<.001$) and in food intake ($p<.001$). Values represent mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

4.3.2 *Behaviour, cognition and motor tasks*

4.3.2.1 *Open field*

At 3 days postsurgery, all sham and all stroke mice moved a shorter distance (Sham: $F(1,12)=137.1$, $p<0.001$; Stroke: $F(1,12)=116.2$, $p<0.001$) (Figure 4.3A) and with a lower velocity (Sham: $F(1,12)=99.4$, $p<0.001$; Stroke: $F(1,12)=116.5$, $p<0.001$) in the arena (Figure 4.3B), compared to baseline. At 21 days post- surgery, sham animals walked more in the open field compared to 3 days post- surgery ($F(1,12)=1\ .1$, $p<0.002$), however stroke animals did not (Figure 4.3A). No effects of diet were observed in distance moved and velocity in the open field.

Anxiety and exploration were assessed by tracking the position of the mice in the open field (center, corners, periphery). Compared to baseline, stroke mice visited the center ($F(1,12)=60.7$, $p<0.001$), periphery ($F(1,12)=230.2$, $p<0.001$) and corners ($F(1,12)=85.5$, $p<0.001$) less frequently at 3 days post-stroke (Figure 4.3C), but showed no change in time spent at all locations (Figure 4.3D). Sham animals also visited the periphery ($F(1,12)= 3.4$, $p<0.001$) and corners ($F(1,12)=1$ 2.0, $p<0.001$) less frequently at 3 days post-stroke compared to baseline (Figure 4.3C), however they spent less time in the corners ($F(1,12)=6.7$, $p<0.024$) at 3 days (Figure 4.3D). No diets effects were observed in these parameters.

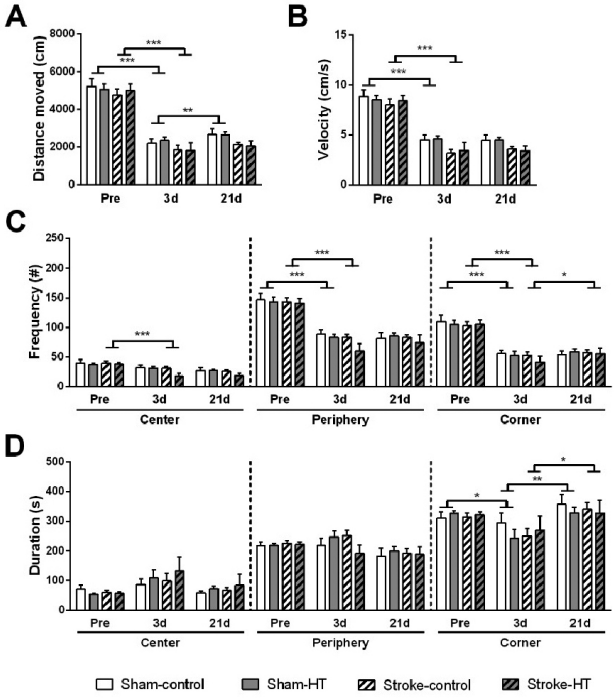


Figure 4.3: Activity, anxiety and explorative behavior measured in open field prior to stroke, and at 3 and 21 days post-stroke. Locomotion was assessed by evaluating (A) the distance moved and (B) the velocity. Anxiety and exploration were evaluated by tracking (C) the frequency and (D) the time spent in the different zones in the arena (center, periphery and corner). (A,B) At 3 days postsurgery, all sham and all stroke mice moved a shorter distance (Sham: $p<0.001$; Stroke: $p<0.001$) with lower velocity (Sham: $p<0.001$; Stroke: $p<0.001$) compared to baseline. At 21 days post-stroke, sham animals walked more in the open field compared to 3 days post-stroke animals ($p<0.002$). (C-D) Stroke mice visited the center ($p<0.001$), periphery ($p<0.001$) and corners ($p<0.001$) less frequently at 3 days post-stroke. Sham animals also visited the periphery ($p<0.001$) and corners ($p<0.001$) less frequently at 3 days post-stroke compared to baseline, however they spent less time in the corners ($p<0.024$) at 3 days. Values represent mean ± SEM. # $0.05<p<0.08$, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

The frequency with which the mice engaged in different types of exploratory behavior was manually scored (Figure 4.4). Compared to baseline, all mice showed decreased frequency of wall leaning (Sham: $F(1,12)=64.5$, $p<0.001$; Stroke: $F(1,12)=258.1$, $p<0.001$) and walking (Sham: $F(1,12)=26.7$, $p<0.001$; Stroke: $F(1,12)=157.3$, $p<0.001$) and also spent less time performing these behaviors (*Wall leaning*: Sham: $F(1,12)=123.0$, $p<0.001$; Stroke: $F(1,12)=8.1$, $p<0.001$; *Walking*: Sham: $F(1,12)=84.2$, $p<0.001$; Stroke: $F(1,12)=40.3$, $p<0.001$) at 3 days post-stroke, while they spent more time sitting (Sham: $F(1,12)=172.4$, $p<0.001$; Stroke: $F(1,12)=62.3$, $p<0.001$) and grooming (Sham: $F(1,12)=6.2$, $p<0.028$). At baseline and 3 days post-stroke, sham-HT mice groomed less frequently than sham-control mice ($F(1,12)=5.6$, $p<0.036$; *not shown in graph*). Additionally, stroke mice also reared less frequently at 3 days compared to pre-stroke ($F(1,12)=8.6$, $p<0.012$). At 21 days post-stroke, walking frequency ($F(1,12)=6.4$, $p<0.026$) was even further decreased in sham mice, whereas stroke mice showed no change in walking frequency or duration, but did show increased rearing ($F(1,12)=8.6$, $p<0.012$) and wall leaning frequency ($F(1,12)= 7.2$, $p<0.020$). Sham mice groomed more frequently at 21 days compared to 3 days post-stroke ($F(1,12)=12.3$, $p<0.004$). No additional diet effects were found for these parameters (Figure 4.4).

4.3.2.2 *Grip test*

Forelimb strength of the mice was quantified with the grip test, by letting the mice grip a small trapeze connected to a grip strength meter. Fore- and hind limb strength was determined in a similar fashion, using a grid instead of a trapeze. At week 2, only sham mice showed a lower grip strength on the grid compared to pre-surgery ($F(1,12)=13.0$, $p<0.004$) (Figure 4.5A). Stroke mice showed no significant decreased grip strength on either the trapeze or grid 2 weeks after stroke compared to baseline. A surgery effect was observed at week 2, shown by weaker grip strength in forelimbs in stroke-control mice compared to sham-control mice ($F(1,9)=17.3$, $p<0.002$) (Figure 4.5A). At 4 weeks postsurgery, sham mice show a decreased forelimb grip strength compared to 2 weeks postsurgery ($F(1,10)=26.0$, $p<0.001$) (Figure 4.5A). Interestingly, HT-fed stroke mice demonstrated a higher grip strength on the trapeze at week 2 and 4 compared to stroke control diet-mice ($F(1,12)=49 .0$, $p<0.018$). No diet effects were observed on the grid (Figure 4.5B).

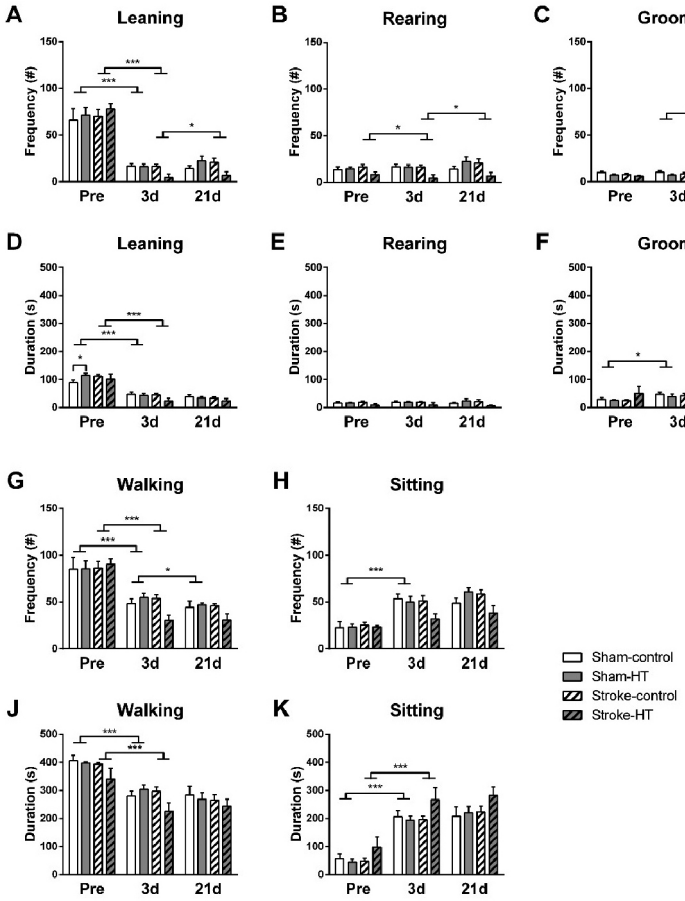


Figure 4.4: Behaviors in the open field. The behaviors of the mice in the arena during the open field were manually scored as another measure of locomotion, activity, and explorative behavior. (A-C, G, H) Frequency and (D- F, J, K) duration of leaning, rearing, grooming, sitting, and walking were quantified presurgery and 3 and 21 days after surgery. (A, G) All mice showed decreased frequency of wall leaning (Sham: $p<0.001$; Stroke: $p<0.001$) and walking (Sham: $p<0.001$; Stroke: $p<0.001$) and (D,J) also spent less time performing these behaviors (Wall leaning: Sham: $p<0.001$; Stroke: $p<0.001$; Walking: Sham: $p<0.001$; Stroke: $p<0.001$) at 3 days post-stroke, (K, F) while they spent more time sitting (Sham: $p<0.001$) and grooming (Sham: $p<0.028$). (C) At baseline and 3 days post-stroke, sham-HT mice groomed less frequently than sham-control mice ($p<0.036$; not shown in graph). (B) Stroke mice also reared less frequently at 3 days compared to pre-stroke ($p<0.012$). (G) At 21 days post- stroke, walking frequency ($p<0.026$) was even further decreased in sham mice, (A,B) whereas stroke show increased rearing frequency ($p<0.012$) and wall leaning frequency ($p<0.020$). (C) Sham mice groomed more frequently at 21 days compared to 3 days post-stroke ($p<0.004$). Values represent mean ± SEM. # $0.05<p<0.08$, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

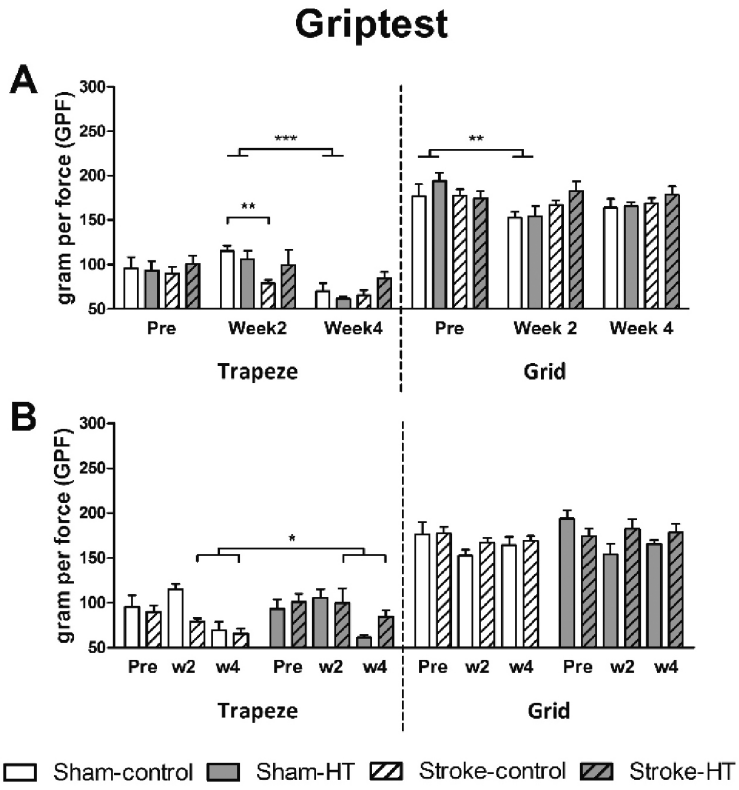


Figure 4.5: Grip strength in the forelimbs (trapeze) and in all four paws (grid) before (pre) and after (week 2 and 4) stroke. (A) Time effects and (B) diet effects on maximum grip strength are shown. (A) At week 2, sham mice showed a lower grip strength on the grid compared to pre-surgery ($p<0.004$). A surgery effect was observed at week 2, shown by weaker grip strength in forelimbs in stroke-control mice compared to sham-control mice ($p<0.002$). At 4 weeks postsurgery, sham mice showed a decreased fore- limb grip strength compared to 2 weeks postsurgery ($p<0.001$). (B) HT- fed stroke mice demonstrated a higher grip strength on the trapeze at week 2 and 4 compared to stroke control diet-mice ($p<0.018$). Values represent mean ± SEM. *p<0.0S, **p<0.01, ***p<0.001.

4.3.2.3 *Pole test*

The pole test was performed to assess motor dysfunction. The time needed to make a full 180 degree turn on the pole and the turning direction was manually scored, and the velocity with which the mice walked down the pole was calculated. There was no change in velocity between baseline measurement and the first measurement after surgery (at 14 days). At 28 days postsurgery, sham mice ($F(1,12)=21.4$, $p<0.001$) and stroke mice ($F(1,9)=5.2$, $p<0.049$) walked

down the pole with a lower velocity compared to 14 days postsurgery (Figure 4.6). Neither time nor diet effects on turning side preference or turning time were observed.

4.3.2.4 *Prepulse inhibition (Ppi)*

The pre-pulse inhibition test was performed to assess sensorimotor gating after stroke. In both surgery and diet groups, no effects could be detected on PPI. No habituation effects were observed, however an overall diet effect was detected. HT-mice showed a higher startle amplitude to the basal and final startle stimulus of 120dB than control-mice ($F(1,22)=8.3$, $p<0.009$) (Figure 4.7).

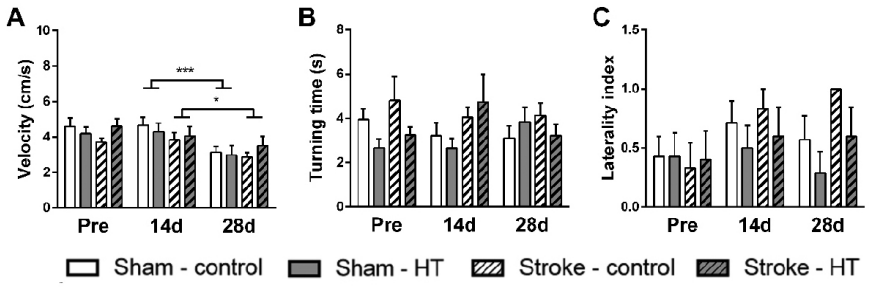


Figure 4.6: Motor coordination assessed pre-stroke and 14 days and 28 days post- stroke by the pole test. (A) Downwards walking velocity. (B) Time to turn around on the pole (turning time) and (C) tendency to turn right vs left (laterally index). (A) At 28 days postsurgery, sham mice ($p<0.001$) and stroke mice ($p<0.049$) walked down the pole with a lower velocity compared to 14 days postsurgery. Values represent mean ± SEM. Values represent mean ± SEM. # $0.05<p<0.08$, *$p<0.05$, ***$p<0.001$

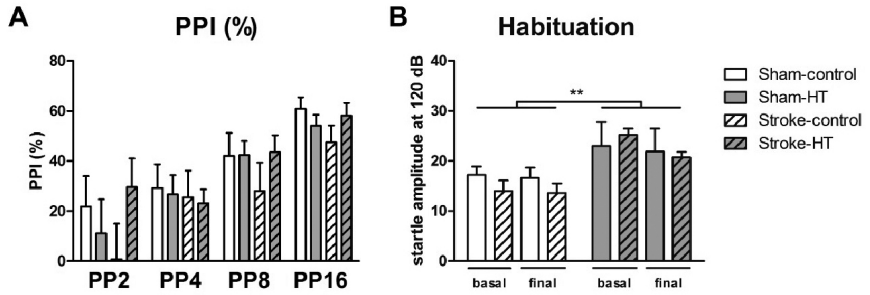


Figure 4.7: Sensorimotor integration measured before and after (16 days) stroke induction by the prepulse inhibition test (PPI). (A) PPI data shown as percentage. (B) Habituation to startle pulse. HT-mice showed a higher startle amplitude to the basal and final startle stimulus of 120dB than control-mice ($p<0.009$). Values represent mean ± SEM. **$p<0.01$.

4.3.2.5 *Novel object recognition test (ORT)*

The object recognition task (ORT) was performed once after stroke to measure short-term memory of the mice. In the 30min trials of the familiarization phase, all discrimination index (DI), recognition index (RI) and preference index (PI) all showed that stroke animals have a preference for object 2 and sham animals a preference for object 1, as they visited it more frequently ($F(1,22)=5.7$, $p<0.026$). However, in stroke animals this effect was largely caused by mice on control diet, as they only showed as PI above 50% (Figure 4.8). In the 30min trials of the test phase, HT-fed animals showed a preference for the novel object and visited it more frequently than control diet-animals ($F(1,22)=6.4$, $p<0.019$), as shown by all indexes (DI, RI and PI) (Figure 4.9).

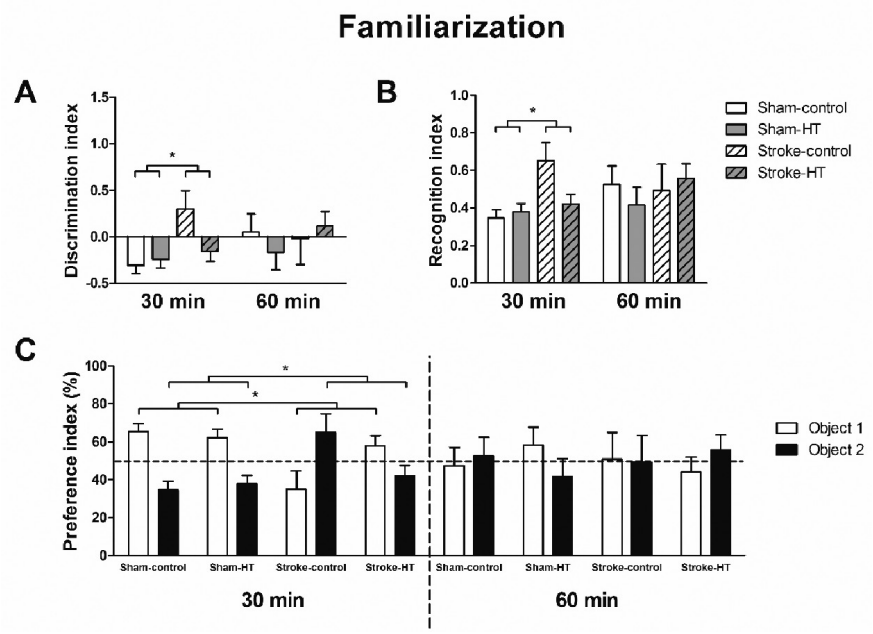


Figure 4.8: Familiarization phase ORT. Short-term memory of sham and stroke mice assessed by the novel object recognition test (ORT). (A) Discrimination index (B) Recognition index and (C) preference index. (A,B,C) Stroke animals had a preference for object 2 and sham animals a preference for object 1 ($p<0.026$). Values represent mean ± SEM. *$p<0.05$.

4.3.2.6 *Digital ventilated cages (DVC) metrics*

See Supplementary results in §4.5

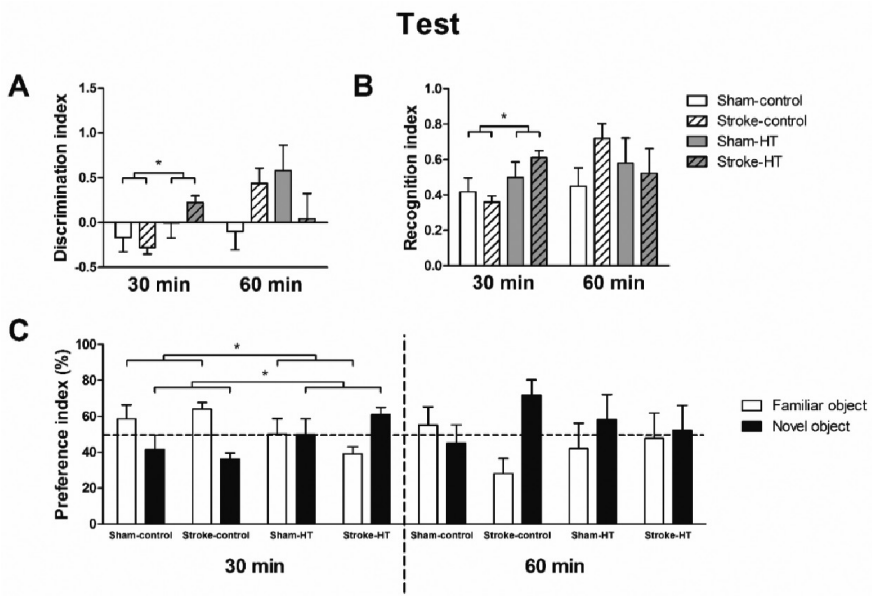


Figure 4.9: Test phase ORT. Short-term memory of sham and stroke mice assessed by the novel object recognition test (ORT). (A) Discrimination index (B) Recognition index and (C) preference index. (A,B,C) HT-fed animals showed a preference for the novel object and visited it more frequently than control diet-animals ($p<0.019$). Values represent mean ± SEM. *$p<0.05$.

4.3.3 *In vivo magnetic resonance imaging (MRI)*

4.3.3.1 *Cerebral Blood Flow (CBF)*

Using an ASL-FAIR technique, CBF was assessed in the lesioned and unlesioned hemisphere at and 35 days post-stroke in the hippocampus, thalamus and cortex (Figure 4.10). At days post-stroke, CBF was lower in all groups in the right cortex (Sham-control: $F(1,6)=24.9$, $p<0.002$; Sham- HT: $F(1,5)=24.5$, $p<0.004$; Stroke-control: $F(1,6)=16.1$, $p<0.00$; Stroke-HT: $F(1,4)=12.6$, $p<0.024$), right hippocampus (Sham-control: $F(1,6)=24.2$, $p<0.003$; Sham-HT: $F(1,5)=50.5$, $p<0.001$; Stroke-control: $F(1,6)= 0.1$, $p<0.001$; Stroke- HT: $F(1,4)=16.3$, $p<0.016$), and right thalamus (Sham-control: $F(1,6)=28.5$, $p<0.002$; Sham-HT: $F(1,5)=262.5$, $p<0.001$; Stroke-control: $F(1,6)=21.2$, $p<0.004$; Stroke-HT: $F(1,3)=10.3$, $p<0.049$) than in the corresponding ROI in the left hemisphere (not shown in figure).

At 35 days post-stroke, almost all groups still displayed a lower CBF in the right cortex (Sham-control: $F(1,6)=14.0$, $p<0.10$; Sham-HT: $F(1,6)=10.8$, $p<0.01$; Stroke-control: $F(1,6)=12.7$, $p<0.012$, Stroke-HT: $F(1,5)=18.9$, $p<0.007$)

and right hippocampus (Sham-control: $F(1,6)=19.5$, $p<0.004$; Sham-HT: $F(1,6)=43.0$, $p<0.001$; Stroke-HT: $F(1,5)=33.5$, $p<0.002$) compared to respectively the left cortex and left hippocampus. In the right thalamus, a lower CBF was also observed in sham animals at 35 days, compared to the left thalamus (Sham- control: $F(1,6)=25.2$, $p<0.002$; Sham-HT: $F(1,6)=38.8$, $p<0.001$). Interestingly, reperfusion was observed in stroke animals in the right thalamus, as demonstrated by the lack of significant difference in CBF between the left and right thalamus at 35 days (not shown in figure).

An overall diet effect was observed in the right hippocampus at and 35 days post-stroke, as shown by an increased CBF in HT-fed sham mice of both surgery groups ($F(1,11)=5.0$, $p<0.046$). Additionally, an increased CBF was also observed in the left cortex of stroke-HT mice compared to stroke-control mice at and 35 days post-stroke ($F(1,10)=5.4$, $p<0.043$) (Figure 4.10)

All stroke animals showed a significantly increased CBF at 35 days post-stroke in the right hippocampus and compared to days post-stroke ($F(1,10)=8.6$, $p<0.015$). In the left hippocampus only HT-fed stroke mice maintained an increased CBF at 35 days compared to control diet-fed mice ($F(1,10)=5.1$, $p<0.048$) (Figure 4.10). In the left thalamus, a decreased CBF was observed at 35 days compared to days post-stroke in stroke-HT mice ($F(1,3)=13.1$, $p<0.036$). Conversely, an increase of CBF was observed in the right thalamus in stroke-control mice at 35 days post-stroke, compared to days post-stroke ($F(1,6)=23.4$, $p<0.003$).

4.3.3.2 *DTI and rs-fMRI*

fractional anisotropy (fa): In sham-control and sham-HT mice, the right hippocampus showed a lower FA than the left hippocampus at 7 days (Sham-control: $F(1,6)=32.1$, $p<0.001$; Sham-HT: $F(1,5)=11.4$, $p<0.020$) and 35 days (Sham-control: $F(1,6)=13.1$, $p<0.011$; Sham-HT: $F(1,5)=49.7$, $p<0.001$) after surgery. In stroke mice, only the control group at days post-stroke showed that FA was lower in the right hippocampus than its contralateral counterpart ($F(1,6)=9.3$, $p<0.022$). In contrast, FA in the right hippocampus increased between days and 35 days after surgery in sham- control mice ($F(1,6)= .4$, $p<0.035$). In the motor cortex, a decreased FA in the right compared to left hemisphere was only observed at 35 days in stroke- control ($F(1,6)=7.6$, $p<0.033$) mice and not in stroke-HT mice. Stroke mice showed an FA increase in the left motor cortex at 35 days post-stroke compared to days post-stroke ($F(1,11)=11.6$, $p<0.006$). However, sham animals showed a decrease of FA in both the left ($F(1,11)=13.7$, $p<0.003$) and right ($F(1,11)=12.8$, $p<0.004$) motor cortex over time (35 days vs.7days) (Figure 4.11A).

MEAN DIFFUSIVITY (MD): In the hippocampus, MD was higher in the lesioned (right) hemisphere than in the unaffected (left) hemisphere at 7 days postsurgery in all groups (Sham-control: $F(1,6)=12.0$, $p<0.013$; Sham- HT: $F(1,5)=25.8$, $p<0.004$; Stroke-control: $F(1,6)=17.4$, $p<0.006$; Stroke- HT: $F(1,5)=6.6$, $p<0.050$), and at 35 days only in sham mice (Sham-control: $F(1,6)=61.6$, $p<0.001$; Sham-HT: $F(1,5)=34.9$, $p<0.002$).

Sham-HT mice showed an increase in MD of the corpus callosum at 35 days compared to days postsurgery ($F(1,5)=8.5$, $p<0.033$). All stroke mice also showed a higher MD in the corpus callosum 35 days compared to days postsurgery ($F(1,11)=10.7$, $p<0.008$).

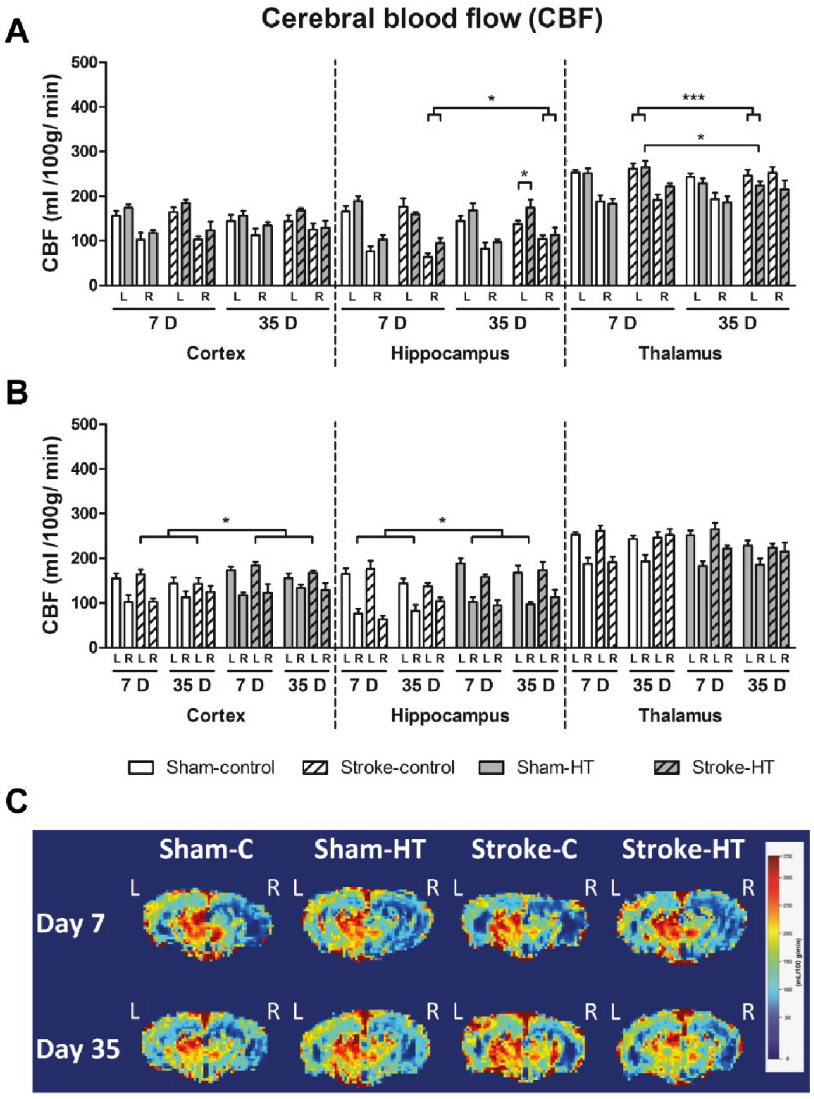
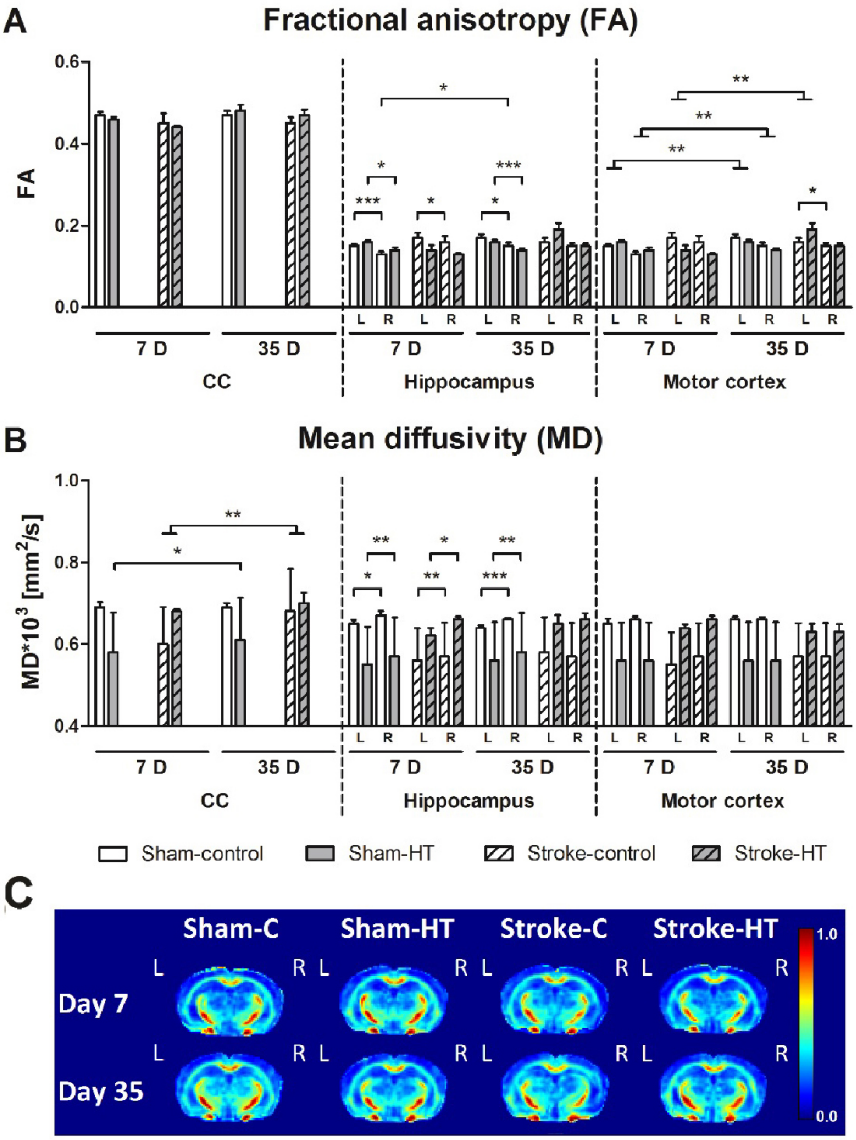


Figure 4.10: CBF was assessed in the lesioned and unlesioned hemisphere at and 35 days post-stroke in the hippocampus, thalamus and cortex. At days post-stroke, CBF was lower in all groups in the right cortex (Sham-control: $p<0.002$; Sham-HT: $p<0.004$; Stroke-control: $p<0.007$; Stroke-HT: $p<0.024$), right hippocampus (Sham-control: $p<0.003$; Sham-HT: $p<0.001$; Stroke-control: $p<0.001$; Stroke-HT: $p<0.016$), and right thalamus (Sham-control: $p<0.002$; Sham-HT: $p<0.001$; Stroke-control: $p<0.004$; Stroke-HT: $p<0.049$) than in the corresponding ROI in the left hemisphere (not shown in figure). At 35 days post-stroke, almost all groups still displayed a lower CBF in the right cortex (Sham-control: $p<0.10$; Sham-HT: $p<0.01$; Stroke- control: $p<0.012$, Stroke-HT: $p<0.007$) and right hippocampus (Sham- control: $p<0.004$; Sham-HT: $p<0.001$; Stroke-HT: $p<0.002$) compared to the left cortex and left hippocampus, respectively (not shown in figure). In the right thalamus, a lower CBF was also observed in sham animals at 35 days, compared to the left thalamus (Sham-control: $p<0.002$; Sham-HT: $p<0.001$) (not shown in figure). CBF was also increased in the left cortex of stroke-HT mice compared to stroke-control mice at and 35 days post-stroke (B, $p<0.043$). All stroke animals showed a significantly increased CBF at 35 days post-stroke in the right hippocampus and compared to days post-stroke (A, $p<0.015$). Notably, CBF in the right hippocampus was increased in HT-fed sham mice of both surgery groups at both and 35 days post-surgery (B, Sham-HT: $p<0.046$). In the left hippocampus only HT-fed stroke mice maintained an increased CBF at 35 days compared to control diet-fed mice ($p<0.048$). In the left thalamus, a decreased CBF was observed at 35 days compared to days post-stroke in stroke-HT mice ($p<0.036$). Conversely, an increase of CBF was observed in the right thalamus in stroke-control mice at 35 days post-stroke, compared to days post- stroke ($p<0.003$). (C) Representative high-resolution voxel-wise analyzed CBF images at and 35 poststroke. Values represent mean ± SEM. Sham-control: n=7, sham-HT: n=6, stroke-control: n=7, stroke-HT:n=4. # $0.05<p<0.08$, *$p<0.05$, ***$p<0.001$.



RSFMRI

Total correlations

Only in stroke mice, HT diet improved FC from day to day 35 after stroke between several cerebral regions: right dorsal hippocampus (DHR) to left motor cortex (MCL, $F(1,8)=8.1$, $p<.022$); DHR to right motor cortex (MCR, $F(1,8)=6.1$, $p<.040$); MCL to right visual cortex (VCR, $F(1,8)=9.4$, $p<.014$); VCR to MCR ($F(1,8)=5.5$, $p<.048$) (Figure 4.12).

Figure 4.11: White matter (WM) and gray matter (GM) integrity as measured by quantitatively assessed diffusion tensor-derived indices at + 35 days poststroke in mice fed HT or Control diet. Fractional anisotropy (FA) (A) and mean diffusivity (MD) (B) were measured for ROI drawn in white (Corpus Callosum, CC) and gray matter (Hippocampus, motor cortex) regions. (A) In sham-control (D, p<0.001; 35D, p<0.011) and sham-HT mice (D, p<0.020; 35D, p<0.001), FA in the right hippocampus was lower than in the left hippocampus at days and 35 days after surgery. Only in stroke-control mice at days post-stroke FA was decreased in the right hippocampus compared to its contralateral counterpart (p<0.022). FA in the right hippocampus also increased between days and 35 days after surgery in sham-control mice (p<0.035). A lowered FA in the right the motor cortex compared to left motor cortex was only found at 35 days in stroke-control (p<0.033) mice. In the left motor cortex, stroke mice had an FA increase in the hemisphere at 35 days post- stroke compared to days post-stroke (p<0.006). Sham animals showed a decrease of FA in both the left (p<0.003) and right (p<0.004) motor cortex over time (35 days vs. days). (B) MD was higher in the right hippocampus than in the left hippocampus at days postsurgery in all groups (Sham-control: p<0.013; Sham-HT: p<0.004; Stroke-control: p<0.006; Stroke-HT: p<0.050), while at 35 days only in sham mice (Sham-control: p<0.001; Sham-HT: p<0.002). Only in stroke-control mice at days postsurgery, a higher MD in the right motor cortex than the left motor cortex was detected (p<0.075). Sham-HT mice had a heightened MD of the corpus callosum at 35 days compared to days postsurgery (p<0.033). All stroke mice also showed a higher MD in the corpus callosum 35 days compared to days postsurgery (p<0.008). (C) Representative high-resolution set of FA images for each dietary group at and 35 days poststroke.

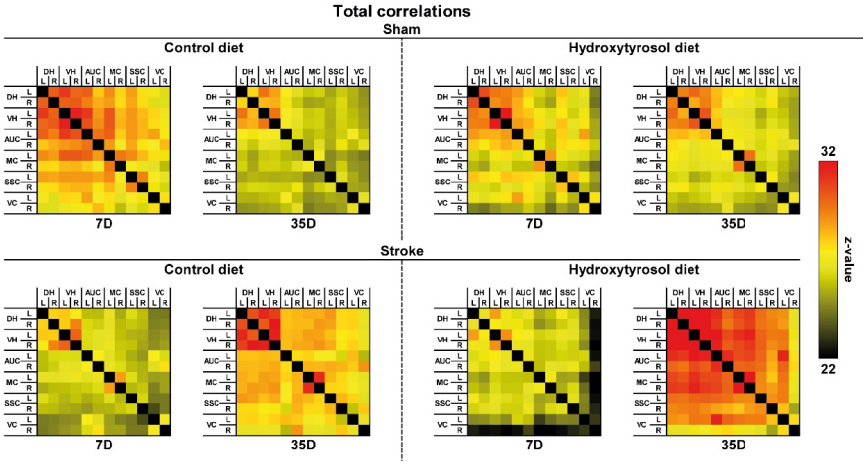


Figure 4.12: Resting-state functional connectivity (FC) based on total (A) and partial correlation analyses (B) in the brains of mice fed HT or Control diet and 35 days poststroke. FC was measured between 12 ROI: dorsal hippocampus (DH), ventral hippocampus (VH), auditory cortex (AU), motor cortex (M1), somatosensory cortex (S1), and visual cortex (V1). Total correlations revealed that HT diet improved FC in stroke mice between several ROI, i.e. right DH to left MC (p<.022); right DH to right M1 (p<.040).

4.3.4. *(Immuno)histochemistry and Biochemical analysis*

4.3.4.1 *DCX Staining*

To visualize immature neurons, we used an anti-DCX antibody as a neurogenesis marker. DCX+ cells were quantified in hippocampus. Here, we found an increased number of DCX+ cells/mm^2 in all stroke mice compared to sham mice ($F(1,23)=8.4$, $p<0.008$). The hippocampus size was reduced significantly in stroke mice without a diet effect ($F(1,23)= 7.0$, $p<0.015$) (Figure 4.13B).

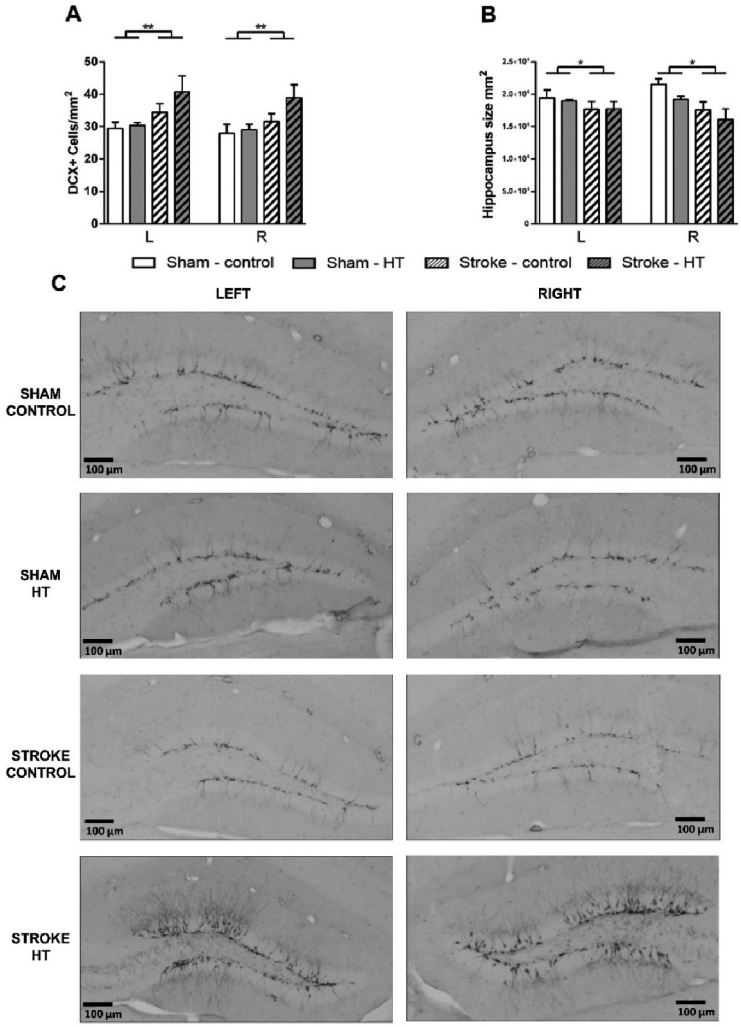


Figure 4.13: Immunohistological stainings for doublecortin (DCX) in hippocampus of the brains of HT and control fed mice 35 days after surgery. (A) All stroke mice showed an increased number of DCX+ cells/mm2 compared to sham mice ($p<0.008$). (B) The hippocampus size was reduced significantly in stroke mice without a diet effect ($p<0.015$). Values represent mean ± SEM.

4.3.4.2 *IBA-I Staining*

All stroke mice showed an higher IBA1+-area (Figure 4.14) than sham mice in the cortex at bregma -1.94 ($F(1,21)=4.5$, $p<0.046$), hippocampus ($F(1,19)=6.6$, $p<0.019$), in both left ($F(1,23)=10.9$, $p<0.002$) and right ($F(1,23)=11.6$, $p<0.002$) thalamus, cortex at bregma 0.62 ($F(1,21)=5.6$, $p<0.027$), and in both left ($F(1,23)=5.0$, $p<0.03$) and right ($F(1,23)=35.8$, $p<0.001$) caudate putamen. Notably, in the corpus callosum only in stroke-control mice a heightened IBA1+-area was found compared to sham-control mice ($F(1,13)=8.9$, $p<0.011$). Moreover, only in stroke mice in the right thalamus ($F(1,12)=11.7$, $p<0.006$) and in the right caudate putamen ($F(1,12)=34.6$, $p<0.001$) IBA1+-area was increased compared to their corresponding left hemispheric part. In the cortex at bregma 0.62 HT-diet lowered IBA1+-area compared to control diet in both sham and stroke mice ($F(1,21)=4.9$, $p<0.039$). While in the corpus callosum IBA1+-area was decreased by HT-diet only in stroke mice ($F(1,12)=6.9$, $p<0.022$).

4.3.4.3 *GLUT-1 Staining*

Vascular density was higher in stroke than shams in the left cortex (Bregma -1.94) ($F(1,23)=17.3$, $p<0.001$) (Figure 4.15). HT stroke mice had a lower vascular density in the left cortex (Bregma -1.94) than control stroke mice ($F(1,23)=4.8$, $p<0.040$, data not shown). Additionally, GLUT-1+-area was increased in stroke mice compared to sham mice in left cortex (Bregma -1.94: $F(1,23)=4.5$, $p<0.046$) and right caudate putamen ($F(1,22)=4.6$, $p<0.043$) (Figure 4.16).

4.3.4.4 *NO and reactive oxygen species (ROS) levels*

NO and ROS levels were determined in serum samples obtained before sacrifice (Figure 4.17). NO production was quantified indirectly by the determination of nitrates/nitrites using an ozone chemiluminescence-based assay. A reduction in NO levels was detected in stroke animals with no evident diet effect ($F(1,23)=12.6$, $p<0.002$). ROS levels were also indirectly quantified by analyzing the level of lipid peroxidation. No changes were detected.

4.3.4.5 *Psd95, Bdnf, and GLUT-1 mRNA expression*

The expression of Bdnf, as a regulator of neurogenesis, and Psd-95, as postsynaptic marker, were determined by qPCR (Figure 4.18). All HT-mice showed an up-regulation in Psd-95 expression without differences between hemispheres ($F(1,23)=6.5$, $p<0.018$). In addition, Bdnf was higher expressed in stroke HT-mice ($F(1,10)=5.6$, $p<0.040$) than in sham HT-mice. The effect of HT in vascular integrity was evaluated by quantifying the mRNA levels of GLUT-1 as a capillary density marker. No changes were detected (data not shown).

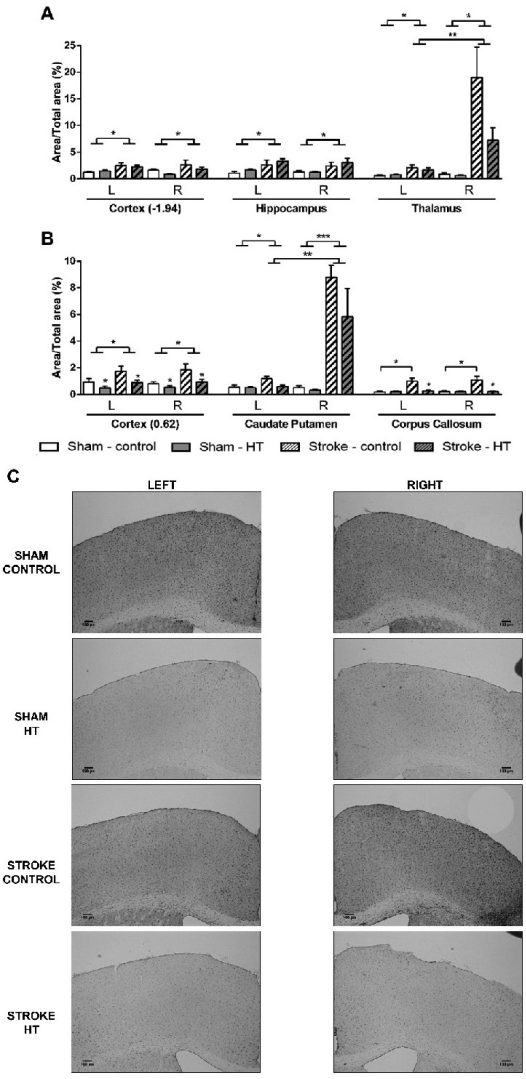


Figure 4.14: Immunohistological stainings for ionized calcium-binding adapter molecule-1 (IBA-1) in brains of HT and control fed mice 35 days after surgery. All stroke mice showed a higher IBA1+-area than sham mice in (A) the cortex (bregma -1.94) ($p<0.046$), hippocampus ($p<0.019$), in both left ($p<0.002$) and right ($p<0.002$) thalamus, (B) cortex (bregma 0.62) ($p<0.027$), and in both left ($p<0.037$) and right ($p<0.001$) caudate putamen. (B) Notably, in the corpus callosum only in stroke-control mice a heightened IBA1+-area was found compared to sham-control mice ($p<0.011$). Moreover, only in stroke mice in the right thalamus ($p<0.006$) and in the right caudate putamen ($p<0.001$) IBA1+-area was increased compared to their corresponding left hemispheric part. In the cortex at bregma 0.62 HT-diet lowered IBA1+-area compared to control diet in both sham and stroke mice ($p<0.039$). While in the corpus callosum IBA1+-area was decreased by HT-diet only in stroke mice ($p<0.022$). (C) Representative images of IBA-1 staining in cortex (bregma 0.62). Values represent mean ± SEM.

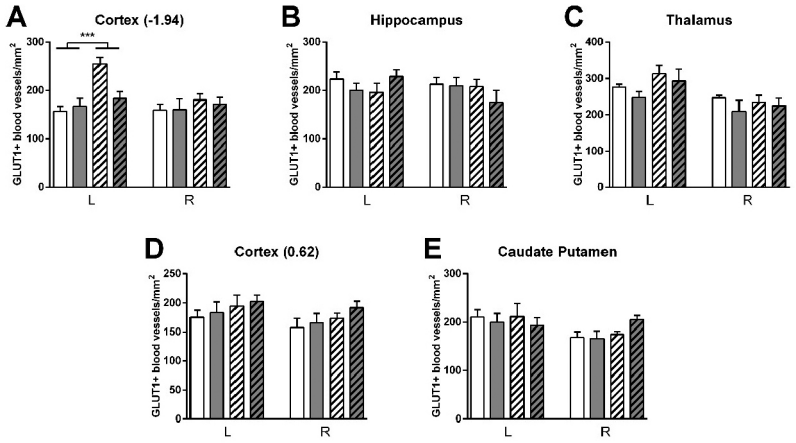


Figure 4.15: Immunohistological stainings for GLUT-1 in brains of HT and control fed mice 35 days after surgery. (A-E) Vascular density was increased in control stroke than control shams in the left cortex (Bregma -1.94) ($p<0.001$). HT stroke mice had a lower vascular density in the left cortex (Bregma 0.62) than control stroke mice ($p<0.040$, data not shown). Values represent mean ± SEM.

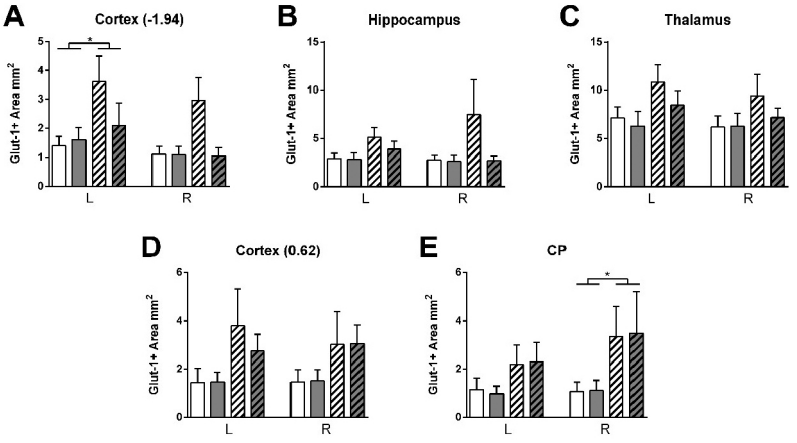


Figure 4.16: Immunohistological stainings for GLUT-1 in brains of HT and control fed mice 35 days after surgery. (A-E) GLUT-1+-area was increased in stroke mice compared to sham mice in left cortex (Bregma -1.94: $p<0.046$) and right caudate putamen ($p<0.043$). Values represent mean ± SEM.

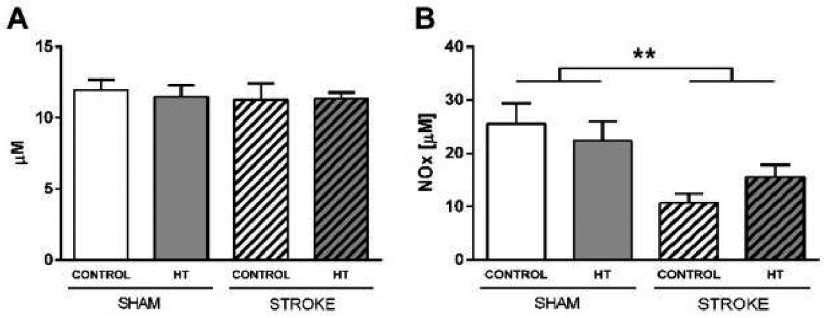


Figure 4.17: ROS and NO levels in serum samples obtained 35 days after surgery. (A) ROS level evaluated by analyzing TBARS (B) NO production quantified by using an ozone chemiluminiscence-based assay. A reduction of NO levels was detected in stroke animals ($p<0.002$). Values represent mean ± SEM.

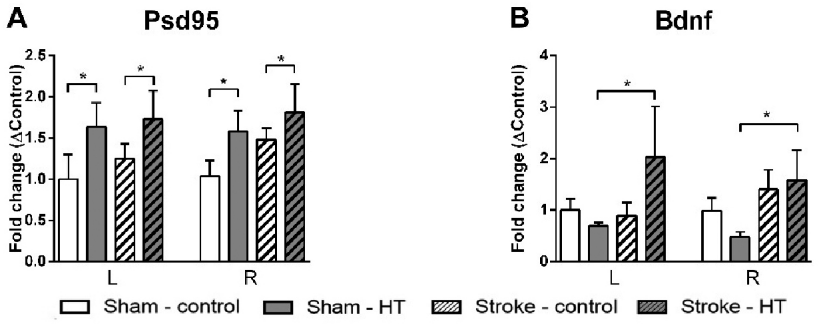


Figure 4.18: mRNA expression of (A) Psd95, (B) Bdnf in frontal parts of the brain 35 days after surgery. (A) All HT-mice showed an up-regulation in Psd- 95 expression without differences between hemispheres ($p<0.018$). (B) Bdnf was higher expressed in stroke HT-mice ($p<0.018$) than in sham HT-mice. Values represent mean ± SEM.

4.4 DISCUSSION

Although ischemic stroke is one of the main causes of death and disability worldwide, the only medical treatment for this disease is reperfusion by using recombinant tissue plasminogen activator or by endovascular thrombectomy with medical devices. However, the risk of hemorrhage, and the narrow therapeutic window makes it urgent to find other treatment options focused not only on reperfusion but on neuroprotection as well. In this sense, it has been previously described that olive oil and an olive leaf extract exert a neuroprotective effect in ischemic rats [241, 273]. Oleuropein, another polyphenol from olive oil and a precursor of HT, has also demonstrated to be neuroprotective in a mouse model of focal cerebral ischemia [274]. However, a comprehensive analysis of the effect of HT as a therapeutic approach in an *in vivo* stroke model is lacking. The present study shows that a HT-diet could be used as a therapeutic approach in stroke recovery by improving: i) learning, short term memory and grip strength, ii) CBF and FC, and iii) different parameters related to neurogenesis and neuroinflammation.

Motor functions, behavior and cognition are severely affected after focal ischemia and decisive in the quality of life of stroke patients [275, 276]. In our previous study, we described that a multicomponent diet, Fortasyn, improved grip strength in male mice [238]. In the current study, we show that HT improved grip strength on the trapeze, highlighting the potential restorative effect of this single dietary compound on motor network connections. Exploring the HT effects on behavior and motor skills, no diet effects were detected neither in the open field, DVC nor the pole test. Notably, in this study DVC were used for the first time to study individual mouse locomotion via calculation of DVC metric measures (activity, walked distance, walked velocity, total turnings, laterality index) during day- and nighttime before and after surgery. This novel approach helped to reveal a decreased nighttime activity in stroke mice 1 week after surgery. Notably, during the nighttime of the second postsurgery week, only stroke mice showed a left turning preference. This latter result is in line with standard behavioral tests like the corner test used in preclinical stroke studies [277, 278]. Short-term memory of mice was also evaluated with the novel object recognition test (ORT). Here, mice on HT-diet visited the novel object more frequently than mice on control-diet. Similarly, in the PPI test, HT-fed mice showed a higher startle amplitude to the basal and final startle stimulus of 120dB control-mice. Altogether, ORT and PPI results suggest that HT improves short-term memory and promotes non-associative learning processes such as habituation, being a promising approach to reduce stroke-associated cognitive deficits.

Moreover, with rsfMRI alterations in neuronal functional architecture, both in animal models and in humans has been found after ischemic stroke [279]. In a human study, it was demonstrated that the alteration of sensorimotor function after a stroke correlated with a loss of interhemispheric connectivity between sensory-motor regions, and that this disruption normalized partially weeks after the infarction [280]. Thus, in patients with stroke, changes in neuronal activity are closely associated with functional recovery. The increase in rsfMRI activity in the supplementary motor cortex, the lateral premotor cortex and the superior parietal cortex in the first 14 days after infarction correlates with an improvement in motor function of the upper extremities during this period [281]. In the present study we also investigated brain diffusivity with DTI as an imaging biomarker for white and gray matter (GM) integrity. Here, we revealed only impaired GM microstructure in the stroke mice on control diet measured by a decreased FA accompanied by an increased MD at seven days poststroke in the right hippocampus and right motor cortex compared to their corresponding left counterpart. This effect was not visible in stroke mice on HT diet which is in line with our previous study in which a multicomponent diet improved functional and structural connectivity after stroke [238].

In our study, the HT-diet improved functional and also structural connectivity between several cerebral regions in the stroke animals. We suggest that these improvements in connectivity could be related to the increase in the grip strength of HT-fed mice as well as with their higher habituation and improved short-term memory described above and on the up-regulation of different neurogenic markers that will be mentioned later.

CBF alterations are also clinically associated with cognitive and motor dysfunction, especially after stroke [282]. In fact, we already described that a post-stroke diet intervention can improve CBF [238, 283]. Other phenolic compounds such as resveratrol have shown to increase CBF in the frontal cortex of healthy humans after a task performance [284]. Moreover, in a rat ischemic model, resveratrol increased hippocampal CBF [285]. Our results show that an acute therapeutic approach with a HT-diet was able to significantly increase CBF in the right hippocampus of all mice on HT-diet, and to mitigate the decreased CBF in the left hippocampus stroke-control mice. Additionally, in the left cortex, HT-diet also increased CBF after stroke. The positive effect of HT on this parameter probably underlies the improvement in short-term memory and learning processes described above.

CBF is linked to a balanced production of NO. The particular effect of NO varies depending on the stage of evolution along the ischemic process and on the cellular source of NO [286, 287]. Of the three NOS isoforms responsible for NO production, the activity of neuronal NOS and inducible NOS results detrimental while endothelial NOS activation is related with neuroprotective effects. We have previously shown in stroke patients that an initial elevation

of NO favors neurological recovery while a latter elevation predicts growth of the infarct volume [288]. In the present study we have observed a significant decrease in serum NO concentration in stroke animals. This decrease has also been reported by ours and other research groups, and may be attributed to the low profile of L-arginine, the NO precursor, in stroke patients [288– 290].

A burst in ROS follows after a stroke insult damaging cellular macromolecules and leading to cell death and tissue loss [291]. HT has been consistently described as an antioxidant compound in several models [292]. Therefore, we evaluated if the HT-diet was able to modulate the oxidative level in serum samples obtained from mice after sacrifice. The fact that no changes were detected in any experimental group, not even between sham and stroke animals, seems to indicate that 35 days after surgery may be too late to detect changes in serum ROS levels. In fact, in a previous study with hypoxic mice we observed that ROS brain levels begin to normalize two hours after the insult [293]. As previously mentioned, further analysis with more animals should be carried out, both in serum and in brain samples, and at time-points closer to surgery to re-evaluate the temporal profile of NO production and the particular isoenzymes of NOS modulated by HT and to analyze the antioxidant capacity of HT-diet after stroke.

HT has shown its anti-inflammatory capacity in different models [294–296] and although its particular effect on microglia-mediated neuroinflammation remained unexplored other phenolic compounds such as oleuropein have been shown to attenuate microglia activation [297]. The decreased level of IBA-1 immunoreactivity 35 days after stroke in the cortex and corpus callosum of mice on the HT-diet corroborates that HT can also reduce the inflammatory environment after stroke. This effect is probably involved in the improved impairments of stroke mice on a HT-diet and points to the interest of carrying out future experiments to deepen into the activity of HT on neuroinflammation.

Neurogenesis is an important process in stroke recovery and a number of therapeutic strategies to promote this process after ischemic events have been investigated with poor outcomes [298]. Stroke insult also involves synaptic degradation which dampens the activity of the CNS. Bdnf is a neurotrophin that regulates synaptic connections, synapse structure, neurotransmitter release and synaptic plasticity [299]. Moreover, Bdnf is required for the induction of neurogenesis and lack of this protein can lead to a lack of neurogenic response in a heterozygous knockout mice model [300]. Additionally, the postsynaptic protein Psd95 is also involved in the regulation of synaptic plasticity and synaptogenesis. Previous studies demonstrate that the administration of olive leaf or oil polyphenol extracts increases Bdnf levels in the olfactory lobes and hippocampus [301, 302]. The synaptogenic potential of HT has been also reported in prenatally stressed rats in which HT prevented the stress-induced downregulation of Bdnf [303]. In accordance, in our study the expression of

Psd95 was significantly induced in all HT-fed mice. Unfortunately, no diet effect was found on amount of DCX+ cells. However, the HT-diet only induced Bdnf expression in stroke HT-mice, suggesting a difference in the response between Bdnf and DCX [304]. Although further analyses at the protein level are necessary, it is remarkable that HT, a single compound, exhibits these promising effects.

4.5 CONCLUSIONS

The data presented here indicate that a post-stroke intervention with a HT-enriched diet favour the recovery after ischemic stroke by ameliorating stroke-associated learning and motor impairments. This effect, probably linked to an increase in CBF, functional and structural connectivity and to its anti-inflammatory and neurogenic potential, makes HT an interesting and safety compound to be further tested in ischemic stroke treatment.

Angle:

$$\alpha = acos\frac{|X_n - X_{n-1}|}{DM_n}$$

where X is x position and DM is direction of motion.

Relative Turn Angle: Change in heading between two consecutive samples

$$RTA_n = HE_n - HE_{n-1}$$

Relative Angular Velocity (synonym, Turn Bias): Change in relative turn angle over time delta

$$RAV_n = \frac{RTA_n}{t_n - t_{n-1}}$$

Laterality Index: Tendency to turn right vs left

$$LI = \frac{\|RTA_R\|}{\|RTA_R\| + \|RTA_L\|}$$

where RTA_R is a right turn angle $\in [30, 90]$, RTA_L is a left turn angle $\in [-30, -90]$, and $LI \in [0, 1]$.

Figure 4.19: A detailed explanation of the calculations on the aforementioned DVC metric measures.

SUPPLEMENTARY MATERIALS: https://www.mdpicom/2072-6643/11/10/2430/s1, Table 4.1. Excluded mice per experiment. Table 4.2. Sequence of the PCR primers used in this study. (Used abbreviations: Bdnf, brain derived neurotrophic factor; GLUT-1, glucose transporter 1; Psd-95, postsynaptic density protein 95; HPRT, hypoxanthine guanine phosphoribosyl transferase; B2M, beta-2 microglobulin). Figure 4.19. A detailed explanation of the calculations on the aforementioned DVC metric measures. Figure 4.20. Individual locomotion via digital ventilated cage (DVC) metrics measures. (A) activity, (B) walked distance, (C,D) walked velocity, (E) total turns and (F,G) laterality index, during day- and nighttime before and after surgery. No diet effects were found on DVC metrics. Notably, several stroke effects were found: i.e., During nighttime, only stroke mice were less active after surgery over time ($p < 0.026$) comparing presurgery with postsurgery week 1. Only during nighttime, stroke mice ($p < 0.028$) showed a left turning preference (laterality) comparing presurgery with postsurgery week.

AUTHOR CONTRIBUTIONS: The authors responsibilities were as follows: Conceptualization, M.A.P.H., E.S., M.W., A.J.K.; Methodology, M.W. and A.J.K.; Formal analysis, J.S., M.W., J.C.; Investigation, J.S., M.W., J.C., V.H.W., V.V., B.G., P.J.D.; Data curation, J.S., M.W., J.C., V.H.W., V.V., B.G., P.J.D.; Writing—original draft preparation, J.S., M.W., J.C., V.H.W.; Writing—review and editing, J.C., M.A.P.H., E.S., M.W., A.J.K.; Visualization,

Table 4.1: Excluded mice per experiment.

Test	Sham-control	Sham-HT	Stroke-control	Stroke-HT
Open Field	0	0	0	0
Grip test	0	0	0	0
Pole test	0	0	2	1
Rotarod	0	0	1	0
Prepulse inhibition	0	1	1	0
Morris water maze	0	0	0	0
Novel object recognition test	0	0	0	0
Digital ventilated cages	0	0	0	0
MRI - Arterial spin labelling	0	1	1	1
MRI - Diffusion tensor imaging	0	1	1	0
Resting state functional MRI	1	1	2	1
qPCR - PSD95 & GLUT-1	0	0	1	1
qPCR - BDNF	0	1	1	1
(Immuno)histochemistry - DCX	0	0	1	0
(Immuno)histochemistry - IBA-1 (0.62)	0	2	1	0
(Immuno)histochemistry - IBA-1 (-1.94)	1	1	0	1
(Immuno)histochemistry - GLUT-1 (0.62)	0	1	2	0
(Immuno)histochemistry - GLUT-1 (-1.94)	0	0	2	0
Serum NO	0	0	1	0
Serum Oxidative Stress Level	0	0	0	0

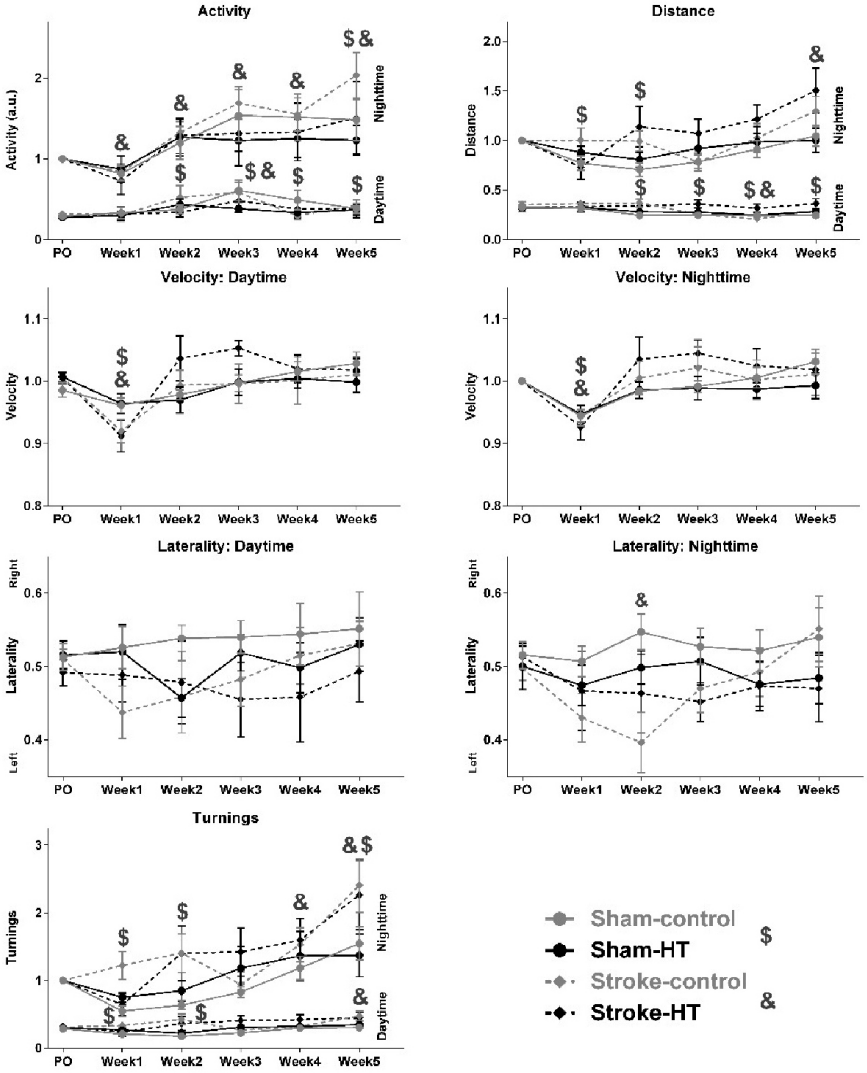


Figure 4.20: Individual locomotion via digital ventilated cage (DVC) metrics measures. (A) activity, (B) walked distance, (C, D) walked velocity, (E) total turnings and (F, G) laterality index, during day- and nighttime before and after surgery. No diet effects were found on DVC metrics. Notably, several stroke effects were found: i.e. During nighttime, only stroke mice were less active after surgery over time ($p<.026$) comparing presurgery with postsurgery week 1. Only during nighttime, stroke mice ($p<.028$) showed a left turning preference (laterality) comparing presurgery with postsurgery week 2.

Table 4.2: Sequence of the PCR Primers Used in This Study. (Used abbreviations: Bdnf, brain derived neurotrophic factor; GLUT-1, glucose transporter 1; Psd-95, postsynaptic density protein 95; HPRT, hypoxanthine guanine phosphoribosyl transferase; B2M, beta-2 microglobulin)

Gene	**Direction**	**Sequence (5' to 3')**
BDNF	Forward	CTTCCTGCATCTGTTGGGGA
	Reverse	TGGTGGAACATTGTGGCTTTG
GLUT1	Forward	GATCCCAGCAGCAAGAAGGT
	Reverse	TAGCCGAACTGCAGTGATCC
PSD95	Forward	TGGATCACAGGGTCGAGAAGA
	Reverse	TTGGCACGGTCTTTGGTAGG
HPRT	Forward	TGATTAGCGATGATGAACCAGGT
	Reverse	AGCAAGTCTTTCAGTCCTGTCC
B2M	Forward	GATGTCAGATATGTCCTTCAGCA
	Reverse	TCACATGTCTCGATCCCAGT

M.W., J.C., V.H.W.; Supervision, M.A.P.H., E.S., M.W., A.J.K.; Funding acquisition, J.C., M.A.P.H., E.S., M.W., A.J.K. All authors approved the final version of the manuscript for submission.

FUNDING: This research was funded by "EMBO Short-Term Fellowship" (Nr.: 142) & "Acción S: Ayudas para estancias breves del Personal Investigador en Formación de la Universidad de Jaén" (Nr.: 554).

ACKNOWLEDGMENTS: Technical and human support provided by CICT of University of Jaén is gratefully acknowledge. The authors thank Seprox Biotech for kindly donating HT.

CONFLICTS OF INTEREST: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Digital ventilated cages (DVC) metrics

DVC were used to study individual locomotion via calculation of DVC metric measures (activity, walked distance, walked velocity, total turnings, laterality index) during day- and nighttime before and after surgery. No diet effects were found on DVC metrics.

Activity

PRESURGERY TO POSTSURGERY WEEK 1: No effects of both types of surgery (stroke and sham) were found on daytime activity. During night- time, only stroke mice were less active after surgery over time ($F(1,12)=5.3$, $p<.040$).

PRESURGERY TO POSTSURGERY WEEK 2: While sham mice were more active ($F(1,12)=6.7$, $p<.024$) during daytime, stroke mice were more active ($F(1,12)=6.3$, $p<.028$) during nighttime.

PRESURGERY TO POSTSURGERY WEEK 3: While during daytime both sham and stroke mice were more active (Sham: $F(1,12)=11.5$, $p<.005$; Stroke: $F(1,12)=5.3$, $p<.040$), during nighttime stroke mice were more active ($F(1,12)=11.4$, $p<.006$).

PRESURGERY TO POSTSURGERY WEEK 4: While during nighttime stroke mice were more active ($F(1,12)=5.3$, $p<.040$), during daytime sham mice were more active ($F(1,12)=5.7$, $p<.035$).

PRESURGERY TO POSTSURGERY WEEK 5: While during nighttime both sham and stroke mice were more active (Sham: $F(1,12)=5.1,7p<.044$; Stroke: $F(1,12)=9.4$, $p<.010$), during daytime sham mice were more active ($F(1,12)=6.7$, $p<.024$).

Walked distance

PRESURGERY TO POSTSURGERY WEEK 1: No effects of both types of surgery (stroke and sham) nor diet effects were found on daytime walked distance. During nighttime, only sham mice walked less after surgery ($F(1,12)=12.0$, $p<.005$).

PRESURGERY TO POSTSURGERY WEEK 2: During both day- and nighttime, sham mice walked less comparing presurgery with post- surgery week 2 (Daytime: $F(1,12)=9.8$, $p<.009$; Nighttime: $F(1,12)=24.0$, $p<.001$).

PRESURGERY TO POSTSURGERY WEEK 3: During daytime sham mice walked less comparing presurgery with postsurgery week 3 ($F(1,12)=10.4$, $p<.007$).

PRESURGERY TO POSTSURGERY WEEK 4: Only during day- time, both sham and stroke mice walked less comparing presurgery with postsurgery week 4 (Sham: $F(1,12)=16.9$, $p<.001$; Stroke: $F(1,12)= 7,3p<.019$).

PRESURGERY TO POSTSURGERY WEEK 5: While during daytime all sham mice walked less ($F(1,12)=12.5$, $p<.004$), during nighttime all stroke mice walked more comparing presurgery with postsurgery week 5 ($F(1,12)=9.6$, $p<.009$).

Walked velocity

PRESURGERY TO POSTSURGERY WEEK 1: During both day- and nighttime, both stroke (Daytime: $F(1,12)=36.4$, $p<.001$; Nighttime: $F(1,12)=21.0$, $p<.001$) and sham (Daytime: $F(1,12)=8.1$, $p<.015$; Nighttime: $F(112)=3\ .0$, $p<.001$) mice walked slower after surgery.

PRESURGERY TO POSTSURGERY WEEK 2-5: No effects were found.

Turnings

PRESURGERY TO POSTSURGERY WEEK 1: During both day- and nighttime, only sham (Daytime: $F(1,12)= .7,4p<.019$; Nighttime: $F(1,12)=42.5$, $p<.001$) mice turned less often after surgery.

PRESURGERY TO POSTSURGERY WEEK 2: During both day- and nighttime, only sham (Daytime: $F(1,12)=12.3$, $p<.004$; Nighttime: $F(1,12)=9.5$, $p<.010$) mice turned less often after surgery.

PRESURGERY TO POSTSURGERY WEEK 3: No effects were revealed.

PRESURGERY TO POSTSURGERY WEEK 4: During nighttime, only stroke mice turned more often after surgery ($F(1,12)=7.8$, $p<.016$).

PRESURGERY TO POSTSURGERY WEEK 5: While during daytime only stroke mice turned more often ($F(1,12)=7.8$, $p<.016$), during nighttime both sham and stroke mice turned more often (Sham: $F(1,12)=5.2$, $p<.041$; Stroke: $F(1,12)=17.5$, $p<.001$).

Laterality

PRESURGERY TO POSTSURGERY WEEK 1: No effects were found.

PRESURGERY TO POSTSURGERY WEEK 2: Only during nighttime, stroke mice ($F(1,12)=6.0$, $p<.031$) showed a left turning preference (laterality).

PRESURGERY TO POSTSURGERY WEEK 3-5: No effects were found.

5

SUMMARIZING DISCUSSION

Contribution of thesis

This thesis introduces a novel software for analysis of 24/7 behavioral data, which we applied to research involving animal models of stroke. We have developed a method for data collection and analysis that opens up a new avenue of research in neuroscience that was not previously accessible, through the application of data mining methods to detect patterns in movement behavior.

Section 1.2 discusses the importance of movement analysis in research, diagnosis and treatment of neurological diseases. It also addresses the shift from traditional methods of locomotion and behavioral analysis towards automated HCM in rodent model research, and the need that this creates for software to manage, process and analyze the resulting data. Section 1.3 provides the technical background relevant to the development of the Traja software.

Chapter 2 presents Traja, a Python package for analysis of trajectory data that was built for use in scientific research. In order to support future developers, an explanation of the software architecture and design is provided.

Then, we demonstrate how Traja may be used in research with mouse models of stroke by analyzing automated HCM data. In Chapter 3, Traja is applied to automated HCM data from mice receiving a multicomponent diet treatment for stroke, Fortasyn. The results demonstrate mild improvement of function, as measured by activity and distance travelled (§3.3.1). In contrast to OF, automated analysis of DVC trajectory data with Traja was able to detect differences in walked distance and velocity in the present female stroke animal model during the post-surgery period [147]. Previously, neither time nor diet differences on locomotion (distance, velocity) have been found in the OF [147].

In Chapter 4, the methods developed in Chapter 3 are extended to a larger study involving treatment of a stroke mouse model with hydroxytyrosol (HT), the major phenolic component of olive oil. Results from using Traja with DVC compared with other motor and behavioral tests in that no diet effects were detected neither in the open field nor the pole test. Traja with DVC were used for the first time to study individual mouse locomotion via calculation of DVC

metric measures (activity, walked distance, walked velocity, total turnings, laterality index) during day- and nighttime before and after surgery. This approach helped to reveal decreased nighttime activity in stroke mice 1 week after surgery, which was not found in the traditional OF test. Additionally, during the nighttime of the second postsurgery week, only stroke mice showed a left turning preference. This result is in line with standard behavioral tests like the corner test used in preclinical stroke studies [277, 278], suggesting that Traja with DVC can be used to supplement traditional tests for observing behavioral phenotypes over broad periods and in high resolution. The use of Traja in this research demonstrates the potential for home cage mouse tracking combined with trajectory data mining and analysis for the use of nutritional interventions to accelerate recovery following stroke in mice.

5.1 ADVANCES IN HCM DATA COLLECTION

Recent technological advances allow for increased automation of some elements of laboratory research. In the case of research with rodents, the most significant change is the shift towards automated HCM for phenotyping. Previously, research using rodent models relied solely on conventional methods of locomotion analysis and behavioral testing. Automated HCM, which allows for constant monitoring of the movement or behavior of rodents in their home environments, has many advantages over these more traditional methods. These include increased efficiency, decrease in interactions between researchers and animals, increased sensitivity and variety of behaviors measured, and the ability to track rodents 24 hours a day.

5.1.1 Improving Animal Welfare in Biological Research

The development of animal laboratory science within Europe over the last century has been associated with increasingly stringent standards for animal welfare. Increasing awareness of animal welfare in research is supported by the general agreement by researchers with the principle of minimizing unnecessary animal suffering in scientific research [305]. Notably, the Three R's (3Rs) are commonly referenced guiding principles for ethical use of animals in testing, derived from Russell and Burch's book "The Principles of Humane Experimental Technique" [306]:

1. **Replacement**: methods that avoid or replace use of animals in testing
2. **Reduction**: methods that provide comparable levels of information from fewer animals
3. **Refinement**: methods that alleviate or minimize potential pain, suffering and distress, and enhance animal welfare for the animals used.

The book highlights the expected improvement of animal welfare with increased efficiency of methods:

It is widely recognized that the humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments. Since the Second World War, in particular, this principle has been increasingly accepted; and the intimate relationship between humanity and efficiency in experimentation will recur constantly as a major theme in the present book.

In particular, methods involving automated analysis of home cage data with HCM are potentially beneficial for *reduction* of animals needed, since they extend the temporal range of behavioral signal gathered. They are also relevant for *refinement*, since they reduce the need for manual handling and stressful experiments. Thus, improvements in efficiency of animal experiments enabled by technological advances such as HCM typically correspond with improved animal welfare.

Efficiency

HCM can greatly reduce the amount of time and manpower needed to complete experiments, since behavior is tracked automatically even without researchers present. This can allow scientists to collect much more data and carry out many more experiments than was previously possible, often at a lower cost [14]. Automated HCM data can additionally be supplemented with the use of traditional behavioral tests to provide further information about animal models.

Animal welfare and reproducibility

It has been shown that the experimenter can affect the outcomes of behavioral tests in rodents [307–309]. Automated HCM can reduce the impact of this potential confounding factor by decreasing the amount of interaction between researchers and animals. This can not only reduce stress for rodents, but also lessen the effects of subjectivity or researcher bias [310]. It can also reduce stress for animals by eliminating the need to frequently remove them from their environments, as the behavioral tracking can rather be carried out in the home cage [311]. For these reasons, automated HCM may help increase the reproducibility of research.

Range of behaviors

In contrast to traditional behavioral tests, automated HCM expands the range of behaviors that can be easily measured in animal models (Table 5.1). For example, in addition to tracking overall activity in their home environments, constant tracking of rodents enables the measurement of how much time is spent on particular behaviors (e.g., unhealthy mice may spend less time grooming than their healthy counterparts) [13]. Many systems of automated HCM also measure food and water consumption. These behaviors have been shown to vary in different rodent disease models [13, 312].

Constant tracking

Unlike traditional locomotion analysis, automated HCM can be used 24 hours a day. Behavioral tests are usually carried out during the workday, which is when mice are least active; automated HCM makes it possible to measure behavior at night when rodents are most active, even when no researchers are in the lab. It also makes it possible to measure changes in animals' circadian rhythm [93]. Additionally, traditional behavioral tests are usually brief and only capture a short snapshot of behavior, while automated HCM allows for tracking of animals for extended periods of time [11]. These factors allow HCM to have increased sensitivity over traditional behavioral tests, and potentially detect very small changes in animals.

Digital Ventilated Cages (DVC, Tecniplast S.p.A., Buguggiate (VA), Italy), the system used for automated HCM in the studies presented in this thesis, provide the particular advantages of space-efficiency (as they can fit in normal home cage racks) and minimal disruption of animals' environment (since DVC, unlike some other automated HCM systems, are identical in size and layout to traditional Individually Ventilated Cages).

Table 5.1: Comparison of rodent behavioral tests.

	Motor / Activity	Motor Coordination	Walked Distance, Velocity	Anxiety / Depression	Turn Preference	Tracking Data
Running Wheel	✓	✗	✓	✗	✗	✗
Rotarod	✗	✓	✗	✗	✗	✗
Pole Test	✗	✓	✗	✗	✗	✗
Corner Test	✗	✓	✓	✓	✓	✗
Elevated Plus Maze	✓	✗	✓	✓	✗	✓
Open Field Test	✓	✗	✓	✓	✓	✓
Morris Water Maze	✗	✗	✓	✗	✗	✓
Radial Arm Maze	✗	✗	✓	✓	✗	✓
Catwalk	✓	✓	✓	✗	✓	✗
Home Cage Monitoring	✓	✗	✓	✓	✓	✓

5.1.2 *HCM Validity*

Validation of DVC

Due to the novelty of the data type (there are no similar datasets available for comparison, and none reported in the literature for stroke models), it is difficult to compare our HCM results with those of other experiments. In this direction,

Validation of Traja

the method for computing walked distance is validated on the Fortasyn dataset against EthoVision XT 14 (Supplementary Figure 1 [147]) to demonstrate results comparable with software widely used for phenotyping mice models of stroke and neurological diseases. The validity of centroid observation in comparison with video detection was previously demonstrated by [92]. Such phenotyping systems do not allow analyzing HCM data sources such as DVC or non-proprietary sources, thus cannot process the 24/7 data collected in this research (Figure 4.1). Therefore, although systems like EthoVision are widely used, they lack customization, transparency and extensibility of open source projects such as Traja which are needed to process novel data sources

(see Table 5.2 for a comparison). Researchers using HCM data derived from non-proprietary sources thus are currently unable to test efficacy of nutritional interventions using off-the-shelf phenotyping software. The results presented here are the first reported for extensive 24/7 home cage monitoring for stroke mouse models with data derived from the DVC system.

As a free, open source toolkit, Traja allows for an improvement in the reproducibility of innovative methodologies (Figure 5.1), such as data-driven model discovery, and can be a useful tool for behavioral research, in particular analysis of locomotion.

In order for the advantages of automated HCM described above to be fully realized, researchers must have a reliable system of organizing and analyzing the large amounts of data generated. This highlights a need for software such as Traja that can fulfill this role.

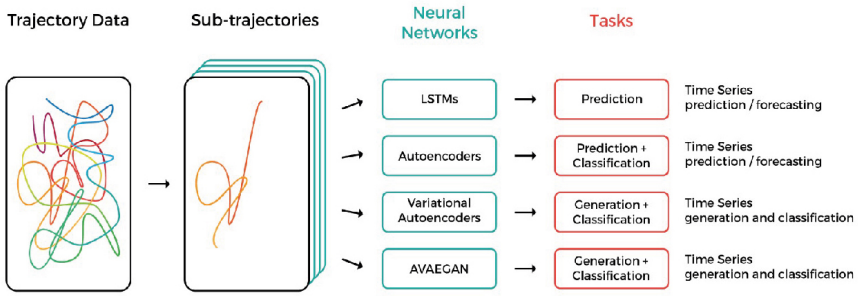


Figure 5.1: Various state of the art neural network architectures for time series data modeling recently implemented in Traja including autoencoder, variational autoencoder [313], and AVAEGAN [314]. Trajectories are decomposed into subtrajectories which can be processed by neural networks specialized for tasks such as prediction, classification and data generation.

5.2 TRAJA AS A TOOL FOR ANIMAL BEHAVIORAL ANALYSIS

see Traja's design principles in Chapter 2

Before Traja, there were no toolkits available in Python for modeling multivariate time-series data with the purpose of deep learning prototyping. The Python programming language is the *de facto* language for machine learning and high throughput data analysis (Figure 5.2). Therefore, it is more valuable to researchers to have software such as Traja written in the multi-purpose language Python as opposed to other popular scientific programming languages such as R or MATLAB. For example, R libraries such as Trajr [108] that allow working with the high throughput DVC data used in this study do not support advanced data mining methods such as prototyping deep learning methods, which require specialized linear algebra libraries, readily available in Python, to manage the massively parallel computations of GPU-accelerated computing (see Table

5.2 for a comparison of several popular libraries). By contrast, Python-based libraries draw on the strength of the Python open source community [315] and the wide range of statistical and numerical analysis packages available, such as PyTorch [192]. Further, the data preprocessing which is required when working with high volume data such as that used in these studies often requires general-purpose computational techniques, such as database querying and storage, parallel processing, memory management, and methods to improve performance - methods which are much easier to implement in Python than in R. Additionally, most state-of-the-art algorithms for time-series data analysis are implemented first in Python before becoming available in R, making Python a natural choice for creating a collaborative, open-source library to support trajectory analysis research.

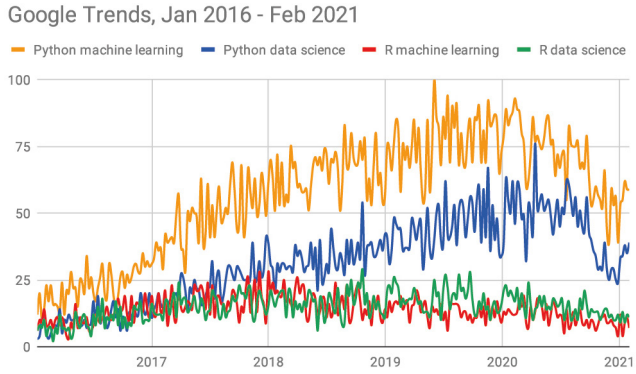


Figure 5.2: Programming language interest over time as indicated by proportion of Google searches [316].

Table 5.2: Representative comparison of software for animal HCM data analysis. Traja is primarily designed for data scientists working with HCM data.

Software	EthoVision	MouseMove	DeepLabCut	Adahabitat	Trajr	Traja
Language	—	Java	Python	Python	R	Python
Open Source License	—	CC 4.0	GPL-3	GPL-3	MIT	MIT
Tracking Hardware	EthoVision Package	Camera	Camera	Any	Any	Any
Deep Learning	Yes	No	Yes	No	No	Yes

Traja includes a high-level application programming interface (API) that enables computing complex functions with a few lines of code. Many computational, preprocessing, and data modeling methods relevant to multivariate time-series data analysis are available, ranging from velocity and acceleration, to complex time series models like recurrent neural networks. The goal of Traja's design was to allow minimal configuration and maximal extensibility. The pandas accessor design pattern allows data scientists as well as scientific programmers new to Python to work with a consistent and well-documented API, and to quickly explore and visualize data.

Advantages of Traja

In addition, Traja is the first library available to animal researchers for prototyping deep learning models for trajectory data (Table 5.2), particularly HCM data (Figure 5.3). Other software available for analysis of data for HCM relies on complex or expensive data recording equipment [104], is limited to black box software [317] or are written in programming languages which are not well-adapted to the goal of automated pattern discovery, such as R [108] and thus less flexible. For example, in recent months at the time of writing Traja has received several feature contributions from the open source community, while most of the other libraries (eg, Trajr, Adehabitat, and MouseMove) have received none.[1]

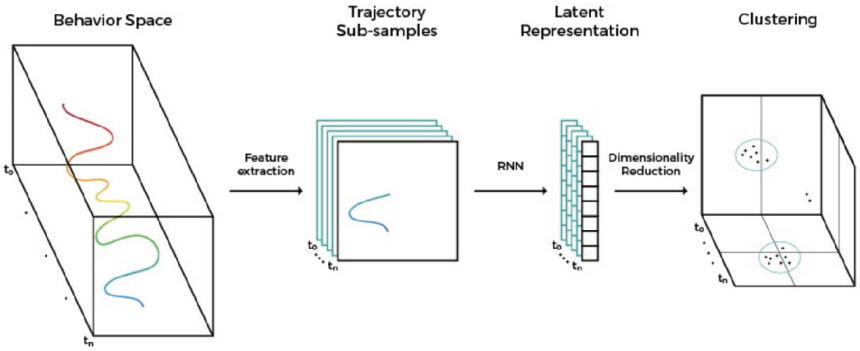


Figure 5.3: Unsupervised learning of behavioral motifs with sequential models such as RNNs can lead to the creation of a library of phenotypes, via methods enabled in Traja (§2.1.9) such as clustering of trajectories in the latent space.

5.2.1 *Limitations of HCM*

As a tool designed for data scientists, applying Traja to DVC data requires some programming skills, which are difficult to acquire without extensive training. This puts it in the same category as other libraries used by ecologists and animal behavior researchers such as MouseMove [318], DeepLabCut [153], Adahabitat [160], and Trajr [108]. Further, as a tool for data analysis, it is still subject to issues in the collection of raw data, also known as the *garbage in, garbage out* principle. Researchers using HCM data for behavioral tests must be trained to handle missing data, for example, due to cage displacements or signal errors, which can affect statistical analysis. Methods such as HCM are not a silver bullet for behavioral analysis and are not immune to the various complexities of managing animal experiments.

Data-driven behavioral analysis

The increasing availability of data derived from sensors is associated with a wider tendency in the sciences towards unsupervised, probabilistic learned categories of behaviors.[2] This is a crucial paradigm shift in the understanding

[1] https://github.com/traja-team/traja/graphs/contributors

[2] A discussion of the "probabilistic revolution" in cognitive science and artificial intelligence is given by [319].

of behavior, and is part of an increasingly greater dependence on probablistic signals, as opposed to direct empirical observations. The ability to combine HCM data from multiple experiments would allow development of a library of phenotypes leveraging probability calculations (Figure 5.3) [320]. For example, an open source software project B-SOiD [154] allows unsupervised (unlabelled) clustering of behaviors from video recordings of mice. As Isaac Newton's laws of motion gradually replaced an Aristotelian conception of physics, reliance on data-driven models of behavior provide reason to hope for improved computational models to aid understanding the world. The reader interested in the treatment of computational modeling in the context of behavioral analysis is directed to [321]. Thus, there exists both a *technological* and *methodological* gap between traditional analysis based on manual behavioral tests and HCM data sources. As discussed in §5.2.1, the trend towards availability and usage of high volume data for phenotyping places greater reliance on researcher's computational and statistical skills in the laboratory. Recommendations for closing the methodological gap are provided in the Concluding Remarks (§5.6). Traja, the software presented in this thesis, helps to bridge these gaps by providing an open source Python toolkit for advanced trajectory data mining to support researchers handling HCM data.

Outlook for HCM software

One major obstacle for the validation of novel methods in automated animal locomotion analysis is the lack of extant experimental data. This presents a chicken-and-egg problem where methods cannot be validated because the raw data is not substantiated and thus not collected. Further improvements in software for HCM are expected as animal tracking, analytical tools, and computational approaches become standardized. Data-driven approaches are generally expected to augment and in some cases supplant hand-crafted approaches in data-heavy fields such as bioinformatics, as the amount of data and processing power continues to increase exponentially.

5.3 TRAJA APPLIED TO MOUSE STROKE MODEL

The impact of stroke on motor behavior such as locomotion has been extensively studied, and indicators such as gait abnormality are evident within the first days after stroke [322]. Mouse models of stroke and other neurological conditions are useful for identifying early treatments, diagnostic indicators, and preventative factors.

Dietary treatment

Diet is a major modifiable risk factor for neurological conditions such as stroke [68, 69, 323]. Nutrients and dietary patterns play an important role in brain development, physiology, and functioning [324–326]. For example, a survey of the literature of dietary fat in rodents on learning and memory identifies the overall deleterious effect of high-fat diets on the brain [327]. Diet also has the potential to be used as a preventative measure or treatment for stroke.

As discussed in Chapters 3 and 4, dietary treatments such as Fortasyn and hydroxytyrosol have been shown to improve health in neurological conditions, in particular via their effects on cardiovascular health. Often, the open field and corner test are used to identify the efficacy of treatments in rodent models, however they are time-intensive and are prone to experimenter bias. For example, open field did not identify changes in post-surgery walked distance and velocity in female stroke animal model during the post-surgery period while analysis of DVC trajectory data did [147]. Also, decreased nighttime activity was identified with DVC and Traja but not traditional OF test in stroke mice 1 week after surgery. Additionally, during the nighttime of the second postsurgery week, only stroke mice showed a left turning preference. A comparison of the outcome of traditional systems with results with HCM and Traja is provided in §5.3.1. Software like Traja augments the ability of researchers to automatically identify patterns in locomotion data, thus supporting the development of novel behavioral tests through advanced data mining (see §5.4 for an overview of planned extensions to Traja) and real-time analysis at virtually unlimited scale [92].

5.3.1 HCM results compared with other behavioral tests

Chapters 3 and 4 demonstrated the application of Traja to position data collected with DVC to enable analysis of the neurobehavioral indicators activity, distance travelled, velocity, total turns, laterality and effect of treatment within a home cage environment. Chapter 3 demonstrated the application of Traja in an experiment over a 1-month period involving dietary intervention with Fortasyn following stroke. Chapter 4 demonstrated analysis of home cage centroids over several months for a similar, more extensive experiment involving a Mediterranean diet-based intervention. HCM data allows more extensive analyses than traditional techniques, since it represents the behavior of the subject 24/7. Derivative methods are thus able to identify subtle patterns which may not be visible to the unaided eye or within the bounds of controlled experiments. In Chapter 4, HCM was carried out over the entire experiment (Figure 4.1), while behavioral tests were performed only occasionally during the inactive phase during the day.

Turn preference

Distinctive of the HCM data approach combined with Traja for analysis is the ability to quantify the home cage activity and laterality. In Chapter 3, as measured with DVC and Traja, we were able to identify that mice showed a left turn preference in the home cage following stroke, whereas this was undetectable with traditional behavioral tests. This turning bias was found exclusively following stroke (Chapter 4) [47] and significant differences were observed following exercise intervention in stroke [96]. These results confirm previous results with corner test where stroke mice exhibited preference towards

the nonimpaired (ipsilateral) side in distal middle cerebral artery occlusion (MCAo) [148, 149] as well as combined distal and proximal MCAo [150], in contrast to sham mice which showed no directional bias. Interestingly, turn bias following surgery was not observed in subjects following *distal* MCAo [63].

Additionally, the temporal resolution of HCM data is much greater than that of the data collected using the open field test, which could potentially allow for the detection of smaller variations in activity during recovery. The results obtained in this research suggest further investigation is warranted for the use of HCM and the automatic phenotyping and behavioral testing of mice in their home cages.

Activity

In Chapter 3, we observed a significant increase in activity with HCM over the 3 weeks following recovery (Figure 3.2), which is assumed to be a standard observation in ischemic stroke mouse models. This behavioral difference during recovery has also been observed by measuring the performance of mice on other behavioral tests such as open field [68]. This suggests that HCM can reliably measure overall activity in rodent models of neurological conditions.

In both the Fortasyn and HT experiments, the diet effects are either subtle or not observed with DVC-based tracking in the home cage, however overall recovery from stroke, as measured by activity and turning bias, was observed. This indicates that HCM combined with software like Traja can be a useful addition to analysis of behavior in research on neurodegenerative diseases such as stroke.

Time intervals in observation

Other methods, such as the open field test, allow the comparison of activity and laterality during experimentation. However, the ability to observe differences in activity and laterality within arbitrary time intervals allows the adaptation of HCM analysis to methodological constraints in a wide range of experiments. A recent survey of continuous monitoring in home cage analysis covered periods ranging from 3 days to 4 weeks [14]. Such extensive monitoring provides more data which can be used to mine behavioral phenotypes which may not manifest in short periods. Further, varying time scales reveal a range of patterns in animal movement, with implications for behavioral phenotyping [328]. Extensive animal tracking allows accessing methods which are only available at broad time scales (days or weeks) and not observable in shorter durations, such as changes in circadian rhythms or overall activity [329].

5.4 OTHER APPLICATIONS OF TRAJA

Traja is demonstrated here as a tool for analyzing behavior of stroke mouse models, however other neuropsychiatric models are also relevant. Behavior is a prognostic indicator for a range of neuropsychiatric rodent models such as anxiety and depression [330, 331].

Applying recent advances in data collection and analysis such as automated HCM to animal models of disease requires balancing the desirable high validity of animal models with practical constraints, particularly for neuropsychiatric models where construct validity is often poorly defined across species [330, 332].

In addition, the same or similar methods which are used for animal locomotion analysis have relevance to other domains such as pedestrian [157] or vehicle [333] traffic modeling, and recent methods such as deep learning-based models which improve learning of long-term dependencies are equally relevant to sequence learning for stock market price forecasting [334] and music generation [335].

While the scientific community has several choices of tools for video-based analysis of mouse behavior, Traja has the potential to change the research landscape for prototyping and collaborating on the development of advanced methods for HCM data analysis. For example, it is currently being extended for visualizing the phase space of the latent variables in a neural ordinary differential equation model of the Fortasyn dataset. Traja presents a unifying standard for data scientific and machine learning approaches with time series data that enables researchers to utilize prior knowledge and focus their efforts on algorithms, modelling and exploration, rather than boilerplate code and software engineering.

Future additions to Traja include fractal methods, such as recurrence plots [336], visualizations of the latent space of recurrent neural networks, and neural ordinary differential equations [141]. As open source software, features are regularly added and bugs are publically tracked in the issue tracker.

5.5 RECOMMENDATIONS FOR FUTURE RESEARCH

As sensors continue to shrink and can be easily implanted in mouse home cages, methods such as HCM can be expected to become standard additions to the neuroscientist toolset. Nutritional and other interventions, which currently take months or years for comparison of efficacy, could potentially yield diagnostic information in real-time with HCM. The data produced by real-time monitoring tools provides unprecedented opportunity to mine patterns relevant to behavior as are found in aging and neurological disease. Some promising areas of research in this direction include statistical and machine learning methods for high-throughput data such as real-time, unsupervised pattern recognition of complex behaviors [125], anomaly detection, and application of advanced behavioral time-series analysis methods such as topological data analysis [337] and manifold learning [338]. Such methods will allow development of a library of behaviors and phenotypes, bringing the potential of data mining to rapidly accelerate drug discovery and research into treatments for diseases such as stroke within the coming years.

5.6 CONCLUDING REMARKS

HCM combined with flexible tools for data mining like Traja are useful additions to the computational neuroscientist's toolbox for preclinical animal research, particularly for stroke mouse behavioral phenotyping. These studies demonstrate the usefulness of applying Traja to DVC data for estimating the effect of nutritional intervention in stroke mice. The results indicate the usefulness of 24/7 HCM data for both confirming and augmenting results found in traditional behavioral tests. The major limitations of this approach are the required programming skills needed to handle the data and the lack of existing public datasets for validating patterns mined from the data. As additional tools enable behavioral data mining such as advanced statistical algorithms and widespread placement of sensors further enable automated behavioral phenotyping, researchers will find increasing opportunities to share their data and collaborate on projects across departments and disciplines.

Shifts from behavioral analysis tests towards data-heavy approaches such as automated HCM reflect a more general shift in scientific research from a top-down to a more bottom-up data-driven approach. The classical scientific method is based on the formation of hypotheses and then the collection of data to support or disprove these hypotheses. Data was historically scarce and often time-consuming and labor-intensive to collect, and the traditional scientific method was designed with this reality in mind. The hypothesis driven model was generally the most effective way to build an understanding of processes in a world with limited data. Nowadays, technological advances have changed the reality of data collection, and data is much more readily and cheaply available than ever before. This abundance necessitates a new approach to scientific research, in which researchers work backwards from copious data to identify trends and conclusions that can be reached [339]. Advances in computing play a vital role in this process, as they enable processing of this data. Thus, software like Traja is at the forefront of this change in scientific research, as it allows for the identification of trends in data without experimenter biases.

With the current pace of technological development, the shift towards data-driven science is likely to only accelerate. Mobile and Internet of Things (IoT) devices are predicted to generate 90 zettabytes of data by 2025, nearly a half of the total data expected to exist at that time [340].

If biological scientists embrace and leverage the new technologies available, this data-heavy approach to research has the potential to revolutionize the field and accelerate the pace of discovery. From a technologically optimistic perspective, one can suppose that, given sufficient data, any pattern which is visible in nature will be able to be modelled using techniques similar to those discussed in this work.

However, data-based research methods do have limitations. For example, they do not necessarily provide insight into the principles underlying observable phenomena, in the same way that a camera pointing out the window can "recognize" repetitive events such as people passing by, but without integrating with other data sources would be rather limited at understanding the underlying causes of the activity.[3] Thus, new data-driven research techniques are most powerful when used alongside traditional methods to augment the current capabilities of biological research.

[3] example from Noam Chomsky [341]

6

APPENDICES

References
Nederlandse samenvatting
Curriculum Vitae
Publications
Acknowledgements
Data Management

REFERENCES

1 Valery L. Feigin et al. “Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015”. In: *The Lancet Neurology* 16.11 (2017)

2 Christopher Bishop. *Pattern Recognition and Machine Learning*. Information Science and Statistics. New York: Springer-Verlag, 2006.

3 Wenlong Tang and Di Su. “Locomotion analysis and its applictions in neurological disorders detection: state-of-art review”. In: *Network Modeling Analysis in Health Informatics and Bioinformat- ics* 2.1 (2013), pp. 1–12.

4 Wenlong Tang, Richard M. Lovering, Joseph A. Roche, Robert J. Bloch, Nagaraj K. Neerchal, and Uri Tasch. “Gait analysis of locomotory impairment in rats before and after neuromuscular injury”. In: *Journal of Neuroscience Methods* 181.2 (2009), pp. 249–256.

5 Wenlong Tang, Paul Yarowsky, and Uri Tasch. “Detecting ALS and Parkinson’s disease in rats through locomotion analysis”. In: *Network Modeling Analysis in Health Informatics and Bioinformatics* 1.1 (2012), pp. 63–68.

6 Brigitte Toro, P Farren, and C Nester. “Review of Observational Gait Assessment in Clinical Practice”. In: *Physiotherapy* 88.11 (2002), p. 01.

7 Wenlong Tang, Kim McDowell, Mark Limsam, Nagaraj K. Neerchal, Paul Yarowsky, and Uri Tasch. “Locomotion analysis of Sprague–Dawley rats before and after injecting 6-OHDA”. In: *Behavioural Brain Research* 210.1 (2010), pp. 131–133.

8 Wenlong Tang, Uri Tasch, Nagaraj K. Neerchal, Liang Zhu, and Paul Yarowsky. “Measuring early pre-symptomatic changes in locomotion of SOD1-G93A rats—A rodent model of amyotrophic lateral sclerosis”. In: *Journal of Neuroscience Methods* 1 6.2 (2009), pp. 254–262.

9 I. Tien, S. D. Glaser, R. Bajcsy, D. S. Goodin, and M. J. Aminoff. “Results of Using a Wireless Inertial Measuring System to Quantify Gait Motions in Control Subjects”. In: *IEEE Transactions on Information Technology in Biomedicine* 14.4 (2010), pp. 904–915.

10 Abdul Saboor, Triin Kask, Alar Kuusik, Muhammad Alam, Yannick Le Moullec, Imran Niazi, Ahmed Zoha, and Rizwan Ahmad. “Latest Research Trends in Gait Analysis Using Wearable Sensors and Machine Learning: A Systematic Review”. In: *IEEE Access* 8 (2020).

11 Alfred Mingrone, Ayal Kaffman, and Arie Kaffman. “The Promise of Automated Home-Cage Monitoring in Improving Translational Utility of Psychiatric Research in Rodents”. In: *Frontiers in Neuroscience* 14 (2020), p. 1341.

12 Lianne Robinson and Gernot Riedel. “Comparison of automated home-cage monitoring systems: Emphasis on feeding behaviour, activity and spatial learning following pharmacological interventions”. In: *Journal of Neuroscience Methods* 234 (2014), pp. 13–25.

13 Claire A. Richardson. “The power of automated behavioural homecage technologies in characterizing disease progression in laboratory mice: A review”. In: *Applied Animal Behaviour Science* 163 (201S), pp. 19–27.

14 Vootele Voikar and Stefano Gaburro. “Three Pillars of Automated Home-Cage Phenotyping of Mice: Novel Findings, Refinement, and Reproducibility Based on Literature and Experience”. In: *Frontiers in Behavioral Neuroscience* 14 (2020), p. 55434.

15 Jonathan P. Balcombe, Neal D. Barnard, and Chad Sandusky. “Laboratory Routines Cause Animal Stress”. In: *Journal of the American Association for Laboratory Animal Science* 43.6 (2004), pp. 42–51.

16 Jennifer A. Teske, Claudio E. Perez-Leighton, Charles J. Billington, and Catherine M. Kotz. “Methodological considerations for measuring spontaneous physical activity in rodents”. In: *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 306.10 (2014), R 14– 21.

17 Pei-Hao Chen, Rong-Long Wang, De-Jyun Liou, and Jin-Siang Shaw. “Gait Disorders in Parkinson’s Disease: Assessment and Management”. In: *International Journal of Gerontology*.4 (2013), pp. 189–193.

18 Seung Min Kim, Dae Hyun Kim, YoungSoon Yang, Sang Won Ha, and Jeong Ho Han. “Gait Patterns in Parkinson’s Disease with or without Cognitive Impairment”. In: *Dementia and Neurocognitive Disorders* 1 .2 (2018), pp. 5 –65.

19 Thomas G. Hampton and Ivo Amende. “Treadmill Gait Analysis Characterizes Gait Alterations in Parkinson’s Disease and Amyotrophic Lateral Sclerosis Mouse Models”. In: *Journal of Motor Behavior* 42.1 (2009), pp. 1–4.

20 G. D. Muir and Ian Q. Whishaw. “Ground reaction forces in locomoting hemi-parkinsonian rats: a definitive test for impairments and compensations”. In: *Experimental Brain Research* 126.3 (1999), pp. 30 –314.

21 Cristina Baiguera, Manuela Alghisi, Annalisa Pinna, Arianna Bellucci, Maria Antonietta De Luca, Lucia Frau, Micaela Morelli, Rosaria Ingrassia, Marina Benarese, Vanessa Porrini, Michele Pellitteri, Giuseppe Bertini, Paolo Francesco Fabene, Sandra Sigala, Maria Grazia Spillantini, Hsiou-Chi Liou, Pier Franco Spano, and Marina Pizzi. “Late-onset Parkinsonism in NFB/c-Rel-deficient mice”. In: *Brain* 135.9 (2012), pp. 2 50–265

22 Katrina L. Paumier, Stacey J. Sukoff Rizzo, Zdenek Berger, Yi Chen, Cathleen Gonzales, Edward Kaftan, Li Li, Susan Lotarski, Michael Monaghan, Wei Shen, Polina Stolyar, Dmytro Vasilyev, Margaret Zaleska, Warren D. Hirst, and John Dunlop. "Behavioral Characterization of AS3T Mice Reveals Early and Late Stage Deficits Related to Parkinson's Disease". In: *PLOS ONE* 8.8 (2013), e70274.

23 B. R. Brooks. "Natural history of ALS: Symptoms, strength, pulmonary function, and disability". In: *Neurology* 4 .Issue 4, Supplement 2 (1996), 15–825 .

24 A. Hirano. "Neuropathology of ALS: An overview". In: *Neurology* 4 .Issue 4, Supplement 2 (1996), 635–665.

25 Jeffrey M. Hausdorff, Apinya Lertratanakul, Merit E. Cudkowicz, Amie L. Peterson, David Kaliton, and Ary L. Goldberger. "Dynamic markers of altered gait rhythm in amyotrophic lateral sclerosis". In: *Journal of Applied Physiology* 88.6 (2000), pp. 2045–2053

26 Sasa Radovanović, Milena Milićev, Stojan Perić, Ivana Basta, Vladimir Kostić, and Zorica Stević. "Gait in amyotrophic lateral sclerosis: Is gait pattern differently affected in spinal and bulbar onset of the disease during dual task walking?" In: *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* 15.7-8 (2014), pp. 488–493 .

27 Yunfeng Wu and Sridhar Krishnan. "Computer-aided analysis of gait rhythm fluctuations in amyotrophic lateral sclerosis". In: *Medical & Biological Engineering & Computing* 4 .11 (2009), p. 1165.

28 Elisabetta Golini, Mara Rigamonti, Fabio Iannello, Carla De Rosa, Ferdinando Scavizzi, Marcello Raspa, and Silvia Mandillo. "A Non- invasive Digital Biomarker for the Detection of Rest Disturbances in the SOD1G93A Mouse Model of ALS". In: *Frontiers in Neuroscience* 14 (2020).

29 S. T. O'Keeffe, H. Kazeem, R. M. Philpott, J. R. Playfer, M. Gosney, and M. Lye. "Gait Disturbance in Alzheimer's Disease: A Clinical Study". In: *Age and Ageing* 25.4 (1996), pp. 313–316.

30 Olivier Beauchet, Gilles Allali, Gilles Berrut, Caroline Hommet, Véronique Dubost, and Frédéric Assal. "Gait analysis in demented subjects: Interests and perspectives". In: *Neuropsychiatric Disease and Treatment* 4.1 (2008), pp. 155–160.

31 Judit España, Lydia Giménez-Llort, Jorge Valero, Alfredo Miñano, Alberto Rábano, José Rodriguez-Alvarez, Frank M. LaFerla, and Car- los A. Saura. "Intraneuronal -Amyloid Accumulation in the Amygdala Enhances Fear and Anxiety in Alzheimer's Disease Transgenic Mice". In: *Biological Psychiatry*. Inflammation in Alzheimer's Disease 6 .6 (2010), pp. 513–521

32 Celine Samaey, An Schreurs, Stijn Stroobants, and Detlef Balschun. "Early Cognitive and Behavioral Deficits in Mouse Models for Tauopathy and Alzheimer's Disease". In: *Frontiers in Aging Neuroscience* 11 (2019).

33 Pau-Choo Chung, Yu-Liang Hsu, Chun-Yao Wang, Chien-Wen Lin, Jeen-Shing Wang, and Ming-Chyi Pai. “Gait analysis for patients with Alzheimer’S disease using a triaxial accelerometer.” In: *2OI2 IEEE International Symposium on Circuits and Systems*. Seoul, Ko- rea (South): IEEE, 2012, pp. 1323–1326.

34 Alina Codita, Astrid Gumucio, Lars Lannfelt, Pär Gellerfors, Bengt Winblad, Abdul H. Mohammed, and Lars N.G. Nilsson. “Impaired behavior of female tg-ArcSwe APP mice in the IntelliCage: A longitudinal study”. In: *Behavioural Brain Research* 215.1 (2010), pp. 83–94 .

35 Norman C. Reynolds, Joel B. Myklebust, Thomas E. Prieto, and Barbara M. Myklebust. “Analysis of gait abnormalities in huntington disease”. In: *Archives of Physical Medicine and Rehabilitation* 80.1 (1999), pp. 59–65 .

36 Francis O Walker. “Huntington’s disease”. In: *The Lancet* 369.955 (200), pp. 218–228.

37 A. Reiner, D.C. Lafferty, H.B. Wang, N. Del Mar, and Y.P. Deng. “The group 2 metabotropic glutamate receptor agonist LY3 9268 rescues neuronal, neurochemical and motor abnormalities in R6/2 Huntington’s disease mice”. In: *Neurobiology of Disease* 4 .1 (2012), pp. 5–91.

38 Fanny Mochel, Brandon Durant, Alexandra Durr, and Raphael Schiffmann. “Altered Dopamine and Serotonin Metabolism in Motorically Asymptomatic R6/2 Mice”. In: *PLOS ONE* 6.3 (2011), e18336.

39 Stephen Oakeshott, Fuat Balci, Igor Filippov, Carol Murphy, Russell Port, David Connor, Ahmad Paintdakhi, Joseph LeSauter, Liliana Menalled, Sylvie Ramboz, Seung Kwak, David Howland, Rae Silver, and Dani Brunner. “Circadian Abnormalities in Motor Activity in a BAC Transgenic Mouse Model of Huntington’s Disease”. In: *PLoS Currents* 3 (2011).

40 Olga Rudenko, Vadim Tkach, Vladimir Berezin, and Elisabeth Bock. “Detection of early behavioral markers of Huntington’s disease in R6/2 mice employing an automated social home cage”. In: *Behavioural Brain Research* 203.2 (2009), pp. 188–199.

41 A. D. Steele, W. S. Jackson, O. D. King, and S. Lindquist. “The power of automated high-resolution behavior analysis revealed by its application to mouse models of Huntington’s and prion diseases”. In: *Proceedings of the National Academy of Sciences* 104.6 (200), pp. 1983– 1988.

42 Nigel I. Wood, Anna O. G. Goodman, Jorien M. M. van der Burg, Véronique Gazeau, Patrik Brundin, Maria Björkqvist, Åsa Petersén, Sarah J. Tabrizi, Roger A. Barker, and A. Jennifer Morton. “Increased thirst and drinking in Huntington’s disease and the R6/2 mouse”. In: *Brain Research Bulletin* 6.1 (2008), pp. 0– 9.

43 Hans-Peter Haring. “Cognitive impairment after stroke:” in: *Current Opinion in Neurology* 15.1 (2002), pp. 9–84.

44 Samar M. Hatem, Geoffroy Saussez, Margaux della Faille, Vincent Prist, Xue Zhang, Delphine Dispa, and Yannick Bleyenheuft. “Rehabilitation of Motor Function after Stroke: A Multiple Systematic Review Focused on Techniques to Stimulate Upper Extremity Recovery”. In: *Frontiers in Human Neuroscience* 10 (2016).

45 Sonja Verstraeten. “Motor and Cognitive Impairment after Stroke: A Common Bond or a Simultaneous Deficit?” In: 1.1 (2016), p. 10.

46 Julien Boudarham, Nicolas Roche, Didier Pradon, Céline Bonnyaud, Djamel Bensmail, and Raphael Zory. “Variations in Kinematics during Clinical Gait Analysis in Stroke Patients”. In: *PLOS ONE* 8.6 (2013), e66421.
.

47 Jesús Calahorra, Justin Shenk, Vera H. Wielenga, Vivienne Verweij, Bram Geenen, Pieter J. Dederen, M. Ángeles Peinado, Eva Siles, Maximilian Wiesmann, and Amanda J. Kiliaan. “Hydroxytyrosol, the Major Phenolic Compound of Olive Oil, as an Acute Therapeutic Strategy after Ischemic Stroke”. In: *Nutrients* 11.10 (2019), p. 2430.

48 Justin Shenk, Klara J. Lohkamp, Maximilian Wiesmann, and Amanda J. Kiliaan. “Automated Analysis of Stroke Mouse Trajectory Data With Traja”. In: *Frontiers in Neuroscience* 14 (2020).

49 Amos D. Korczyn, Veronika Vakhapova, and Lea T. Grinberg. “Vascular dementia”. In: *Journal of the Neurological Sciences* 322.1- 2 (2012), pp. 2–10 .

50 John T O’Brien and Alan Thomas. “Vascular dementia”. In: *The Lancet* 386.10004 (2015), pp. 1698–1706 .

51 G. C. Román, T. K. Tatemichi, T. Erkinjuntti, J. L. Cummings, J. C. Masdeu, J. H. Garcia, L. Amaducci, J. M. Orgogozo, A. Brun, and A. Hofman. “Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop”. In: *Neurology* 43.2 (1993), pp. 250–260.

52 Poornima Venkat, Michael Chopp, and Jieli Chen. “Models and mechanisms of vascular dementia”. In: *Experimental Neurology* 272 (2015), pp. 9 –108.

53 Maximilian Wiesmann, Amanda J Kiliaan, and Jurgen AHR Claassen. “Vascular Aspects of Cognitive Impairment and Dementia”. In: *Journal of Cerebral Blood Flow & Metabolism* 33.11 (2013), pp. 1696–1 06 .

54 Jing Zhou, Jin-Tai Yu, Hui-Fu Wang, Xiang-Fei Meng, Chen-Chen Tan, Jun Wang, Chong Wang, and Lan Tan. “Association between stroke and Alzheimer’s disease: systematic review and meta-analysis”. In: *Journal of Alzheimer’s disease: JAD* 43.2 (2015), pp. 479–489.

55 Joy Adamson, Andy Beswick, and Shah Ebrahim. “Is stroke the most common cause of disability?” In: *Journal of Stroke and Cerebrovascular Diseases* 13.4 (2004), pp. 171–177.

56 Philip B. Gorelick. “The global burden of stroke: persistent and disabling”. In: *The Lancet Neurology* 18.S (2019), pp. 417–418 .

57 Christopher J L Murray et al. “Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010”. In: *The Lancet* 380.9859 (2012), pp. 2197–2223 .

58 Francesco Ferrarello, Valeria Anna Maria Bianchi, Marco Baccini, Gaia Rubbieri, Enrico Mossello, Maria Chiara Cavallini, Niccolò Marchionni, and Mauro Di Bari. “Tools for Observational Gait Analysis in Patients With Stroke: A Systematic Review”. In: *Physical Therapy* 93.12 (2013), pp. 1673–1685.

59 Gunes Yavuzer, Öznur Öken, Atilla Elhan, and Henk J. Stam. “Repeatability of lower limb three-dimensional kinematics in patients with stroke”. In: *Gait & Posture* 2 .1 (2008), pp. 31–35.

60 S. Thomas Carmichael. “Rodent Models of Focal Stroke: Size, Mechanism, and Purpose”. In: *NeuroRx* 2.3 (2005), pp. 396–409.

61 Felix Fluri, Michael K. Schuhmann, and Christoph Kleinschnitz. “Animal models of ischemic stroke and their application in clinical research”. In: *Drug Design, Development and Therapy* 9 (2015), pp. 3445–3454 .

62 Yonggang Wang, Bruno Bontempi, Shwehuey M. Hong, Kala Mehta, Philip R. Weinstein, Gary M. Abrams, and Jialing Liu. “A comprehensive analysis of gait impairment after experimental stroke and the therapeutic effect of environmental enrichment in rats”. In: *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 28.12 (2008), pp. 1936–1950.

63 Judith Lubjuhn, Alexandra Gastens, Gertrud von Wilpert, Panagiotis Bargiotas, Oliver Herrmann, Sasidhar Murikinati, Tamer Rabie, Hugo H. Marti, Hugo Marti, Ivo Amende, Tom G. Hampton, and Markus Schwaninger. “Functional testing in a mouse stroke model induced by occlusion of the distal middle cerebral artery”. In: *Journal of Neuroscience Methods* 184.1 (2009), pp. 95–103.

64 B. J. Jones and D. J. Roberts. “The quantiative measurement of motor incoordination in naive mice using an acelerating rotarod”. In: *The Journalof Pharmacyand Pharmacology* 20.4 (1968), pp. 302– 304.

65 K. Matsuura, H. Kabuto, H. Makino, and N. Ogawa. “Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion”. In: *Journal of Neuroscience Methods* 3.1 (199), pp. 45–48.

66 Valentine Bouët, Thomas Freret, Jérôme Toutain, Didier Divoux, Michel Boulouard, and Pascale Schumann-Bard. “Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse”. In: *Experimental Neurology* 203.2 (2007), pp. 555–567 .

67 R. Morris. “Developments of a water-maze procedure for studying spatial learning in the rat”. In: *Journal of Neuroscience Methods* 11.1 (1984), pp. 47–60

68 Maximilian Wiesmann, Bastian Zinnhardt, Dirk Reinhardt, Sarah Eligehausen, Lydia Wachsmuth, Sven Hermann, Pieter J. Dederen, Marloes Hellwich, Michael T. Kuhlmann, Laus M. Broersen, Arend Heerschap, Andreas H. Jacobs, and Amanda J. Kiliaan. “A specific dietary intervention to restore brain structure and function after ischemic stroke”. In: *Theranostics* .2 (201), pp. 493–512 .

69 Maximilian Wiesmann, Nienke M. Timmer, Bastian Zinnhardt, Dirk Reinhard, Sarah Eligehausen, Anja Königs, Hasnae Ben Jeddi, Pieter J. Dederen, Andreas H. Jacobs, and Amanda J. Kiliaan. “Ef- fect of a multinutrient intervention after ischemic stroke in female CS Bl/6 mice”. In: *Journal of Neurochemistry* 144.S (2018), pp. 549– 564 .

70 Matthias Endres and Ulrich Dirnagl. “Ischemia and stroke”. In: *Advances in Experimental Medicine and Biology* S13 (2002), pp. 455– 473.

71 Odilo Engel, Sabine Kolodziej, Ulrich Dirnagl, and Vincent Prinz. “Modeling stroke in mice middle cerebral artery occlusion with the filament model”. In: *Journal of Visualized Experiments: JoVE* 4 (2011) .

72 A Costa, K Walsh, and M Davisson. “Motor dysfunction in a mouse model for Down syndrome”. In: *Physiology & Behavior* 68.1-2 (1999), pp. 211–220.

73 R. J. Full and M. S. Tu. “Mechanics of six-legged runners”. In: *Journal of Experimental Biology* 148.1 (1990), pp. 129–146.

74 Gillian D Muir and Ian Q Whishaw. “Complete locomotor recovery following corticospinal tract lesions: measurement of ground reaction forces during overground locomotion in rats”. In: *Behavioural Brain Research* 103.1 (1999), pp. 45–53.

75 K Clarke and J Still. “Development and consistency of gait in the mouse”. In: *Physiology & Behavior* 3.1-2 (2001), pp. 159–164.

76 Thomas G. Hampton, Melissa R. Stasko, Ajit Kale, Ivo Amende, and Alberto C. S. Costa. “Gait dynamics in trisomic mice: quantitative neurological traits of Down syndrome”. In: *Physiology & Behavior* 82.2-3 (2004), pp. 381–389.

77 Shuxin Li, Ji-Eun Kim, Stephane Budel, Thomas G. Hampton, and Stephen M. Strittmatter. “Transgenic inhibition of Nogo-66 receptor function allows axonal sprouting and improved locomotion after spinal injury”. In: *Molecular and Cellular Neuroscience* 29.1 (2005), pp. 26–39.

78 Christine M. Wooley, Roger B. Sher, Ajit Kale, Wayne N. Frankel, Gregory A. Cox, and Kevin L. Seburn. “Gait analysis detects early changes in transgenic SOD1(G93A) mice”. In: *Muscle & Nerve* 32.1 (2005), pp. 43–50.

79 Ying-Ju Chen, Fu-Chou Cheng, Meei-Ling Sheu, Hong-Lin Su, Chun-Jung Chen, Jason Sheehan, and Hung-Chuan Pan. "Detection of subtle neurological alterations by the Catwalk XT gait analysis system". In: *Journal of NeuroEngineering and Rehabilitation* 11.1 (2014), p. 62.

80 Sabine Herold, Prateek Kumar, Klaus Jung, Irina Graf, Henrike Menkhoff, Xenia Schulz, Mathias Bähr, and Katharina Hein. "Cat-Walk gait analysis in a rat model of multiple sclerosis". In: *BMC Neuroscience* 1 .1 (2016), p. 8.

81 Johannes Walter, Olga Kovalenko, Alexander Younsi, Martin Grutza, Andreas Unterberg, and Klaus Zweckberger. "The CatWalk XT® is a valid tool for objective assessment of motor function in the acute phase after controlled cortical impact in mice". In: *Behavioural Brain Research* 392 (2020), p. 112680.

82 Cornelis JT van Uden and Marcus P. Besser. "Test-retest reliability of temporal and spatial gait characteristics measured with an instrumented walkway system (GAITRite®)". In: *BMC Musculoskeletal Disorders* 5.1 (2004), p. 13).

83 Edward S Sazonov, George Fulk, James Hill, Yves Schutz, and Raymond Browning. "Monitoring of Posture Allocations and Activities by a Shoe-Based Wearable Sensor". In: *IEEE Transactions on Biomed- ical Engineering* S8.4 (2011), pp. 983–990

84 P. Lopez-Meyer, G. D. Fulk, and E. S. Sazonov. "Automatic Detection of Temporal Gait Parameters in Poststroke Individuals". In: *IEEE Transactions on Information Technology in Biomedicine* 1S.4 (2011), pp. 594–601.

85 Nadezhda Sazonova, Raymond C. Browning, and Edward Sazonov. "Accurate Prediction of Energy Expenditure Using a Shoe-Based Activity Monitor". In: *Medicine & Science in Sports & Exercise* 43. (2011), pp. 1312–1321.

86 Nadezhda A. Sazonova, Raymond Browning, and Edward S. Sazonov. "Prediction of Bodyweight and Energy Expenditure Using Point Pressure and Foot Acceleration Measurements". In: *The Open Biomedical Engineering Journal* S.1 (2011).

87 S. Bamberg, A.Y. Benbasat, D.M. Scarborough, D.E. Krebs, and J.A. Paradiso. "Gait Analysis Using a Shoe-Integrated Wireless Sensor System". In: *IEEE Transactions on Information Technology in Biomedicine* 12.4 (2008), pp. 413–423

88 Fokke B. van Meulen, Dirk Weenk, Edwin H. F. van Asseldonk, H. Martin Schepers, Peter H. Veltink, and Jaap H. Buurke. "Analysis of Balance during Functional Walking in Stroke Survivors". In: *PLOS ONE* 11.11 (2016), e0166 89

89 V Maynard, A.M.O Bakheit, J Oldham, and J Freeman. "Intra-rater and inter-rater reliability of gait measurements with CODA mpx30 motion analysis system". In: *Gait & Posture* 1 .1 (2003), pp. 59–67.

90 Kenneth Monaghan, Eamonn Delahunt, and Brian Caulfield. “Increasing the number of gait trial recordings maximises intra-rater reliability of the CODA motion analysis system”. In: *Gait & Posture* 25.2 (200), pp. 303–315

91 Liane Hobson, Rasneer S. Bains, Simon Greenaway, Sara Wells, and Patrick M. Nolan. “Phenotyping in Mice Using Continuous Home Cage Monitoring and Ultrasonic Vocalization Recordings”. In: *Current Protocols in Mouse Biology* 10.3 (2020) .

92 Fabio Iannello. “Non-intrusive high throughput automated data collection from the home cage”. In: *Heliyon* 5.4 (2019), e01454.

93 Karin Pernold, F. Iannello, B. E. Low, M. Rigamonti, G. Rosati, F. Scavizzi, J. Wang, M. Raspa, M. V. Wiles, and B. Ulfhake. “Towards large scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of CS BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique”. In: *PLoS ONE* 14.2 (2019).

94 Rasneer S. Bains, Heather L. Cater, Rowland R. Sillito, Agisilaos Chartsias, Duncan Sneddon, Danilo Concas, Piia Keskivali-Bond, Timothy C. Lukins, Sara Wells, Abraham Acevedo Arozena, Patrick M. Nolan, and J. Douglas Armstrong. “Analysis of Individual Mouse Activity in Group Housed Animals of Different Inbred Strains using a Novel Automated Home Cage Analysis System”. In: *Frontiers in Behavioral Neuroscience* 10 (2016).

95 Pieter M. Goltstein, Sandra Reinert, Annet Glas, Tobias Bonhoeffer, and Mark Hübener. “Food and water restriction lead to differential learning behaviors in a head-fixed two-choice visual discrimination task for mice”. In: *PloS One* 13.9 (2018), e0204066.

96 Klara J. Lohkamp, Amanda J. Kiliaan, Justin Shenk, Vivienne Verweij, and Maximilian Wiesmann. “The Impact of Voluntary Exercise on Stroke Recovery”. In: *Frontiers in Neuroscience* 07 (2021).

97 Fabrizio Grieco, Briana J. Bernstein, Barbara Biemans, Lior Bikovski, C. Joseph Burnett, Jesse D. Cushman, Elsbeth A. van Dam, Sydney A. Fry, Bar Richmond-Hacham, Judith R. Homberg, Martien J. H. Kas, Helmut W. Kessels, Bastijn Koopmans, Michael J. Krashes, Vaishnav Krishnan, Sreemathi Logan, Maarten Loos, Katharine E. McCann, Qendresa Parduzi, Chaim G. Pick, Thomas D. Prevot, Gernot Riedel, Lianne Robinson, Mina Sadighi, August B. Smit, William Sonntag, Reinko F. Roelofs, Ruud A.J. Tegelenbosch, and Lucas P.J.J. Noldus. “Measuring Behavior in the Home Cage: Study Design, Applications, Challenges, and Perspectives”. In: *Frontiers in Behavioral Neuroscience* 15 (2021).

98 John Samuel Bass, Anney H. Tuo, Linh T. Ton, Miranda J. Jankovic, Paarth K. Kapadia, Catharina Schirmer, and Vaishnav Krishnan. “On the Digital Psychopharmacology of Valproic Acid in Mice”. In: *Frontiers in Neuroscience* 14 (2020), p. 594612.

99 Shanshan Guo, Yiru Huang, Yan Zhang, He Huang, Shangyu Hong, and Tiemin Liu. "Impacts of exercise interventions on different diseases and organ functions in mice". In: *Journal of Sport and Health Science* 9.1 (2020), pp. 53– 37.

100 Fabrice de Chaumont, Elodie Ey, Nicolas Torquet, Thibault Lagache, Stéphane Dallongeville, Albane Imbert, Thierry Legou, Anne-Marie Le Sourd, Philippe Faure, Thomas Bourgeron, and Jean-Christophe Olivo-Marin. "Real-time analysis of the behaviour of groups of mice via a depth-sensing camera and machine learning". In: *Nature Biomedical Engineering* 3.11 (2019), pp. 930–942.

101 Vadim Alexandrov, Dani Brunner, Taleen Hanania, and Emer Leahy. "High-throughput analysis of behavior for drug discovery". In: *European Journal of Pharmacology* 50 (2015), pp. 82–89.

102 Tatiana Peleh, Xuesheng Bai, Martien J. H. Kas, and Bastian Hengerer. "RFID-supported video tracking for automated analysis of social behaviour in groups of mice". In: *Journal of Neuroscience Methods* 325 (2019), p. 108323.

103 Yair Shemesh, Yehezkel Sztainberg, Oren Forkosh, Tamar Shlapobersky, Alon Chen, and Elad Schneidman. "High-order social interactions in groups of mice". In: *eLife* 2 (2013), e00 59.

104 Hueihan Jhuang, Estibaliz Garrote, Xinlin Yu, Vinita Khilnani, Tomaso Poggio, Andrew D. Steele, and Thomas Serre. "Automated home-cage behavioural phenotyping of mice". In: *Nature Communications* 1.1 (2010), p. 68.

105 Elsbeth A. van Dam, Johanneke E. van der Harst, Cajo J. F. ter Braak, Ruud A. J. Tegelenbosch, Berry M. Spruijt, and Lucas P. J. J. Noldus. "An automated system for the recognition of various specific rat behaviours". In: *Journal of Neuroscience Methods* 218.2 (2013), pp. 214–224.

106 Charles V. Vorhees and Michael T. Williams. "Assessing spatial learning and memory in rodents". In: *ILAR journal* 55.2 (2014), pp. 310– 332.

107 Cathy O'Neill and Rachel Schutt. *Doing Data Science*. O'Reilly Media, Inc., 2013 .

108 Donald James McLean and Marta A. Skowron Volponi. "trajr: An R package for characterisation of animal trajectories". In: *Ethology* 124.6 (2018), pp. 440–448 .

109 Harvey J. Miller, Somayeh Dodge, Jennifer Miller, and Gil Bohrer. "Towards an integrated science of movement: converging research on animal movement ecology and human mobility science". In: *International Journal of Geographical Information Science* 33.5 (2019), pp. 855–8 67.

110 Clément Calenge, Stéphane Dray, and Manuela Royer-Carenzi. "The concept of animals' trajectories from a data analysis perspective". In: *Ecological Informatics* 4.1 (2009), pp. 34–41.

111 Urška Demšar, Kevin Buchin, Francesca Cagnacci, Kamran Safi, Bettina Speckmann, Nico Van de Weghe, Daniel Weiskopf, and Robert Weibel. "Analysis and visualisation of movement: an interdisciplinary review". In: *Movement Ecology* 3.1 (2015), p. 5.

112 Wullianallur Raghupathi and Viju Raghupathi. "Big data analytics in healthcare: promise and potential". In: *Health information science and systems* 2.1 (2014), p. 3 .

113 Surya Nepal, Rajiv Ranjan, and Kim-Kwang Raymond Choo. "Trustworthy processing of healthcare big data in hybrid clouds". In: *IEEE Cloud Computing* 2.2 (2015), pp. 8–84.

114 Michael R. Stratton, Peter J. Campbell, and P. Andrew Futreal. "The cancer genome". In: *Nature* 458. 239 (2009), pp. 19– 24.

115 Jay Shendure and Hanlee Ji. "Next-generation DNA sequencing". In: *Nature Biotechnology* 26.10 (2008), pp. 1135–1145.

116 Michael L. Metzker. "Sequencing technologies the next generation". In: *Nature Reviews. Genetics* 11.1 (2010), pp. 31–46.

117 Clifford Lynch. "Big data: How do your data grow?" In: *Nature* 455. 209 (2008), pp. 28–29.

118 Sabyasachi Dash, Sushil Kumar Shakyawar, Mohit Sharma, and Sandeep Kaushik. "Big data in healthcare: management, analysis and future prospects". In: *Journal of Big Data* 6.1 (2019), p. 54.

119 Usama Fayyad, Gregory Piatetsky-Shapiro, and Padhraic Smyth. "The KDD process for extracting useful knowledge from volumes of data". In: *Communications of the ACM* 39.11 (1996), pp. 27–34

120 Oded Maimon and Lior Rokach. "Introduction to Knowledge Discovery and Data Mining". In: *Data Mining and Knowledge Discovery Handbook*. Ed. by Oded Maimon and Lior Rokach. Boston, MA: Springer US, 2010, pp. 1–15.

121 Di Wang, Tomio Miwa, and Takayuki Morikawa. "Big Trajectory Data Mining: A Survey of Methods, Applications, and Services". In: *Sensors (Basel, Switzerland)* 20.16 (2020), p. 4571.

122 Tsubasa Hirakawa, Takayoshi Yamashita, Toru Tamaki, Hironobu Fujiyoshi, Yuta Umezu, Ichiro Takeuchi, Sakiko Matsumoto, and Ken Yoda. "Can AI predict animal movements? Filling gaps in animal trajectories using inverse reinforcement learning". In: *Ecosphere* 9.10 (2018), e0244.

123 Takuya Maekawa, Kazuya Ohara, Yizhe Zhang, Matasaburo Fukutomi, Sakiko Matsumoto, Kentarou Matsumura, Hisashi Shidara, Shuhei J. Yamazaki, Ryusuke Fujisawa, Kaoru Ide, Naohisa Nagaya, Koji Yamazaki, Shinsuke Koike, Takahisa Miyatake, Koutarou D. Kimura, Hiroto Ogawa, Susumu Takahashi, and Ken Yoda. "Deep learning-assisted comparative analysis of animal trajectories with DeepHL". In: *Nature Communications* 11.1 (2020), p. 5316.

124 Xinqing Li, Tanguy Tresor Sindihebura, Lei Zhou, Carlos M. Duarte, Daniel P. Costa, Mark A. Hindell, Clive McMahon, Mônica M. C. Muelbert, Xiangliang Zhang, and Chengbin Peng. "A prediction and imputation method for marine animal movement data". In: *PeerJ. Computer Science* (2021), e656.

125 Alexander I. Hsu and Eric A. Yttri. "B-SOiD: An Open Source Unsupervised Algorithm for Discovery of Spontaneous Behaviors". In: *bioRxiv* (2019), p. 0271.

126 Dhanushi A. Wijeyakulasuriya, Elizabeth W. Eisenhauer, Benjamin A. Shaby, and Ephraim M. Hanks. "Machine learning for modeling animal movement". In: *PLOS ONE* 1S. (2020), pp. 1–30

127 Javad Amirian, Jean-Bernard Hayet, and Julien Pettre. "Social Ways: Learning Multi-Modal Distributions of Pedestrian Trajectories with GANs". In: *arXiv:I9O4. O95O7 [cs]* (2019).

128 Marloes Arts, Ihor Smal, Maarten W. Paul, Claire Wyman, and Erik Meijering. "Particle Mobility Analysis Using Deep Learning and the Moment Scaling Spectrum". In: *Scientific Reports* 9.1 (2019), p. 17160.

129 Danilo Bzdok, Naomi Altman, and Martin Krzywinski. "Statistics versus machine learning". In: *Nature Methods* 15.4 (2018), pp. 233– 234.

130 Ahmed M. Alaa and Mihaela van der Schaar. "Forecasting Individualized Disease Trajectories using Interpretable Deep Learning". In: *arXiv:I8IO.IO489 [cs, stat]* (2018).

131 Tien T. Tang, Janice A. Zawaski, Kathleen N. Francis, Amina A. Qutub, and M. Waleed Gaber. "Image-based Classification of Tumor Type and Growth Rate using Machine Learning: a preclinical study". In: *Scientijic Reports* 9.1 (2019), p. 12529.

312 Michael B. Schultz, Alice E. Kane, Sarah J. Mitchell, Michael R. MacArthur, Elisa Warner, David S. Vogel, James R. Mitchell, Susan E. Howlett, Michael S. Bonkowski, and David A. Sinclair. "Age and life expectancy clocks based on machine learning analysis of mouse frailty". In: *Nature Communications* 11.1 (2020), p. 4618.

133 Jacopo Acquarelli, Monica Bianchini, and Elena Marchiori. "Discovering Potential Clinical Profiles of Multiple Sclerosis from Clinical and Pathological Free Text Data with Constrained Non-negative Matrix Factorization." In: *Applications of Evolutionary Computation*. Ed. by Giovanni Squillero and Paolo Burelli. Lecture Notes in Computer Science. Cham: Springer International Publishing, 2016, pp. 169–183 .

134 Esra Zihni, Vince Istvan Madai, Michelle Livne, Ivana Galinovic, Ahmed A. Khalil, Jochen B. Fiebach, and Dietmar Frey. "Opening the black box of artificial intelligence for clinical decision support: A study predicting stroke outcome". In: *PLOS ONE* 1S.4 (2020), e0231166.

135 Geert Litjens, Thijs Kooi, Babak Ehteshami Bejnordi, Arnaud Arindra Adiyoso Setio, Francesco Ciompi, Mohsen Ghafoorian, Jeroen A. W. M. van der Laak, Bram van Ginneken, and Clara I. Sánchez. "A Survey on Deep Learning in Medical Image Analysis". In: *CoRR* abs/1 02.05 4 (2017).

136 Athanasios Voulodimos, Nikolaos Doulamis, Anastasios Doulamis, and Eftychios Protopapadakis. "Deep learning for computer vision: A brief review". In: *Computtion al intelligence and neuroscience* 2018 (2018).

137 Daniele Ravì, Charence Wong, Fani Deligianni, Melissa Berthelot, Javier Andreu-Perez, Benny Lo, and Guang-Zhong Yang. "Deep learning for health informatics". In: *IEEE journal of biomedical and health informatics* 21.1 (2016), pp. 4–21.

138 Marcel A. J. van Gerven, Katja Seeliger, Umut Güçlü, and Yağmur Güçlütürk. "Current Advances in Neural Decoding". In: *Explainable AI: Interpreting, Explaining and Visualizing Deep Learning*. Ed. by Wojciech Samek, Grégoire Montavon, Andrea Vedaldi, Lars Kai Hansen, and Klaus-Robert Müller. Lecture Notes in Computer Science. Cham: Springer International Publishing, 2019, pp. 379-394 .

139 Geert Litjens, Thijs Kooi, Babak Ehteshami Bejnordi, Arnaud Arindra Adiyoso Setio, Francesco Ciompi, Mohsen Ghafoorian, Jeroen A. W. M. van der Laak, Bram van Ginneken, and Clara I. Sánchez. "A Survey on Deep Learning in Medical Image Analysis". In: *CoRR* abs/1 02.05 4 (2017).

140 Simon Haykin. *Neural Networks: A Comprehensive Foundation*. 2nd. USA: Prentice Hall PTR, 1998.

141 Ricky T. Q. Chen, Yulia Rubanova, Jesse Bettencourt, and David Duvenaud. "Neural Ordinary Differential Equations". In: *arX- iv:I8O6.O7366 [cs, stat]* (2019) .

142 Hiromu Monai, Xiaowen Wang, Kazuko Yahagi, Nanhong Lou, Humberto Mestre, Qiwu Xu, Yoichiro Abe, Masato Yasui, Youichi Iwai, Maiken Nedergaard, and Hajime Hirase. "Adrenergic receptor antagonism induces neuroprotection and facilitates recovery from acute ischemic stroke". In: *Proceedings of the National Academy of Sciences* 116.22 (2019), pp. 11010–11019.

143 Liang Han, Qiang Cai, Daofeng Tian, Derek K. Kong, Xingchun Gou, Zeming Chen, Stephen M. Strittmatter, Zuoheng Wang, Kevin N. Sheth, and Jiangbing Zhou. "Targeted drug delivery to ischemic stroke via chlorotoxin-anchored, lexiscan-loaded nanoparticles". In: *Nanomedicine: Nanotechnology, Biology and Medicine* 12.7 (2016), pp. 1833–1842.

144 Maximilian Wiesmann, Valerio Zerbi, Diane Jansen, Roy Haast, Dieter Lütjohann, Laus M. Broersen, Arend Heerschap, and Amanda J. Kiliaan. "A Dietary Treatment Improves Cerebral Blood Flow and Brain Connectivity in Aging apoE4 Mice". In: *Neural Plasticity* 2016 (2016), p. 6846 21.

145 Anita Graser, Esteban Zimányi, and Krishna Chaitanya Bommakanti. "From Simple Features to Moving Features and Beyond?" In: (2020).

146 Md Mahbub Alam, Luis Torgo, and Albert Bifet. "A Survey on Spatio-temporal Data Analytics Systems". In: (2021).

147 Maximilian Wiesmann, Nienke M. Timmer, Bastian Zinnhardt, Dirk Reinhard, Sarah Eligehausen, Anja Königs, Hasnae Ben Jeddi, Pieter J. Dederen, Andreas H. Jacobs, and Amanda J. Kiliaan. "Effect of a multinutrient intervention after ischemic stroke in female CS Bl/6 mice". In: *Journal of Neurochemistry* 144.5 (2018), pp. 549– 564.

148 Li Zhang, Timothy Schallert, Zheng Gang Zhang, Quan Jiang, Polly Arniego, Qingjiang Li, Mei Lu, and Michael Chopp. "A test for detecting long-term sensorimotor dysfunction in the mouse after focal cerebral ischemia". In: *Journal of Neuroscience Methods* 11 .2 (2002), pp. 207–214.

149 Jiukuan Hao, Alexander Mdzinarishvili, Thomas J. Abbruscato, Jochen Klein, Werner J. Geldenhuys, Cornelis J. Van der Schyf, and Ulrich Bickel. "Neuroprotection in mice by NGP1-01 after transient focal brain ischemia". In: *Brain Research* 1196 (2008), pp. 113–120 .

150 Sin-Young Park, Subash Marasini, Geu-Hee Kim, Taeyun Ku, Chulhee Choi, Min-Young Park, Eun-Hee Kim, Young-Don Lee, Haeyoung Suh-Kim, and Sung-Soo Kim. "A Method for Generate a Mouse Model of Stroke: Evaluation of Parameters for Blood Flow, Behavior, and Survival". In: *Experimental Neurobiology* 23.1 (2014), pp. 104–114.

151 Yu Zheng. "Trajectory Data Mining: An Overview". In: *ACM Transaction on Intelligent Systems and Technology* (2015).

152 A. J. Spink, R. A. J. Tegelenbosch, M. O. S. Buma, and L. P. J. J. Noldus. "The EthoVision video tracking system—A tool for behavioral phenotyping of transgenic mice". In: *Physiology & Behavior* 73.5 (2001), pp. 31– 44.

153 Alexander Mathis, Pranav Mamidanna, Kevin M. Cury, Taiga Abe, Venkatesh N. Murthy, Mackenzie W. Mathis, and Matthias Bethge. "DeepLabCut: markerless pose estimation of user-defined body parts with deep learning". In: *Nature Neuroscience* (2018).

154 Alexander I. Hsu and Eric A. Yttri. "B-SOiD: An Open Source Un- supervised Algorithm for Discovery of Spontaneous Behaviors". In: *bioRxiv* (2020).

155 sgoldenlab, Jia Jie Choong, Simon Nilsson, Aasiya Islam, and sophihwang26. *sgoldenlab/simba: SimBA: release vI.3*. Version v1.3. 2021.

156 Junwei Liang, Lu Jiang, Juan Carlos Niebles, Alexander Hauptmann, and Li Fei-Fei. "Peeking into the Future: Predicting Future Person Activities and Locations in Videos". In: *arXiv:I9O2.O3748 [cs]* (2019).

157 Rohan Chandra, Uttaran Bhattacharya, Aniket Bera, and Dinesh Manocha. "TraPHic: Trajectory Prediction in Dense and Heterogeneous Traffic Using Weighted Interactions". In: *arXiv:I8I2.O4767 [cs]* (2019).

158 Wes McKinney. "Data Structures for Statistical Computing in Python." In: *Proceedings of the 9th Python in Science Conference*. Ed. by Stéfan van der Walt and Jarrod Millman. 2010, pp. 56 -61.

159 The pandas development team. *pandas-dev/pandas: Pandas*. Version latest. 2020.

160 C. Calenge. "The package adehabitat for the R software: tool for the analysis of space and habitat use by animals". In: *Ecological Modelling* 19 (2006), p. 1035.

161 Sean Gillies et al. *Shapely: manipulation and analysis of geometric objects*. toblerity.org, 2007.

162 Anita Graser. "MovingPandas: Efficient Structures for Movement Data in Python". In: *GI_Forum 2OI9,* Volume , (2019), pp. 54–68.

163 Vivek Hari Sridhar. *vivekhsridhar/tracktor: Tracktor*. Version tracktor. 201.

164 J. D. Hunter. "Matplotlib: A 2D graphics environment". In: *Computing in Science & Engineering* 9.3 (2007), pp. 90–95.

165 Michael L. Waskom. "seaborn: statistical data visualization". In: *Journal of Open Source Software* 6.60 (2021), p. 3021.

166 Charles R. Harris, K. Jarrod Millman, St'efan J. van der Walt, Ralf Gommers, Pauli Virtanen, David Cournapeau, Eric Wieser, Julian Taylor, Sebastian Berg, Nathaniel J. Smith, Robert Kern, Matti Picus, Stephan Hoyer, Marten H. van Kerkwijk, Matthew Brett, Allan Haldane, Jaime Fern'andez del Rio, Mark Wiebe, Pearu Peterson, Pierre G'erard-Marchant, Kevin Sheppard, Tyler Reddy, Warren Weckesser, Hameer Abbasi, Christoph Gohlke, and Travis E. Oliphant. "Array programming with NumPy". In: *Nature* 585. 825 (2020), pp. 357 – 362

167 Ronaldo G. Morato, Jeffrey J. Thompson, Agustin Paviolo, Jesus A. de La Torre, Fernando Lima, Roy T. McBride, Rogerio C. Paula, Laury Cullen, Leandro Silveira, Daniel L. Z. Kantek, Emiliano E. Ramalho, Louise Maranhão, Mario Haberfeld, Denis A. Sana, Rodrigo A. Medellin, Eduardo Carrillo, Victor Montalvo, Octavio Monroy- Vilchis, Paula Cruz, Anah T. Jacomo, Natalia M. Torres, Giselle B. Alves, Ivonne Cassaigne, Ron Thompson, Carolina Saens-Bolanos, Juan Carlos Cruz, Luiz D. Alfaro, Isabel Hagnauer, Xavier Marina da Silva, Alexandre Vogliotti, Marcela F. D. Moraes, Selma S. Miyazaki, Thadeu D. C. Pereira, Gediendson R. Araujo, Leanes Cruz da Silva, Lucas Leuzinger, Marina M. Carvalho, Lilian Rampin, Leonardo Sartorello, Howard Quigley, Fernando Tortato, Rafael Hoogesteijn, Peter G. Crawshaw, Allison L. Devlin, Joares A. May, Fernando C. C. de Azevedo, Henrique V. B. Concone, Veronica A. Quiroga, Sebastian A. Costa, Juan P. Arrabal, Ezequiel Vanderhoeven, Yamil E. Di Blanco, Alexandre M. C. Lopes, Cynthia E. Widmer, and Milton Cezar Ribeiro. "Jaguar movement database: a GPS-based movement dataset of an apex predator in the Neotropics". In: *Ecology* 99. (2018), pp. 1691–1691.

168 Jan Bosch, Helena Holmström Olsson, and Ivica Crnkovic. *Engineering AI Systems: A Research Agenda*. Chap. 2021.

169 P. M. Kareiva and N. Shigesada. "Analyzing insect movement as a correlated random walk". In: *Oecologia* 56.2-3 (1983), pp. 234–238.

170 Joel Grus. *Data Science from Scratch: First Principles with Python*. Beijing: O'Reilly, 2015.

171 Xintong Wang. "Modeling Trajectory as Image: Convolutional Neural Networks for Multi-scale Taxi Trajectory Prediction". In: (2017),

172 Hoyoung Jeung, Heng Tao Shen, and Xiaofang Zhou. "Mining Trajectory Patterns Using Hidden Markov Models". In: *Data Warehousing and Knowledge Discovery*. Ed. by Il Yeal Song, Johann Eder, and Tho Manh Nguyen. Vol. 4654. Berlin, Heidelberg: Springer Berlin Heidelberg, 200 , pp. 470–480.

173 Abraham. Savitzky and M. J. E. Golay. "Smoothing and Differentiation of Data by Simplified Least Squares Procedures." In: *Analytical Chemistry* 36.8 (1964), pp. 162 –1639 .

174 J. Marcus Rowcliffe, Chris Carbone, Roland Kays, Bart Kranstauber, and Patrick A. Jansen. "Bias in estimating animal travel distance: the effect of sampling frequency". In: *Methods in Ecology and Evolution* 3.4 (2012), pp. 653–662.

175 Shane R. DE Solla, Russell Bonduriansky, and Ronald J. Brooks. "Eliminating autocorrelation reduces biological relevance of home range estimates". In: *Journal of Animal Ecology* 68.2 (1999), pp. 221– 234.

176 Michael J. Noonan, Christen H. Fleming, Thomas S. Akre, Jonathan Drescher-Lehman, Eliezer Gurarie, Autumn-Lynn Harrison, Roland Kays, and Justin M. Calabrese. "Scale-insensitive estimation of speed and distance traveled from animal tracking data". In: *Movement Ecology* .1 (2019), p. 35.

177 Gerard P. Van Galen, Robert R. Van Doorn, and Lambert R. Schomaker. "Effects of motor programming on the power spectral density function of finger and wrist movements". In: *Journal of Experimental Psychology: Human Perception and Performance* 16.4 (1990), pp. 55– 65.

178 Kang Huang, Yaning Han, Ke Chen, Hongli Pan, Wenling Yi, Xiaoxi Li, Siyuan Liu, Pengfei Wei, and Liping Wang. "Mapping Mouse Behavior with an Unsupervised Spatio-temporal Sequence Decomposition Framework". In: *bioRxiv* (2020) .

179 Peter Bak, Florian Mansmann, Halldor Janetzko, and Daniel Keim. "Spatiotemporal Analysis of Sensor Logs using Growth Ring Maps". In: *IEEE transactions on visualization and computer graphics* 17 (2009), pp. 913–207.

180 CE. Rasmussen and CKI. Williams. *Gaussian Processes for Machine Learning*. Adaptive Computation and Machine Learning. Cambridge, MA, USA: Biologische Kybernetik, 2006.

181 Gregory E Cox, George Kachergis, and Richard M Shiffrin. "Gaussian Process Regression for Trajectory Analysis." In: 2012, p. 67.

182 Bryan E. Kerster, Theo Rhodes, and Christopher T. Kello. "Spatial memory in foraging games". In: *Cognition* 148 (2016), pp. 85–96.

183 Stephanie Huette, Christopher Kello, Theo Rhodes, and Michael Spivey. "Drawing from Memory: Hand-Eye Coordination at Multiple Scales". In: *PloS one* 8 (2013), e58464.

184 Jessica M. Ross, Anne S. Warlaumont, Drew H. Abney, Lillian
M. Rigoli, and Ramesh Balasubramaniam. "Influence of musical groove on postural sway". In: *Journal of Experimental Psychology: Human Perception and Performance* 42.3 (2016), pp. 308–319.

185 Kevin Shockley, Marie-Vee Santana, and Carol Fowler. "Mutual Interpersonal Postural Constraints are Involved in Cooperative Conversation". In: *Journal of experimental psychology. Humanperception and performance* 29 (2003), pp. 326–32.

186 Felipe Marcel Neves, Ricardo Luiz Viana, and Marcio Roberto Pie. "Recurrence analysis of ant activity patterns". In: *PLOS ONE* 12.10 (2017), pp. 1–15.

187 Carolyn Ayers, Paul Armsworth, and Berry Brosi. "Determinism as a statistical metric for ecologically important recurrent behaviors with trapline foraging as a case study". In: *Behavioral Ecology and Sociobiology* 69 (2015).

188 Toby A. Patterson, Alison Parton, Roland Langrock, Paul G. Blackwell, Len Thomas, and Ruth King. “Statistical modelling of individ- ual animal movement: an overview of key methods and a discussion of practical challenges”. In: *AStA Advances in Statistical Analysis* 101.4 (201), pp. 399–438.

189 Alastair Franke, Terry Caelli, and Robert J Hudson. “Analysis of movements and behavior of caribou (Rangifer tarandus) using hidden Markov models”. In: *Ecological Modelling* 1 3.2 (2004), pp. 259– 270.

190 Hajo Holzmann, Axel Munk, Max Suster, and Walter Zucchini. “Hidden Markov models for circular and linear-circular time series”. In: *Environmental and Ecological Statistics* 13.3 (2006), pp. 325–347.

191 Toby A. Patterson, J. Paige Eveson, Jason R. Hartog, Karen Evans, Scott Cooper, Matt Lansdell, Alistair J. Hobday, and Campbell R. Davies. “Migration dynamics of juvenile southern bluefin tuna”. In: *Scientijic Reports* 8.1 (2018), pp. 1–10.

192 Adam Paszke, Sam Gross, Francisco Massa, Adam Lerer, James Bradbury, Gregory Chanan, Trevor Killeen, Zeming Lin, Natalia Gimelshein, Luca Antiga, Alban Desmaison, Andreas Kopf, Edward Yang, Zachary DeVito, Martin Raison, Alykhan Tejani, Sasank Chilamkurthy, Benoit Steiner, Lu Fang, Junjie Bai, and Soumith Chintala. “PyTorch: An Imperative Style, High-Performance Deep Learning Library”. In: *Advances in Neural Information Processing Systems 32*. Ed. by H. Wallach, H. Larochelle, A. Beygelzimer, F. d’Alché-Buc, E. Fox, and R. Garnett. Curran Associates, Inc., 2019, pp. 8024–8035.

193 Peter J. Huber. “Robust Estimation of a Location Parameter”. In: *Annals of Mathematical Statistics* 37.1 (1964), pp. 73–101

194 A. Arpteg, B. Brinne, Luka Crnkovic-Friis, and J. Bosch. “Software Engineering Challenges of Deep Learning”. In: *2OI8 44th Euromicro Conference on Software Engineering and Advanced Applications (SEAA)* (2018), pp. 50–59.

195 Tomas Mikolov, Ilya Sutskever, Kai Chen, Greg Corrado, and Jeffrey Dean. *Distributed Representations of Words and Phrases and their Compositionality*. 2013.

196 Grégory Barrière, Hugues Leblond, Janyne Provencher, and Serge Rossignol. “Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries”. In: *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 28.15 (2008), pp. 3976–3987.

197 N. J. Tester, D. R. Howland, K. V. Day, S. P. Suter, A. Cantrell, and A. L. Behrman. “Device use, locomotor training and the presence of arm swing during treadmill walking after spinal cord injury”. In: *Spinal Cord* 49.3 (2011), pp. 451–456.

198 Nicole J. Tester, Hugues Barbeau, Dena R. Howland, Amy Cantrell, and Andrea L. Behrman. “Arm and leg coordination during treadmill walking in individuals with motor incomplete spinal cord injury: a preliminary study”. In: *Gait & Posture* 36.1 (2012), pp. 49–55.

199 M. E. Morris, R. Iansek, T. A. Matyas, and J. J. Summers. “Stride length regulation in Parkinson’s disease. Normalization strategies and underlying mechanisms”. In: *Brain: A Journal of Neurology* 119 (Pt 2) (1996), pp. 551–568.

200 M. E. Morris, F. E. Huxham, J. McGinley, and R. Iansek. “Gait disorders and gait rehabilitation in Parkinson’s disease”. In: *Advances in Neurology* 8 (2001), pp. 347–361

201 Ivo Amende, Ajit Kale, Scott McCue, Scott Glazier, James P. Mor- gan, and Thomas G. Hampton. “Gait dynamics in mouse models of Parkinson’s disease and Huntington’s disease”. In: *Journal of Neuro- engineering and Rehabilitation* 2 (2005), p. 207.

202 Thomas G. Hampton, Melissa R. Stasko, Ajit Kale, Ivo Amende, and Alberto C. S. Costa. “Gait dynamics in trisomic mice: quantitative neurological traits of Down syndrome”. In: *Physiology & Behavior* 82.2-3 (2004), pp. 381–389.

203 Yann Herault, Jean M Delabar, Elizabeth MC Fisher, Victor LJ Tybulewicz, Eugene Yu, and Veronique Brault. “Rodent models in Down syndrome research: impact and future opportunities”. In: *Disease models & mechanisms* 10.10 (201), pp. 1165–1186.

204 Willias Masocha and Subramanian S. Parvathy. “Assessment of weight bearing changes and pharmacological antinociception in mice with LPS-induced monoarthritis using the Catwalk gait analysis system”. In: *Life Sciences* 8S.11-12 (2009), pp. 462–469.

205 G. W. M. Bothe, V. J. Bolivar, M. J. Vedder, and J. G. Geistfeld. “Genetic and behavioral differences among five inbred mouse strains commonly used in the production of transgenic and knockout mice”. In: *Genes, Brain and Behavior* 3.3 (2004), pp. 149–175

206 Steven A. Crone, Guisheng Zhong, Ronald Harris-Warrick, and Kamal Sharma. “In Mice Lacking V2a Interneurons, Gait Depends on Speed of Locomotion”. In: *Journal of Neuroscience* 29.21 (2009), pp. 098– 109.

207 Angelo Encarnacion, Nobutaka Horie, Hadar Keren-Gill, Tonya M. Bliss, Gary K. Steinberg, and Mehrdad Shamloo. “Long-term behavioral assessment of function in an experimental model for ischemic stroke”. In: *Journal of Neuroscience Methods* 196.2 (2011), pp.724 –725.

208 Susann Hetze, Christine Römer, Carena Teufelhart, Andreas Meisel, and Odilo Engel. “Gait analysis as a method for assessing neurological outcome in a mouse model of stroke”. In: *Journal of Neuroscience Methods* 206.1 (2012), pp. –14.

209 Fabrice de Chaumont, Elodie Ey, Nicolas Torquet, Thibault Lagache, Stéphane Dallongeville, Albane Imbert, Thierry Legou, Anne-Marie Le Sourd, Philippe Faure, Thomas Bourgeron, and Jean-Christophe Olivo-Marin. "Live Mouse Tracker: real-time behavioral analysis of groups of mice". In: *bioRxiv* (2018), p. 345132.

210 Yoav Benjamini, Dina Lipkind, Guy Horev, Ehud Fonio, Neri Kafkafi, and Ilan Golani. "Ten ways to improve the quality of descriptions of whole-animal movement". In: *Neuroscience & Biobehavioral Reviews* 34.8 (2010), pp. 1351–1365.

211 Mustafa Balkaya, Jan M. Kröber, Andre Rex, and Matthias Endres. "Assessing post-stroke behavior in mouse models of focal ischemia". In: *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 33.3 (2013), pp. 330–338.

212 G. Casadesus, B. Shukitt-Hale, and J. A. Joseph. "Automated measurement of age-related changes in the locomotor response to environmental novelty and home-cage activity". In: *Mechanisms of Ageing and Development* 122.15 (2001), pp. 188 –189.

213 Jeremy D. Bailoo, Martin O. Bohlen, and Douglas Wahlsten. "The precision of video and photocell tracking systems and the elimination of tracking errors with infrared backlighting". In: *Journal of Neuroscience Methods* 188.1 (2010), pp. 45–52 ..

214 Fergal Dunne, Ambrose O'Halloran, and John P. Kelly. "Development of a home cage locomotor tracking system capable of detecting the stimulant and sedative properties of drugs in rats". In: *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 31. (200), pp. 1456–1463.

215 L. P. Noldus, A. J. Spink, and R. A. Tegelenbosch. "EthoVision: a versatile video tracking system for automation of behavioral experiments". In: *Behavior research methods, instruments, & computers : a journal of the Psychonomic Society, Inc* 33.3 (2001), pp. 398–414.

216 Sheldon L Reeves, Kelsey E Fleming, Lin Zhang, and Annalisa Scimemi. "M-Track: a new software for automated detection of grooming trajectories in mice". In: *PLoS computational biology* 12.9 (2016).

217 A. J. Spink, R. A. J. Tegelenbosch, M. O. S. Buma, and L. P. J. J. Noldus. "The EthoVision video tracking system—A tool for behavioral phenotyping of transgenic mice". In: *Physiology & Behavior* 73.5 (2001), pp. 31– 44.

218 Surjeet Singh, Edgar Bermudez-Contreras, Mojtaba Nazari, Robert J. Sutherland, and Majid H. Mohajerani. "Low-cost solution for rodent home-cage behaviour monitoring". In: *PLOS ONE* 14.8 (2019), e0220 51.

219 Donald James McLean and Marta A. Skowron Volponi. “trajr: An R package for characterisation of animal trajectories”. In: *Ethology* 124.6 (2018), pp. 440–448.

220 Rohan Chandra, Uttaran Bhattacharya, Aniket Bera, and Dinesh Manocha. “TraPHic: Trajectory Prediction in Dense and Hetero- geneous Traffic Using Weighted Interactions”. In: *arXiv:I8I2.O4767 [cs]* (2018).

221 Fabio Iannello. “Non-intrusive high throughput automated data collection from the home cage”. In: *Heliyon* 5.4 (2019), e01454.

222 Karin Pernold, F. Iannello, B. E. Low, M. Rigamonti, G. Rosati, F. Scavizzi, J. Wang, M. Raspa, M. V. Wiles, and B. Ulfhake. “Towards large scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of CS BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique”. In: *PLoS ONE* 14.2 (2019).

223 Jesús Calahorra, Justin Shenk, Vera H. Wielenga, Vivienne Verweij, Bram Geenen, Pieter J. Dederen, M. Ángeles Peinado, Eva Siles, Max- imilian Wiesmann, and Amanda J. Kiliaan. “Hydroxytyrosol, the Major Phenolic Compound of Olive Oil, as an Acute Therapeutic Strategy after Ischemic Stroke”. In: *Nutrients* 11.10 (2019), p. 2430 .

224 Hannah J. Williams, Mark D. Holton, Emily L. C. Shepard, Nicola Largey, Brad Norman, Peter G. Ryan, Olivier Duriez, Michael Scantlebury, Flavio Quintana, Elizabeth A. Magowan, Nikki J. Marks, Abdulaziz N. Alagaili, Nigel C. Bennett, and Rory P. Wilson. “Identification of animal movement patterns using tri-axial magnetometry”. In: *Movement Ecology* 5 (2017).

225 Odilo Engel, Sabine Kolodziej, Ulrich Dirnagl, and Vincent Prinz. “Modeling stroke in mice middle cerebral artery occlusion with the filament model”. In: *Journal of Visualized Experiments: JoVE* 4 (2011).

226 Maximilian Wiesmann, Bastian Zinnhardt, Dirk Reinhardt, Sarah Eligehausen, Lydia Wachsmuth, Sven Hermann, Pieter J. Dederen, Marloes Hellwich, Michael T. Kuhlmann, Laus M. Broersen, Arend Heerschap, Andreas H. Jacobs, and Amanda J. Kiliaan. “A specific dietary intervention to restore brain structure and function after ischemic stroke”. In: *Theranostics* .2 (201), pp. 493–512.

227 Marc Fisher, Giora Feuerstein, David W. Howells, Patricia D. Hurn, Thomas A. Kent, Sean I. Savitz, and Eng H. Lo. “Update of the Stroke Therapy Academic Industry Roundtable Preclinical Recommendations”. In: *Stroke; a journal of cerebral circulation* 40.6 (2009), pp. 2244–2250.

228 Carol Kilkenny, William J. Browne, Innes C. Cuthill, Michael Emerson, and Douglas G. Altman. “Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research”. In: *PLOS Biology* 8.6 (2010), e1000412.

229 Raquel da Silva Aragão, Marco Aurélio Benedetti Rodrigues, Karla Mônica Ferraz Teixeira de Barros, Sebastião Rogério Freitas Silva, Ana Elisa Toscano, Ricardo Emmanuel de Souza, and Raul Manhães- de Castro. "Automatic system for analysis of locomotor activity in rodents—A reproducibility study". In: *Journal of Neuroscience Methods* 195.2 (2011), pp. 216–221.

230 Melanie Nichols, Nick Townsend, Ramon Luengo-Fernandez, Jose Leal, Alastair Gray, Pete Scarborough, and Mike Rayner. "European cardiovascular disease statistics 2012". In: (2012).

231 Fabio Di Domenico, Gabriella Casalena, Jia Jia, Rukhsana Sultana, Eugenio Barone, Jian Cai, William M Pierce, Chiara Cini, Cesare Mancuso, and Marzia Perluigi. "Sex differences in brain proteomes of neuron-specific STAT3-null mice after cerebral ischemia/reperfusion". In: *Journal of neurochemistry* 121.4 (2012), pp. 680–692 .

232 H Jokinen, S Melkas, R Ylikoski, T Pohjasvaara, M Kaste, T Erkinjuntti, and M Hietanen. "Post-stroke cognitive impairment is com- mon even after successful clinical recovery". In: *European Journal of Neurology* 22.9 (201S), pp. 1288–1294.

233 E Auriel and NM Bornstein. "Neuroprotection in acute ischemic stroke–current status". In: *Journal of cellular and molecular medicine* 14.9 (2010), pp. 2200–2202 .

234 Valerio Zerbi, Diane Jansen, Maximilian Wiesmann, Xiaotian Fang, Laus M Broersen, Andor Veltien, Arend Heerschap, and Amanda J Kiliaan. "Multinutrient diets improve cerebral perfusion and neuroprotection in a murine model of Alzheimer's disease". In: *Neurobiology of aging* 35.3 (2014), pp. 600–613.

235 Bruce H Dobkin and Andrew Dorsch. "New evidence for therapies in stroke rehabilitation". In: *Currentatherosclerosis reports* 15.6 (2013), p. 331.

236 María Irene Ayuso, Rafael Gonzalo-Gobernado, and Joan Montaner. "Neuroprotective diets for stroke". In: *Neurochemistry international* 10 (201), pp. 4–10.

237 Maximilian Wiesmann, Nienke M Timmer, Bastian Zinnhardt, Dirk Reinhard, Sarah Eligehausen, Anja Königs, Hasnae Ben Jeddi, Pieter J Dederen, Andreas H Jacobs, and Amanda J Kiliaan. "Effect of a multinutrient intervention after ischemic stroke in female CS Bl/6 mice". In: *Journal of neurochemistry* 144.5 (2018), pp. 549–564.

238 Maximilian Wiesmann, Bastian Zinnhardt, Dirk Reinhardt, Sarah Eligehausen, Lydia Wachsmuth, Sven Hermann, Pieter J Dederen, Marloes Hellwich, Michael T Kuhlmann, and Laus M Broersen. "A specific dietary intervention to restore brain structure and function after ischemic stroke". In: *Theranostics* .2 (201), p. 493 .

239 Miguel A Martínez-González, Ligia J Dominguez, and Miguel Delgado-Rodríguez. "Olive oil consumption and risk of CHD and/or stroke: a meta-analysis of case–control, cohort and intervention studies". In: *British Journal of Nutrition* 112.2 (2014), pp. 248– 259.

240 Ramón Estruch, Emilio Ros, Jordi Salas-Salvadó, Maria-Isabel Covas, Dolores Corella, Fernando Arós, Enrique Gómez-Gracia, Valentina Ruiz-Gutiérrez, Miquel Fiol, and José Lapetra. "Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts". In: *New England Journal of Medicine* (2018).

241 Fatemeh Mohagheghi, Mohammad Reza Bigdeli, Bahram Rasoulian, Ali Asghar Zeinanloo, and Ali Khoshbaten. "Dietary virgin olive oil reduces blood brain barrier permeability, brain edema, and brain injury in rats subjected to ischemia-reperfusion". In: *The Scientijic World Journal* 10 (2010), pp. 1180–1191.

242 Zahra Rabiei, Mohammad R Bigdeli, and Bahram Rasoulian. "Neuroprotection of dietary virgin olive oil on brain lipidomics during stroke". In: *Current neurovascular research* 10.3 (2013), pp. 231–237.

243 Natalia Lausada, Nathalie Arnal, Mariana Astiz, María Cristina Marín, Juan Manuel Lofeudo, Pablo Stringa, María J Tacconi de Alaniz, Nelva Tacconi de Gómez Dumm, Graciela Hurtado de Catalfo, and Norma Cristalli de Piñero. "Dietary fats significantly in- fluence the survival of penumbral neurons in a rat model of chronic ischemic by modifying lipid mediators, inflammatory biomarkers, NOS production, and redox-dependent apoptotic signals". In: *Nu- trition* 31.11-12 (2015), pp. 1430–1442.

244 Sergio Granados-Principal, José L Quiles, Cesar L Ramirez-Tortosa, Pedro Sanchez-Rovira, and M Carmen Ramirez-Tortosa. "Hydroxy- tyrosol: from laboratory investigations to future clinical trials". In: *Nutrition reviews* 68.4 (2010), pp. 191–206 .

245 Karina De la Torre-Carbot, Olga Jauregui, Eva Gimeno, Ana I Castel- lote, Rosa M Lamuela-Raventós, and M Carmen López-Sabater. "Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS". In: *Journal of agricultural and food chemistry* 53.11 (2005), pp. 4331– 4340.

246 María Robles-Almazan, Mario Pulido-Moran, Jorge Moreno- Fernandez, Cesar Ramirez-Tortosa, Carmen Rodriguez-Garcia, Jose L Quiles, and MCarmen Ramirez-Tortosa. "Hydroxytyrosol: Bioavailability, toxicity, and clinical applications". In: *Food Research International* (2017).

247 José Antonio González-Correa, María Dolores Navas, Juan An- tonio Lopez-Villodres, Mariana Trujillo, José Luis Espartero, and José Pedro De La Cruz. "Neuroprotective effect of hydroxyty- rosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia–reoxygenation". In: *Neuroscience letters* 446.2-3 (2008), pp. 143–146.

248 Susana Cabrerizo, José Pedro De La Cruz, Juan Antonio López- Villodres, Javier Muñoz-Marín, Ana Guerrero, José Julio Reyes, María Teresa Labajos, and José Antonio González-Correa. "Role of the inhibition of oxidative stress and inflammatory mediators in the neuroprotective effects of hydroxytyrosol in rat brain slices subjected to hypoxia reoxygenation". In: *The Journal of nutritional biochemistry* 24.12 (2013), pp. 2152–2157.

249 JP De La Cruz, MI Ruiz-Moreno, A Guerrero, JA López-Villodres, JJ Reyes, JL Espartero, MT Labajos, and JA González-Correa. "Role of the catechol group in the antioxidant and neuroprotective effects of virgin olive oil components in rat brain". In: *The Journal of nutri- tional biochemistry* 26.5 (201S), pp. 549–555.

250 Jose Pedro De La Cruz, Maria Isabel Ruiz-Moreno, Ana Guerrero, Jose Julio Reyes, Adela Benitez-Guerrero, Jose Luis Espartero, and Jose Antonio Gonzalez-Correa. "Differences in the neuroprotective effect of orally administered virgin olive oil (Olea europaea) polyphenols tyrosol and hydroxytyrosol in rats". In: *Journal of agricultural and food chemistry* 63.25 (2015), pp. 5957 –5963.

251 Jose Julio Reyes, Beatriz Villanueva, Juan Antonio Lopez-Villodres, Jose Pedro De La Cruz, Lidia Romero, Maria Dolores Rodriguez- Perez, Guillermo Rodriguez-Gutierrez, Juan Fernandez-Bolanos, and Jose Antonio Gonzalez-Correa. "Neuroprotective effect of hydroxy- tyrosol in experimental diabetes mellitus". In: *Journal of agricultural and food chemistry* 65.22 (2017), pp. 4378–4383 .

252 Jareca M. Giles, Julia W. Whitaker, Sheryl S. Moy, and Craig A. Fletcher. "Effect of Environmental Enrichment on Aggression in BALB/cJ and BALB/cByJ Mice Monitored by Using an Automated System". In: *Journal of the American Association for Laboratory Animal Science* 5 .3 (2018), pp. 236–243.

253 Karin Pernold, F. Iannello, B. E. Low, M. Rigamonti, G. Rosati, F. Scavizzi, J. Wang, M. Raspa, M. V. Wiles, and B. Ulfhake. "Towards large scale automated cage monitoring - Diurnal rhythm and impact of interventions on in-cage activity of CS BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique". In: *PloS one* 14.2 (2019), e0211063–e0211063.

254 João Tomé-Carneiro, M Carmen Crespo, Estefanía García-Calvo, José L Luque-García, Alberto Dávalos, and Francesco Visioli. "Proteomic evaluation of mouse adipose tissue and liver following hydroxytyrosol supplementation". In: *Food and Chemical Toxicology* 10 (201), pp. 329–338.

255 E Giordano, A Dávalos, and F Visioli. "Chronic hydroxytyrosol feeding modulates glutathione-mediated oxido-reduction pathways in adipose tissue: A nutrigenomic study". In: *Nutrition, Metabolism and Cardiovascular Diseases* 24.10 (2014), pp. 1144–1150.

256 Joao Tomé-Carneiro, María Carmen Crespo, Eduardo Iglesias- Gutierrez, Roberto Martín, Judit Gil-Zamorano, Cristina Tomas- Zapico, Emma Burgos-Ramos, Carlos Correa, Diego Gómez-Coronado, and Miguel A Lasunción. "Hydroxytyrosol supplementation modulates the expression of miRNAs in rodents and in humans". In: *The Journalofnutritionalbiochemistry* 34 (2016), pp. 146– 155.

257 Femke Streijger, Frank Oerlemans, Bart A Ellenbroek, Carolina R Jost, Bé Wieringa, and Catharina EEM Van der Zee. "Structural and behavioural consequences of double deficiency for creatine kinases BCK and UbCKmit". In: *Behavioural brain research* 15 .2 (2005), pp. 219–234.

258 Carola IF Janssen, Valerio Zerbi, Martina PC Mutsaers, Bas SW de Jong, Maximilian Wiesmann, Ilse AC Arnoldussen, Bram Geenen, Arend Heerschap, Frits AJ Muskiet, and Zeina E Jouni. "Impact of dietary n-3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing CS BL/6J mice". In: *The Journal of nutritional biochemistry* 26.1 (2015), pp. 24–35.

259 M Antunes and Grazyna Biala. "The novel object recognition memory: neurobiology, test procedure, and its modifications". In: *Cognitive processing* 13.2 (2012), pp. 93–110.

260 George Paxinos and Keith BJ Franklin. *The mouse brain in stereo- taxic coordinates*. Gulf professional publishing, 2004.

261 L. A. Harsan, D. Paul, S. Schnell, B. W. Kreher, J. Hennig, J. F. Staiger, and D. von Elverfeldt. "In vivo diffusion tensor magnetic resonance imaging and fiber tracking of the mouse brain". In: *NMR in biomedicine* 23. (2010), pp. 884–96.

262 Diane Jansen, Valerio Zerbi, Ilse A. C. Arnoldussen, Maximilian Wiesmann, Anne Rijpma, Xiaotian T. Fang, Pieter J. Dederen, Martina P. C. Mutsaers, Laus M. Broersen, Dieter Lütjohann, Malgorzata Miller, Leo A. B. Joosten, Arend Heerschap, and Amanda J. Kili- aan. "Effects of Specific Multi-Nutrient Enriched Diets on Cerebral Metabolism, Cognition and Neuropathology in AβPPswe-P51dE9 Mice". In: *PLoS ONE* 8.9 (2013), e 5393.

263 Diane Jansen, Valerio Zerbi, CarolaI F. Janssen, Daan van Rooij, Bastian Zinnhardt, PieterJ Dederen, AlanJ Wright, LausM Broersen, Dieter Lütjohann, Arend Heerschap, and AmandaJ Kiliaan. "Impact of a multi-nutrient diet on cognition, brain metabolism, hemodynamics, and plasticity in apoE4 carrier and apoE knockout mice". In: *Brain Structure and Function* 219.5 (2014), pp. 1841–1868.

264 M. P. Zwiers. "Patching cardiac and head motion artefacts in diffusion-weighted images". In: *Neuroimage* 53.2 (2010), pp. 565– 577.

265 George Paxinos and Keith BJ Franklin. *The mouse brain in stereo- taxic coordinates / George Paxinos, Keith Franklin*. London : Academic, 2004.

266 Stephen Sawiak, N. Wood, G. Williams, A. Morton, and T. Carpenter. "SPMMouse: a new toolbox for SPM in the animal brain". In: *ISMRM 17th Scientijic Meeting & Exhibition* (2009), pp. 18–24.

267 Elisabeth Jonckers, Johan Van Audekerke, Geofrey De Visscher, Annemie Van der Linden, and Marleen Verhoye. "Functional connectivity fMRI of the rodent brain: comparison of functional connectivity networks in rat and mouse". In: *PloS one* 6.4 (2011), e18876.

268 Carola IF Janssen, Diane Jansen, Martina PC Mutsaers, Pieter JWC Dederen, Bram Geenen, Monique T Mulder, and Amanda J Kiliaan. "The effect of a high-fat diet on brain plasticity, inflammation and cognition in female ApoE4-knockin and ApoE-knockout mice". In: *PLoS One* 11.5 (2016), e015530.

269 JC Lopez-Ramos, R Martinez-Romero, F Molina, A Cañuelo, E Martínez-Lara, E Siles, and MA Peinado. "Evidence of a decrease in nitric oxide-storage molecules following acute hypoxia and/or hypobaria, by means of chemiluminescence analysis". In: *Nitric Oxide* 13.1 (2005), pp. 62–67.

270 MA Peinado, JC Lopez-Ramos, MV Camacho, FJ Molina, R Martínez-Romero, R Hernández, E Siles, E Martínez-Lara, ML Del Moral, and JA Pedrosa. "Age and sex-related serum changes in nitric oxide: correlations with serological markers". In: *International journal of cardiology* 121.1 (2007), pp. 88–90.

271 Robert S Braman and Steven A Hendrix. "Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection". In: *Analytical Chemistry* 61.24 (1989), pp. 2715–2718.

272 John A Buege and Steven D Aust. "[30] Microsomal lipid peroxidation." In: *Methods in enzymology*. Vol. 52. Elsevier, 1978, pp. 302–310.

273 Fatemeh Mohagheghi, Mohammad Reza Bigdeli, Bahram Rasoulian, Payman Hashemi, and Marzyeh Rashidi Pour. "The neuroprotective effect of olive leaf extract is related to improved blood–brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia". In: *Phytomedicine* 18.2-3 (2011), pp. 170–175

274 Hailong Yu, Peipei Liu, Hui Tang, Jian Jing, Xiang Lv, Lanlan Chen, Li Jiang, Jun Xu, and Jun Li. "Oleuropein, a natural extract from plants, offers neuroprotection in focal cerebral ischemia/reperfusion injury in mice". In: *European journal of pharmacology* S (2016), pp. 113–119.

275 Mustafa Balkaya, Jan M Kröber, Andre Rex, and Matthias Endres. "Assessing post-stroke behavior in mouse models of focal ischemia". In: *Journalof Cerebral Blood Flow& Metabolism* 33.3 (2013), pp. 330– 338

276 Pauline Schaapsmeerders, Noortje AM Maaijwee, Ewoud J van Dijk, Loes CA Rutten-Jacobs, Renate M Arntz, Hennie C Schoonderwaldt, Lucille DA Dorresteijn, Roy PC Kessels, and Frank-Erik de Leeuw. "Long-term cognitive impairment after first-ever ischemic stroke in young adults". In: *Stroke* 44.6 (2013), pp. 1621–1628.

277 Sin-Young Park, Subash Marasini, Geu-Hee Kim, Taeyun Ku, Chulhee Choi, Min-Young Park, Eun-Hee Kim, Young-Don Lee, Haeyoung Suh-Kim, and Sung-Soo Kim. "A method for generating a mouse model of stroke: evaluation of parameters for blood flow, behavior, and survival [corrected]". In: *Experimental neurobiology* 23.1 (2014), pp. 104–114.

278 Hannes Kroll, Greg Zaharchuk, Thomas Christen, Jeremy Heit, and Michael Iv. "Resting State BOLD MRI for Perfusion and Ischemia". In: *Topics in magnetic resonance imaging: TMRI* 26.2 (2017), p. 91.

279 Alex R Carter, Serguei V Astafiev, Catherine E Lang, Lisa T Con- nor, Jennifer Rengachary, Michael J Strube, Daniel LW Pope, Gordon L Shulman, and Maurizio Corbetta. "Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke". In: *Annals of neurology* 6 .3 (2010), pp. 365–375.

280 Anne K Rehme, Gereon R Fink, D Yves von Cramon, and Christian Grefkes. "The role of the contralesional motor cortex for motor recovery in the early days after stroke assessed with longitudinal FMRI". In: *Cerebral cortex* 21.4 (2010), pp. 56– 68.

281 Shigehiko Ogoh. "Relationship between cognitive function and regulation of cerebral blood flow". In: *The Journal of Physiological Sciences* 6 .3 (201), pp. 345–351.

282 Maximilian Wiesmann, Valerio Zerbi, Diane Jansen, Roy Haast, Dieter Lütjohann, Laus M Broersen, Arend Heerschap, and Amanda J Kiliaan. "A dietary treatment improves cerebral blood flow and brain connectivity in aging apoE4 mice". In: *Neural plasticity* 2016 (2016).

283 David O Kennedy, Emma L Wightman, Jonathon L Reay, Georg Li- etz, Edward J Okello, Anthea Wilde, and Crystal F Haskell. "Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation". In: *The American journal of clinical nutrition* 91.6 (2010), pp. 1590–1597.

284 Kwok Tung Lu, Robin YY Chiou, Li Ging Chen, Ming Hsiang Chen, Wan Ting Tseng, Hsiang Tsang Hsieh, and Yi Ling Yang. "Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation". In: *Journal of agricultural and food chemistry* 54.8 (2006), pp. 3126–3131.

285 J Rodrigo, AP Fernandez, J Serrano, MA Peinado, and A Martinez. "The role of free radicals in cerebral hypoxia and ischemia". In: *Free Radical Biology and Medicine* 39.1 (2005), pp. 26–50.

286 E Martinez-Lara, AR Canuelo, E Siles, R Hernandez, ML Del Moral, S Blanco, JA Pedrosa, J Rodrigo, and MA Peinado. "Constitutive nitric oxide synthases are responsible for the nitric oxide production in the ischemic aged cerebral cortex". In: *Brain research* 1054.1 (2005), pp. 88–94.

287 Marta Serrano-Ponz, Carmen Rodrigo-Gasqué, Eva Siles, Esther Martínez-Lara, Laura Ochoa-Callejero, and Alfredo Martínez. "Temporal profiles of blood pressure, circulating nitric oxide, and adrenomedullin as predictors of clinical outcome in acute ischemic stroke patients". In: *Molecularmedicine reports* 13.5 (2016), pp. 3724– 3734.

288 Parveen A Rashid, Angela Whitehurst, Nigel Lawson, and Philip MW Bath. "Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome". In: *Journal of Stroke and Cerebrovascular Diseases* 12.2 (2003), pp. 82–8.

289 Acar Abdullah, Varol Ssefer, Uzar Ertugrul, Evliyaoglu Osman, Akil Esref, Cevik Mehmet Ugur, Arikanoglu Adalet, Yucel Yavuz, Ekici Faysal, and Tasdemir Nebahat. "Evaluation of serum oxidant/antioxidant balance in patients with acute stroke". In: *J Pak Med Assoc* 63.5 (2013), pp. 790–3.

290 Ramón Rodrigo, Rodrigo Fernández-Gajardo, Rodrigo Gutiér- rez, Jose Manuel Matamala, Rodrigo Carrasco, Andrés Miranda- Merchak, and Walter Feuerhake. "Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities". In: *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 12.5 (2013), pp. 698– 714.

291 Francisca Echeverría, Macarena Ortiz, Rodrigo Valenzuela, and Luis Videla. "Hydroxytyrosol and cytoprotection: a projection for clinical interventions". In: *International journal of molecular sciences* 18.5 (2017), p. 930.

292 Rubén Martínez-Romero, Ana Cañuelo, Esther Martínez-Lara, Francisco Javier Oliver, Sara Cárdenas, and Eva Siles. "Poly (ADP- ribose) polymerase-1 modulation of in vivo response of brain hypoxia- inducible factor-1 to hypoxia/reoxygenation is mediated by nitric oxide and factor inhibiting HIF". In: *Journal of neurochemistry* 111.1 (2009), pp. 150–159.

293 Nathalie Richard, Sabine Arnold, Ulrich Hoeller, Claus Kilpert, Karin Wertz, and Joseph Schwager. "Hydroxytyrosol is the major anti-inflammatory compound in aqueous olive extracts and impairs cytokine and chemokine production in macrophages". In: *Planta medica* .1 (2011), pp. 1890–1897.

294 Claudio Pirozzi, Adriano Lama, Raffaele Simeoli, Orlando Paciello, Teresa Bruna Pagano, Maria Pina Mollica, Francesca Di Guida, Roberto Russo, Salvatore Magliocca, and Roberto Berni Canani. "Hydroxytyrosol prevents metabolic impairment reducing hepatic inflammation and restoring duodenal integrity in a rat model of NAFLD". In: *The Journal of nutritional biochemistry* 30 (2016), pp. 108–115.

295 Sergio Lopez, Sergio Montserrat-de la Paz, Ricardo Lucas, Beatriz Bermudez, Rocio Abia, Juan C Morales, and Francisco JG Muriana. "Effect of metabolites of hydroxytyrosol on protection against oxidative stress and inflammation in human endothelial cells". In: *Journal of functional foods* 29 (2017), pp. 238–24.

296 Ruth Hornedo-Ortega, Ana B Cerezo, Rocío M de Pablos, Stéphanie Krisa, Tristan Richard, M Carmen García-Parrilla, and Ana M Troncoso. "Phenolic compounds characteristic of the Mediterranean diet in mitigating microglia-mediated neuroinflammation". In: *Frontiers in cellular neuroscience* 12 (2018).

297 Seong-Ho Koh and Hyun-Hee Park. "Neurogenesis in stroke recovery". In: *Translational stroke research* 8.1 (201), pp. 3–13.

298 Minseok Song, Keri Martinowich, and Francis S Lee. "BDNF at the synapse: why location matters". In: *Molecular psychiatry* 22.10 (2017), p. 1370.

299 Chiara Rossi, Andrea Angelucci, Laura Costantin, Chiara Braschi, Mario Mazzantini, Francesco Babbini, Maria Elena Fabbri, Lino Tessarollo, Lamberto Maffei, and Nicoletta Berardi. "Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment". In: *European Journal of Neuroscience* 24. (2006), pp. 18S0–1856.

300 Valentina Carito, Alessandro Venditti, Armandodoriano Bianco, Mauro Ceccanti, Anna Maria Serrilli, George Chaldakov, Luigi Tarani, Sara De Nicolò, and Marco Fiore. "Effects of olive leaf polyphenols on male mouse brain NGF, BDNF and their receptors TrkA, TrkB and p 5". In: *Natural product research* 28.22 (2014), pp. 1970–1984.

301 Sara De Nicoló, Luigi Tarani, Mauro Ceccanti, Mariateresa Maldini, Fausta Natella, Andrea Vania, George N Chaldakov, and Marco Fiore. "Effects of olive polyphenols administration on nerve growth factor and brain-derived neurotrophic factor in the mouse brain". In: *Nutrition* 29.4 (2013), pp. 681–68.

302 Adi Zheng, Hao Li, Ke Cao, Jie Xu, Xuan Zou, Yuan Li, Cong Chen, Jiankang Liu, and Zhihui Feng. "Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring". In: *The Journal of nutritional biochemistry* 26.2 (2015), pp. 190–199.

303 Fabio Gualtieri, Catherine Brégère, Grace C Laws, Elena A Armstrong, Nicholas J Wylie, Theo T Moxham, Raphael Guzman, Timothy Boswell, and Tom V Smulders. "Effects of environmental en- richment on doublecortin and BDNF expression along the dorsoventral axis of the dentate gyrus". In: *Frontiers in neuroscience* 11 (2017), p. 488.

304 F. Gualtieri, C. Brégère, G. C. Laws, E. A. Armstrong, N. J. Wylie, T. T. Moxham, R. Guzman, T. Boswell, and T. V. Smulders. "Effects of Environmental Enrichment on Doublecortin and BDNF Expression along the Dorso-Ventral Axis of the Dentate Gyrus". In: *Front Neurosci* 11 (201), p. 488.

305 Jerrold Tannenbaum and B Taylor Bennett. "Russell and Burch's 3Rs Then and Now: The Need for Clarity in Definition and Purpose". In: *Journal of the American Association for Laboratory Animal Science : JAALAS* 54.2 (2015), pp. 120–132.

306 William Moy Stratton Russell and Rex Leonard Burch. *The principles of humane experimental technique*. Methuen, 1959.

307 L. Lewejohann, C. Reinhard, A. Schrewe, J. Brandewiede, A. Haemisch, N. Görtz, M. Schachner, and N. Sachser. "Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests". In: *Genes, Brain and Behavior* 5.1 (2006), pp. 64–72.

308 Robert E. Sorge, Loren J. Martin, Kelsey A. Isbester, Susana G. Sotocinal, Sarah Rosen, Alexander H. Tuttle, Jeffrey S. Wieskopf, Erinn L. Acland, Anastassia Dokova, Basil Kadoura, Philip Leger, Josiane C. S. Mapplebeck, Martina McPhail, Ada Delaney, Gustaf Wigerblad, Alan P. Schumann, Tammie Quinn, Johannes Frasnelli, Camilla I. Svensson, Wendy F. Sternberg, and Jeffrey S. Mogil. "Olfactory exposure to males, including men, causes stress and related analgesia in rodents". In: *Nature Methods* 11.6 (2014), pp. 629–632.

309 Katja S van Driel and Janet C Talling. "Familiarity increases consistency in animal tests". In: *Behavioural Brain Research* 159.2 (2005), pp. 243–245.

310 Berry M. Spruijt and Leonie DeVisser. "Advanced behavioural screening: automated home cage ethology". In: *Drug Discovery Today: Technologies* 3.2 (2006), pp. 231–23.

311 Berry M. Spruijt, Suzanne M. Peters, Raymond C. de Heer, Helen H.J. Pothuizen, and Johanneke E. van der Harst. "Reproducibility and relevance of future behavioral sciences should benefit from a cross fertilization of past recommendations and today's technology: "Back to the future"". In: *Journal of Neuroscience Methods* 234 (2014), pp. 2–12.

312 Lianne Robinson, Andrea Plano, Stuart Cobb, and Gernot Riedel. "Long-term home cage activity scans reveal lowered exploratory behaviour in symptomatic female Rett mice". In: *Behavioural Brain Research* 250 (2013), pp. 148–156.

313 Xinyu Chen, Jiajie Xu, Rui Zhou, Wei Chen, Junhua Fang, and Chengfei Liu. "TrajVAE: A Variational AutoEncoder model for trajectory generation". In: *Neurocomputing* 428 (2021), pp. 332–339.

314 Anders Boesen Lindbo Larsen, Søren Kaae Sønderby, Hugo Larochelle, and Ole Winther. "Autoencoding beyond pixels using a learned similarity metric". In: *arXiv:I5I2.O93OO [cs, stat]* (2016).

315 Jim Brittain, Mariana Cendon, Jennifer Nizzi, and John Pleis. "Data Scientist's Analysis Toolbox: Comparison of Python, R, and SAS Performance". In: *SMU Data Science Review* 1.2 (2018).

316 *Google Trends*. 2021

317 L. P. Noldus, A. J. Spink, and R. A. Tegelenbosch. "EthoVision: a versatile video tracking system for automation of behavioral experiments". In: *Behavior research methods, instruments, & computers : a journal of the Psychonomic Society, Inc* 33.3 (2001), pp. 398–414.

318 Andre L. Samson, Lining Ju, Hyun Ah Kim, Shenpeng R. Zhang, Jessica A. A. Lee, Sharelle A. Sturgeon, Christopher G. Sobey, Shaun P. Jackson, and Simone M. Schoenwaelder. "MouseMove: an open source program for semi-automated analysis of movement and cognitive testing in rodents". In: *Scientific reports* 5 (2015), p. 16171.

319 Nick Chater, Joshua B. Tenenbaum, and Alan Yuille. "Probabilistic models of cognition: where next?" In: *Trends in Cognitive Sciences* 10. (2006), pp. 292–293.

320 Ernst Sigmund Brun Eric Ulfhake, Amanda J Kiliaan, and Jan-Bas Prins. *Home-Cage Monitoring and Its Effects on Research Capability and Outcomes*. 2019.

321 Robert C Wilson and Anne GE Collins. "Ten simple rules for the computational modeling of behavioral data". In: *eLife* 8 (2019). Ed. by Timothy E Behrens, e4954.

322 J. M. Veerbeek, E. E. H. Van Wegen, B. C. Harmeling–Van der Wel, and G. Kwakkel. "Is Accurate Prediction of Gait in Non- ambulatory Stroke Patients Possible Within 2 Hours Poststroke?: The EPOS Study". In: *Neurorehabilitation and Neural Repair* 25.3 (2011), pp. 268–274.

323 Graeme J. Hankey. "The Role of Nutrition in the Risk and Burden of Stroke: An Update of the Evidence". In: *Stroke* 48.11 (2017), pp. 3168– 3174.

324 Fernando Gómez-Pinilla. "Brain foods: the effects of nutrients on brain function". In: *Nature reviews. Neuroscience* 9. (2008), pp. 568–578.

325 Marta K. Zamroziewicz and Aron K. Barbey. "Nutritional Cognitive Neuroscience: Innovations for Healthy Brain Aging". In: *Frontiers in Neuroscience* 10.

326 Kiran V. Sandhu, Eoin Sherwin, Harriët Schellekens, Catherine Stanton, Timothy G. Dinan, and John F. Cryan. "Feeding the microbiotagut-brain axis: diet, microbiome, and neuropsychiatry". In: *Translational Research: The Journal of Laboratory and Clinical Medicine* 179 (2017), pp. 223–244.

327 Paul D. Loprinzi, Pamela Ponce, Liye Zou, and Hong Li. "The Counteracting Effects of Exercise on High-Fat Diet-Induced Memory Im- pairment: A Systematic Review". In: *Brain Sciences* 9.6 (2019).

328 Eliezer Gurarie, Otso Ovaskainen, Associate Editor: Volker Grimm, and Editor: Mark A. McPeek. "Characteristic Spatial and Temporal Scales Unify Models of Animal Movement". In: *The American Naturalist* 178.1 (2011), pp. 113–123.

329 L. de Visser, R. van den Bos, W. W. Kuurman, M. J. H. Kas, and B. M. Spruijt. "Novel approach to the behavioural characterization of inbred mice: automated home cage observations". In: *Genes, Brain, and Behavior* 5.6 (2006), pp. 458–466.

330 Anders Hånell and Niklas Marklund. "Structured evaluation of rodent behavioral tests used in drug discovery research". In: *Frontiers in Behavioral Neuroscience* 8 (2014).

331 Ahmed Eltokhi, Barbara Kurpiers, and Claudia Pitzer. "Behavioral tests assessing neuropsychiatric phenotypes in adolescent mice reveal strain- and sex-specific effects". In: *Scientijic Reports* 10.1 (2020), p. 11263.

332 Catherine Belzung and Maël Lemoine. "Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression". In: *Biology of Mood & Anxiety Disorders* 1.1 (2011), p.79.

333 Yu Zheng. "Trajectory Data Mining: An Overview". In: *ACM Transaction on Intelligent Systems and Technology* (2015).

334 H. Li, Y. Cui, S. Wang, J. Liu, J. Qin, and Y. Yang. "Multivariate Financial Time-Series Prediction With Certified Robustness". In: *IEEE Access* 8 (2020), pp. 109133–109143.

335 Kratarth Goel, Raunaq Vohra, and J. K. Sahoo. "Polyphonic Music Generation by Modeling Temporal Dependencies Using a RNN- DBN." In: *Artificial Neural Networks and Machine Learning – ICANN 2014*. Ed. by Stefan Wermter, Cornelius Weber, Włodzisław Duch, Timo Honkela, Petia Koprinkova-Hristova, Sven Magg, Günther Palm, and Alessandro E. P. Villa. Lecture Notes in Computer Science. Cham: Springer International Publishing, 2014, pp. 217–224.

336 Justin Shenk, Wolf Byttner, Saranraj Nambusubramaniyan, and Alexander Zoeller. "Traja: A Python toolbox for animal trajectory analysis". In: *Journal of Open Source Software* 6.63 (2021), p. 3202.

337 Ashleigh Thomas, Kathleen Bates, Alex Elchesen, Iryna Hartsock, Hang Lu, and Peter Bubenik. “Topological data analysis of C. elegans locomotion and behavior”. In: *Frontiers in Artificial Intelligence* 4 (2021), p. 668395.

338 Sachit Butail, Erik M. Bollt, and Maurizio Porfiri. “Analysis and classification of collective behavior using generative modeling and nonlinear manifold learning”. In: *Journal of Theoretical Biology* 336 (2013), pp. 185–199.

339 Francisco J. Montáns, Francisco Chinesta, Rafael Gómez-Bombarelli, and J. Nathan Kutz. “Data-driven modeling and learning in science and engineering”. In: *Comptes Rendus Mécanique*. Data-Based Engineering Science and Technology 34 .11 (2019), pp. 845–855.

340 IDC (International Data Corporation). *Data Age 2025*. Tech. rep. 2019.

341 Yarden Katz. *Noam Chomsky on Where Artificial Intelligence Went Wrong*. 2012.

342 Rasmus Diederichsen. “Live Introspection for Neural Network Training.” MA thesis. Osnabrück, Germany: University of Osnabrück, 2019.

343 Leslie Lamport. *LaTeX: ADocument Preparation System*. Reading, MA: Addison–Wesley Pub.\ Co., 1986.

344 Guido van Rossum. *Python tutorial*. Technical Report CS-R9S26. Amsterdam: Centrum voor Wiskunde en Informatica (CWI), Amsterdam, 1995.

345 Wes McKinney et al. “Data structures for statistical computing in python.” In: *Proceedings of the 9th Python in Science Conference*. Vol. 445. Austin, TX. 2010, pp. 51–56.

346 Kelsey Jordahl. *GeoPandas: Python tools for geographic data*. https://github.com/geopandas/geopandas. 2014.

347 conda-forge community. *The conda-forge Project: Community-based Software Distribution Built on the condaPackage FormatandEcosys- tem*. 2015.

SUMMARY

This thesis introduces a novel, freely available software, Traja, which was developed for the analysis of homecage trajectory data from mouse models of stroke.

§1.2 presents the biological context of the thesis and addresses the importance of movement analysis in research, diagnosis and treatment of neurological diseases. We also discuss the shift from traditional methods of locomotion and behavioral analysis towards automated HCM in research with rodent models, and the need that this creates for software to manage, process and analyze the resulting data. §1.3 provides the technical background relevant to the development of the Traja software.

Chapter 2 presents Traja, a Python package built for analyzing trajectory data. It provides an explanation of the software architecture and design and demonstrate various methods useful for preprocessing, analyzing, and modeling trajectory data, using data derived from the study on multinutrient intervention after ischemic stroke in mice [48]. It was published as *Traja: A Python toolbox for animal trajectory analysis* in "Journal of Open Source Software" [336].

Then, we demonstrate how Traja may be used in research with mouse models of stroke by analyzing mouse homecage trajectory data. Chapter 3 is based on the paper *Automated Analysis of Stroke Mouse Trajectory Data With Traja* [48], which was published in "Frontiers in Neuroscience". In this study, we used Traja to analyze trajectory data from stroke-induced mice receiving a fortified diet treatment, Fortasyn. We demonstrated the usability of Traja for analysis of mouse positions in terms of activity (§3.3.1), distance travelled (§3.3.2), velocity (§3.3.3), and turns and laterality (§3.3.4). Chapter 4 is based on the paper *Hydroxytyrosol, the major phenolic compound of olive oil as acute therapeutic strategy after ischemic stroke*, which was published in "Nutrients" [47]. Here, we extended the methods developed in Chapter 3 to a larger study involving treatment of a stroke mouse model with Hydroxytyrosol (HT) also referred to as a Mediterranean diet. We used Traja to analyze the effects of light phase and HT treatment on activity, distance travelled, walking velocity, total turnings, and laterality index 24/7 (§4.5). The results of these two studies indicate that Traja can be successfully applied to trajectory data mining and analysis, providing insight to researchers and demonstrating the potential for home cage mouse tracking in neurological research.

Finally, Chapter 5 provides a unifying discussion for the thesis. It addresses recent advances in technology used for behavioral analysis, particularly automated home cage monitoring, and how these create a need for software like Traja. We also further discuss the capabilities and advantages of Traja, and the applications of the software presented in this thesis. Finally, we consider other possible applications of Traja and reflect on how the software fits into the changing landscape of scientific research today.

NEDERLANDSE SAMENVATTING

Dit proefschrift introduceert een nieuwe, vrij beschikbare software, Traja, die ontwikkeld is voor de analyse van homecage trajectory data van muismodellen voor beroerte.

§1.2 presenteert de biologische context van het proefschrift en gaat in op het belang van bewegingsanalyse voor onderzoek, diagnose en behandeling van neurologische aandoeningen. De verschuiving van traditionele methodes van bewegingsanalyse en gedragsanalyse naar geautomatiseerde HCM in onderzoek met knaagdiermodellen, en de behoefte die hierdoor ontstaat aan software om de resulterende gegevens te beheren, te verwerken en te analyseren worden besproken. §1.3 geeft de technische achtergrond die relevant is voor de ontwikkeling van de Traja software.

Hoofdstuk 2 presenteert Traja, een Python pakket gebouwd voor het analyseren van looppatronen. Het geeft uitleg over de architectuur en het ontwerp van de software en demonstreert verschillende methoden die nuttig zijn voor het voorbewerken, analyseren en modelleren van looppatronen, aan de hand van gegevens uit de studie over multinutriënten interventie na ischemische beroerte bij muizen.

Vervolgens demonstreren we hoe Traja kan worden gebruikt in onderzoek met muismodellen voor stroke door het analyseren van gegevens over het looppatroon in muizenkooien. Hoofdstuk 3 is gebaseerd op het gepubliceerde artikel *Automated Analysis of Stroke Mouse Trajectory Data With Traja* [48]. In deze studie gebruikten we Traja om traject gegevens van stroke-geïnduceerde muizen op een multicomponenten dieet interventie, te analyseren. We hebben de bruikbaarheid van Traja aangetoond voor de analyse van muisposities in termen van activiteit (§3.3.1), afgelegde afstand (§3.3.2), snelheid (§3.3.3), en draaiingen en lateraliteit (§3.3.4). Hoofdstuk 4 is gebaseerd op het gepubliceerde artikel *Hydroxytyrosol, the major phenolic compound of olive oil as acute therapeutic strategy after ischemic stroke* [47]. Hier hebben we de methoden ontwikkeld in Hoofdstuk 3 naar een grotere studie waarbij de behandeling van een beroerte muismodel met Hydroxytyrosol (HT) ook wel aangeduid als een mediterraan dieet. We gebruikten Traja om de effecten van HT behandeling op activiteit, afgelegde afstand, loopsnelheid, totale draaiingen, en lateraliteitsindex 24/7 te analyseren (§4.5). De resultaten van deze twee studies tonen aan dat Traja met succes kan worden toegepast voor traject datamining en analyse, hierbij inzicht verschaffend aan onderzoekers en de potentie aantonend van thuiskooi muistracking in neurologisch onderzoek.

De discussie in het proefschrift behandelt recente ontwikkelingen in technologie die worden gebruikt voor gedragsanalyse, in het bijzonder geautomatiseerde thuiskooimonitoring, en hoe deze een behoefte creëren aan software zoals Traja. We bespreken verder de mogelijkheden en voordelen van Traja, en de toepassingen van de software die in dit proefschrift worden gepresenteerd. Tenslotte staan we stil bij andere mogelijke toepassingen van Traja en denken we na over hoe de software past in het veranderende landschap van het wetenschappelijk onderzoek van vandaag.

CURRICULUM VITEA

Justin Shenk was born on the 30th of June 1985 in San Antonio, Texas. He obtained his Bache- lor in Biology at the University of Texas at San Antonio in 2006. In 2008 he obtained his Master's degree in Biology under the supervision of Prof. George Perry with a thesis on the reduction of neural pathologies in Alzheimer's disease with nutritional supplements. After managing the electron microscopy lab and working in a molecular biology lab, he worked at the University of Texas Health Science Center Research Imaging Institute analyzing brain activation patterns and developing software for neuropsychological experiments of speech and movement disorders.

In the following years he travelled the world, studied languages, and maintained several positions as a Biology and Chemistry lecturer, English teacher, journalist, and social entrepreneur. In 2016 he began studies at the University of Osnabrück in Germany, where he completed his second Master's degree in Cognitive Science in 2018. There he taught several courses on deep learning within the Artificial Intelligence (AI) Research Group. Justin completed his thesis with the AI software company Peltarion in Stockholm, Sweden, entitled "Live Guidance of Neural Network Architecture Design." He worked at Peltarion as an AI Research Engineer and Data Scientist. He presented his computer vision deep learning applications as a consultant for Intel at several international conferences including International Conference on Machine Learning (ICML), Neural Information Processing Systems (NeurIPS), and Conference on Computer Vision and Pattern Recognition (CVPR).

In 2018 Justin began a PhD project under the supervision of Professor Amanda Kiliaan at Radboud University Medical Center with the objective of exploring and analyzing 24/7 home cage trajectory data for hundreds of mice.

He is active in open source software development and founded and maintains several open source projects, including Traja, FER and Delve. He served as a reviewer for the *Journal of Open Source Software* and Associate Editor for the *Journal of Open Research Software*. In 2019 and 2020 he mentored software engineering projects for OpenCV's international Google Summer of Code program and has remotely supervised several bachelor's and master's theses on AI.

Currently, he serves as Managing Director of VisioLab GmbH, an AI software company, teaches university courses, and continues research on deep learning in Berlin.

LIST OF PUBLICATIONS

IN THIS THESIS

Justin Shenk, Wolf Byttner, Saranraj Nambusubramaniyan, and Alexander Zoeller. "Traja: A Python toolbox for animal trajectory analysis". In: *Journal of Open Source Software* 6.63 (2021), p. 3202.

Justin Shenk, Klara J. Lohkamp, Maximilian Wiesmann, and Amanda J. Kiliaan. "Automated Analysis of Stroke Mouse Trajectory Data With Traja". In: *Frontiers in Neuroscience* 14 (2020).

Justin Shenk, Jesús Calahorra, Vera H. Wielenga, Vivienne Verweij, Bram Geenen, Pieter J. Dederen, M. Ángeles Peinado, Eva Siles, Maximilian Wiesmann, and Amanda J. Kiliaan. "Hydroxytyrosol, the Major Phenolic Compound of Olive Oil, as an Acute Therapeutic Strategy after Ischemic Stroke". In: *Nutrients* 11.10 (2019), p. 2430.

NOT IN THIS THESIS

Justin Shenk, Mats L. Richter, and Wolf Byttner. "Delve: Neural Network Feature Variance Analysis". In: *Journal of Open Source Software* .69 (2022), p. 3992.

Kyle S. Herman and Justin Shenk. "Pattern Discovery for climate and environmental policy indicators". In: *Environmental Science & Policy* 120 (2021), pp. 89–98.

Klara J. Lohkamp, Amanda J. Kiliaan, Justin Shenk, Vivienne Verweij, and Maximilian Wiesmann. "The Impact of Voluntary Exercise on Stroke Recovery". In: *Frontiers in Neuroscience* 15 (2021), p. 856.

Mats L. Richter, Wolf Byttner, Ulf Krumnack, Anna Wiedenroth, Ludwig Schallner, and Justin Shenk. "(Input) Size Matters for CNN Classifiers." In: *Artificial Neural Networks and Machine Learning – ICANN 2021*. Ed. by Igor Farkaš, Paolo Masulli, Sebastian Otte, and Stefan Wermter. Lecture Notes in Computer Science. Cham: Springer International Publishing, 2021, pp. 133–144.

Xiongwei Zhu, Rudy J Castellani, Paula I Moreira, Gjumrakch Aliev, Justin C Shenk, Sandra L Siedlak, Peggy LR Harris, Hisashi Fujioka, Lawrence M Sayre, Pamela A Szweda, et al. "Hydroxynonenal- generated crosslinking fluorophore accumulation in Alzheimer disease reveals a dichotomy of protein turnover". In: *Free Radical Biology and Medicine* 52.3 (2012), pp. 699–704.

RJ Castellani, Paula Moreira, Gjumrakch Aliev, Justin C Shenk, Sandra L Siedlak, Peggy LR Harris, Lawrence M Sayre, LI Szweda, X Zhu, MA Smith, et al. "Hydroxynonenal-Generated Crosslinking Fluorophore and Mitochondria-derived Lipoic Acid Accumulation in Alzheimer Disease Reveal a Dichotomy of Protein Turnover". In: *Microscopy and Microanalysis* 16.52 (2010), pp. 1014–1015.

Justin C Shenk, Jiankang Liu, Kathryn Fischbach, Kui Xu, Michel Puchowicz, Mark E Obrenovich, Eldar Gasimov, Ludis Morales Alvarez, Bruce N Ames, Joseph C LaManna, et al. "The effect of acetyl-L-carnitine and R-α-lipoic acid treatment in ApoE4 mouse as a model of human Alzheimer's disease". In: *Journal of the Neurological Sciences* 283.1-2 (2009), pp. 199–206.

Gjumrakch Aliev, Jiankang Liu, Justin C Shenk, Kathryn Fischbach, Gerardo J Pacheco, Shu G Chen, Mark E Obrenovich, Walter F Ward, Arlan G Richardson, Mark A Smith, et al. "Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats". In: *Journal of Cellular and Molecular Medicine* 13.2 (2009), pp. 320–333.

Mark E Obrenovich, Ludis A Morales, Celia J Cobb, Justin C Shenk, Gina M Méndez, Kathryn Fischbach, Mark A Smith, Eldar K Qasimov, George Perry, and Gjumrakch Aliev. "Insights into cerebrovascular complications and Alzheimer disease through the selective loss of GRK2 regulation". In: *Journal of Cellular and Molecular Medicine* 13.5 (2009), pp. 853–865.

Gjumrakch Aliev, Celia Cobb, Gerry Pacheco, Justin C Shenk, Paula I Moreira, Kathryn Fischbach, Ludis Morales, Edgar Gasimov, and George Perry. "The Role of Oxidative Stress and Vasoactive Substances in the Pathophysiology of Alzheimer's Disease". In: *BioMarkers for Early Diagnosis of Alzheimer's Disease* (2008), pp. 241–26S.

Gjumrakch Aliev, Eldar Gasimov, Mark E Obrenovich, Kathryn Fischbach, Justin C Shenk, Mark A Smith, and George Perry. Appendices16 "Atherosclerotic lesions and mitochondria DNA deletions in brain microvessels: implication in the pathogenesis of Alzheimer's disease". In: *Vascular Health and Risk Management* 4.3 (2008), p. 21.

Gjumrakch Aliev, Mark E Obrenovich, V Prakash Reddy, Justin C Shenk, Paula I Moreira, Akihiko Nunomura, Xiongwei Zhu, Mark A Smith, and George Perry. "Antioxidant therapy in Alzheimer's disease: theory and practice". In: *Mini reviews in medicinal chemistry*
8.13 (2008), p. 1395.

George Perry, Paula I Moreira, Maria S Santos, Catarina R Oliveira, Justin C Shenk, Akihiko Nunomura, Mark A Smith, and Xiongwei Zhu. "Alzheimer disease and the role of free radicals in the pathogenesis of the disease". In: *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* .1 (2008), pp. 3–10.

Paula I Moreira, Akihiko Nunomura, Masao Nakamura, Atsushi Takeda, Justin C Shenk, Gjumrakch Aliev, Mark A Smith, and George Perry. "Nucleic acid oxidation in Alzheimer disease". In: *Free Radical Biology and Medicine* 44.8 (2008), pp. 1493–1505.

Gjumrakch Aliev, Justin C Shenk, Kathryn Fischbach, and George Perry. “Stem cell niches as clinical targets: the future of anti-ischemic therapy?” In: *Nature Clinical Practice Cardiovascular Medicine* S.9 (2008), pp. 590–591.

G Aliev, JP Miller, DW Leifer, ME Obrenovich, JC Shenk, MA Smith, JC Lamanna, G Perry, DW Lust, and AR Cohen. “Ultrastructural analysis of a murine model of congenital hydrocephalus produced by overexpression of transforming growth factor-beta1 in the central nervous system”. In: *Journal of Submicroscopic Cytolology and Pathology* 38.2-3 (2006), pp. 85–91.

OTHER PUBLICATION (NON PEER REVIEW)

Justin Shenk, Mats L Richter, Wolf Byttner, Anders Arpteg, and Mikael Huss. “Feature Space Saturation during Training”. In: *arXiv preprint arXiv:2OO6.O8679* (2020).

Justin Shenk, Mats L Richter, Anders Arpteg, and Mikael Huss. “Spectral analysis of latent representations”. In: *arXiv preprint arX- iv:I9O7.O8589* (2019).

OTHER SOFTWARE PUBLISHED

Other software published during PhD (www.github.com/ justinshenk):

- *FER:* Facial expression recognition library with a deep neural network
- *Posture Pal:* Computer vision-based posture monitor application
- *Simages:* Detection of similar images in a dataset with autoencoders
- *Delve:* Analysis of deep neural network representations live during training

This appendix lists all code and documentation contributions made to other projects during development.

OPEN SOURCE CONTRIBUTIONS

Table .3: Contributions to third-party projects

Project	Contribution
epysurv	Maintainer, added continuous integration (CI) pipeline, commit d62fba6892db0efd7239fdcd3959f2771cc82aa0
rpy2	Add microsecond support when converting from pandas (commit hash f6c7416b4e87a922b5cba3d2177e4bcd18584197)
pyCeterisParibus	Add Mac OSX support for webbrowser (commit hash97c07a2718e c2ca0f1ea9f9a27f6a94c5816608)
Coach	Fix installation by adding force flag to library symbolic link
moveHMM	Documentation improvements
autoKeras	Documentation improvements

OPEN SOURCE ACKNOWLEDGEMENTS

The following presents a non-exhaustive list of open source tools used in creating the software, experiments and this document.[1]:

1. LaTeX for typesetting of this document [343]. This includes creators of various packages as well as the base template[2] developed by André Miede.

2. Python [344] and the NumFocus developer community (Matplotlib [164], Numpy [344], pandas [345], scipy [158], etc.).

3. PyTorch [192] for allowing prototyping of deep neural networks with a painless, Pythonic API.

4. GeoPandas [346] for demonstrating the capabilities of the pandas accessor functionality and sharing code for their continuous integration pipeline.

5. conda-forge [347] and the community for support developing a continuous integration pipeline and packaging support on Windows, Mac and Linux.

6. Trajr [108] R package inspired several implementations used in Traja.

1 Inspiration for this section comes from Rasmus Diederichsen's thesis [342]

2 http://bitbucket.org/amiede/classicthesis

ACKNOWLEDGEMENTS

First, I wish to thank my dissertation committee for their willingness to sit on the committee and to read this far. I am deeply grateful for the support of my supervisor Professor Amanda Kiliaan whose expertise provided an essential background for this thesis. Without her constant trust, and sometimes, gentle prodding, this thesis would not have been possible. Likewise, Maximilian Wiesmann's infinite patience and his crucial guidance with the analyses performed in Chapters 3 and 4 was essential for this work. I am also thankful for Kai-Uwe Kühnberger's keen eye for details in reading the manuscript and helpful corrections.

I would like to extend my sincere thanks to the amazing colleagues I befriended during my year with Peltarion in Stockholm, in particular Anders Arpteg for supporting me with combining my work responsibilities with doctoral research. Thanks to Veine Haglund for guidance with the data analysis.

Within the Radboud UMC Anatomy department, I have found a second home and am especially grateful for the regular feedback and support from Klara Lohkamp.

I would like to thank Fabio Iannello from Tecniplast for generously providing the data used in these studies and for sharing his valuable time during our visit to his facilities in Varese.

The academic freedom living in Germany was made fruitful due to mental and emotional support of advisors and friends. Special thanks to Laura Terlau for her personal and emotional support during the challenging first year of this thesis. Within VisioLab, I am especially honored by the encouragement and support I received from Tim Niekamp and Thiago Goldschmidt. Many thanks to Rasmus Diederichsen and Natalia Bielczyk for their helpful feedback to this thesis.

The completion of this thesis would not have been possible without the unwavering support and improvements by Hannah Payette Peterson.

DATA MANAGEMENT

All animal experiments described in this thesis were carried out in accordance with international European ethical standards (European Directive 2010/63/EU) and were approved and pre-registered by the Animal Ethics Committee (called the Dierexperimentencommissie; DEC, RU-DEC 2014- 1 1 & RU-DEC 2017-0021) of the Radboud University Medical Center (Radboudumc) and reported according to the ARRIVE guidelines. All applicable international, national, and institutional guidelines for the care and use of animals were followed. Our studies were also in concurrence with the European regulations on ethics and responsible conduct regarding scientific communication. The research data presented in this thesis and obtained during this PhD at the department of Medical Imaging, Anatomy (Radboudumc) were archived according to Findable, Accessible, Interoperable and Reusable (FAIR) principles.

Findable All raw and processed digital data described in this thesis are stored on the server of the Department of Medical Imaging, Anatomy (Radboudumc) and backed-up daily on the local Radboudumc server. The data can be found at the Department of Medical Imaging, Anatomy, (Radboudumc).

Accessible The data and protocols described in this thesis are included in published articles and can be obtained on request from the Department of Medical Imaging, Anatomy, Radboudumc, Nijmegen, the Netherlands. The digital data of chapter 2, 3, and 4 are also stored on the departments' NAS and could be made available after contacting the corresponding author.

Interoperable The data presented in this thesis are documented in a formal, accessible, shared, and broadly applicable language for knowledge representation.

Reusable The data shown in this thesis are adequately documented to be reusable for further research and analysis. The data will be saved for 15 years after termination of the study (July 1, 2031). To ensure interpretability of the data, all filenames, primary and secondary data, metadata, descriptive files and program code and scripts used to provide the final results are documented along with the data.

DONDERS GRADUATE SCHOOL FOR COGNITIVE NEUROSCIENCE

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna, etc. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.

For more information on the DGCN as well as past and upcoming de- fenses please visit: http://www.ru.nl/donders/graduate-school/ donders-graduate/