griPASTA: A hacked kitchen scale-based system for quantification of grip strength in rodents

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**Abstract**

Assessment of neuromuscular function is critical for understanding pathophysiological changes related to motor system dysfunction in animal models of different diseases. Among a number of methods used for quantification of grip performance in rodents, gauge-based grip strength meters seem to provide the most reliable results, however such instruments are unaffordable by many laboratories. Here we demonstrate how to build a grip strength apparatus for rodents from scratch using a digital kitchen scale, an empty cage and a microcontroller, with both hardware and software being completely open-source to enable maximal modularity and flexibility of the instrument in concordance with the principles of open-source bioinstrumentation. Furthermore, we test the griPASTA system for assessment of increased muscular rigidity in the proof-of-concept experiment in the rat model of Parkinson's disease induced by intrastriatal administration of 6-hydroxydopamine (6-OHDA). Finally, the importance of bioinstrumental customization is demonstrated by exploiting the ability of griPASTA to provide raw data and enable calculation of grip strength trial speed, a variable known to affect the grip strength measurements that is not standardly measured by the commercial grip strength meters.

**Keywords**

open-source; grip strength; neuromuscular function; animal models; Parkinson's disease; 6-ohda; rigidity

## Introduction

Assessment of muscle strength is critical for understanding neuromuscular function in rodents. As a plethora of rodent models demonstrate impaired neuromuscular function, sensitive methods for objective quantification of muscle strength is a topic of interest for many research groups. Over the years, numerous tests have been proposed for in vivo muscle strength assessment such as inverted screen (Kondziella, 1964), scale collector weight lifting (Deacon, 2013), wire hang (Hoffman & Winder, 2016), treadmill (Beatriz Castro, 2017), vertical pole (Matsuura et al., 1997), swimming endurance and grip strength test (Takeshita et al., 2017). The grip strength test is favored by most as it is fast, convenient and introduces less stress in comparison with other tests (Takeshita et al., 2017). Its importance has been recognized by authorities so the test is included both in the US Environmental Protection Agency Functional Observational Battery (US EPA FOB) and OECD guidelines for neurobehavioral toxicity screening (Moser, 2011). Several commercial strain gauge-based grip strength meters suitable for precise and objective quantification of grip strength are available on the market, however they are unaffordable by many laboratories. As a consequence, researchers usually opt out for cheap semi-quantitative solutions for grip performance evaluation. We believe money should not stand in the way of scientific accuracy (or science in general) so we designed a simple open-source platform that combines affordability, availability and simplicity of semi-quantitative grip strength tests, and precision, robustness and informativeness of the quantitative strain gauge-based methods.

Commercially available equipment for measurement of grip strength in rodents exploits strain gauges, sensors that convert force into a change in electrical resistance that can be easily measured. The same force-to-resistance conversion method is unknowingly used by many every day as cheap commercial kitchen scales exploit the very same principle for weight estimation. We have previously proposed a method for assessment of acoustic startle response and prepulse inhibition sensorimotor gating phenomenon in rodents that exploits the same principles (Virag et al., 2020). Based on the same open source platform we present a solution for quantification of grip strength in experimental animals with a step-by-step guide on how to build it by transforming the kitchen scale.

We hypothesize that cheap open-source solutions such as griPASTA might be particularly valuable in specific sensitive experimental settings where the modularity of the open-source devices enables the researchers to address specific methodological problems that would otherwise remain overlooked due to the limited flexibility of the black box solutions. For this reason, we exploited the ability of griPASTA to provide raw data to test for the potential confounding effect of the trial speed in our proof-of-concept (POC) experiment in a rat model of Parkinson's disease (PD). This variable would remain largely ignored if a commercial apparatus was used for the grip strength assessment in the same scenario, although previous research identified trial speed as a factor that might affect the grip strength measurements (Maurissen et al., 2003). griPASTA was tested in a real world experimental setting for the assessment of muscular rigidity in the rat model of PD induced by intrastriatal administration of 6-hydroxydopamine (6-OHDA). Muscular rigidity has been considered as an important parameter in animal models of PD, however it remains

underrepresented in the literature due to the challenging methodology associated with rigidity assessment in rodents (Meredith & Kang, 2006). Different tests have been proposed for the purpose, such as the lateral displacement test (Lindner et al., 1999). Still, researchers often opt for other tests more closely related to the grip strength, such as the grasping test, for their better discriminatory power (Ma et al., 2014; Sotnikova et al., 2005). We hypothesized that quantitative grip strength assessment by the means of griPASTA might be able to detect an increment in muscular rigidity in 6-OHDA-treated rats because quantitative grip strength assessment offers several methodological advantages over the grasping test. The most important differences between these two tests is that by means of griPASTA it is easier to control for the confounding effect of body weight, body weight-grasping outcome linearity, cut-off determination and ceiling effects (Liu & Wang, 2020). We hypothesized that the benefit of modularity and the ability to access raw data in the open-source griPASTA setup might enable us to detect the effect even though previous reports indicated that intrastriatal lesions induce much less pronounced rigidity in comparison with nigrostriatal ones (Jeyasingham et al., 2001).

## Materials and Methods

### *Construction of griPASTA Hardware*

A setup for measurement of grip strength in rodents consists of two main parts: A hacked digital kitchen scale connected to a computer (Virag et al., 2020) and a weighted mouse cage used as a grip test platform (Fig 1. A-C).

### *A hacked kitchen scale*

For detailed instructions on how to hack a kitchen scale and connect it to a computer as well as for free open-source software packages for data extraction and visualization please see Virag et al. (Virag et al., 2020). In brief, a digital kitchen scale is opened and strain gauge load cells are identified and connected to a readily-available HX711 circuit board modified by reconnecting the RATE pin to VCC to enable 80 samples per second sampling rate mode of the integrated circuit. The HX711 is connected to a microcontroller that acts as a communication bridge between the kitchen scale and the computer through a USB connection. We used a NodeMCU ESP-32S board, but other Arduino microcontroller boards may be used just as well, including AVR-based ones.

### *A grip test platform*

In order to extract grip strength data from a kitchen scale, we constructed a grip platform for rats. We wanted to use something readily available in most laboratories equipped for experiments with laboratory animals, so we used a mouse cage with a cage lid being used as a test surface and several boxes of old histological slides used as a weight to stabilize the platform. In order to reduce the variability of animal gripping patterns, duct tape was placed on part of the cage lid making only central grids available for the animals to hold onto. The whole system is fixed onto the PASTA platform with duct tape or reusable putty-like pressure-sensitive adhesive (eg. Patafix, UHU, Germany).

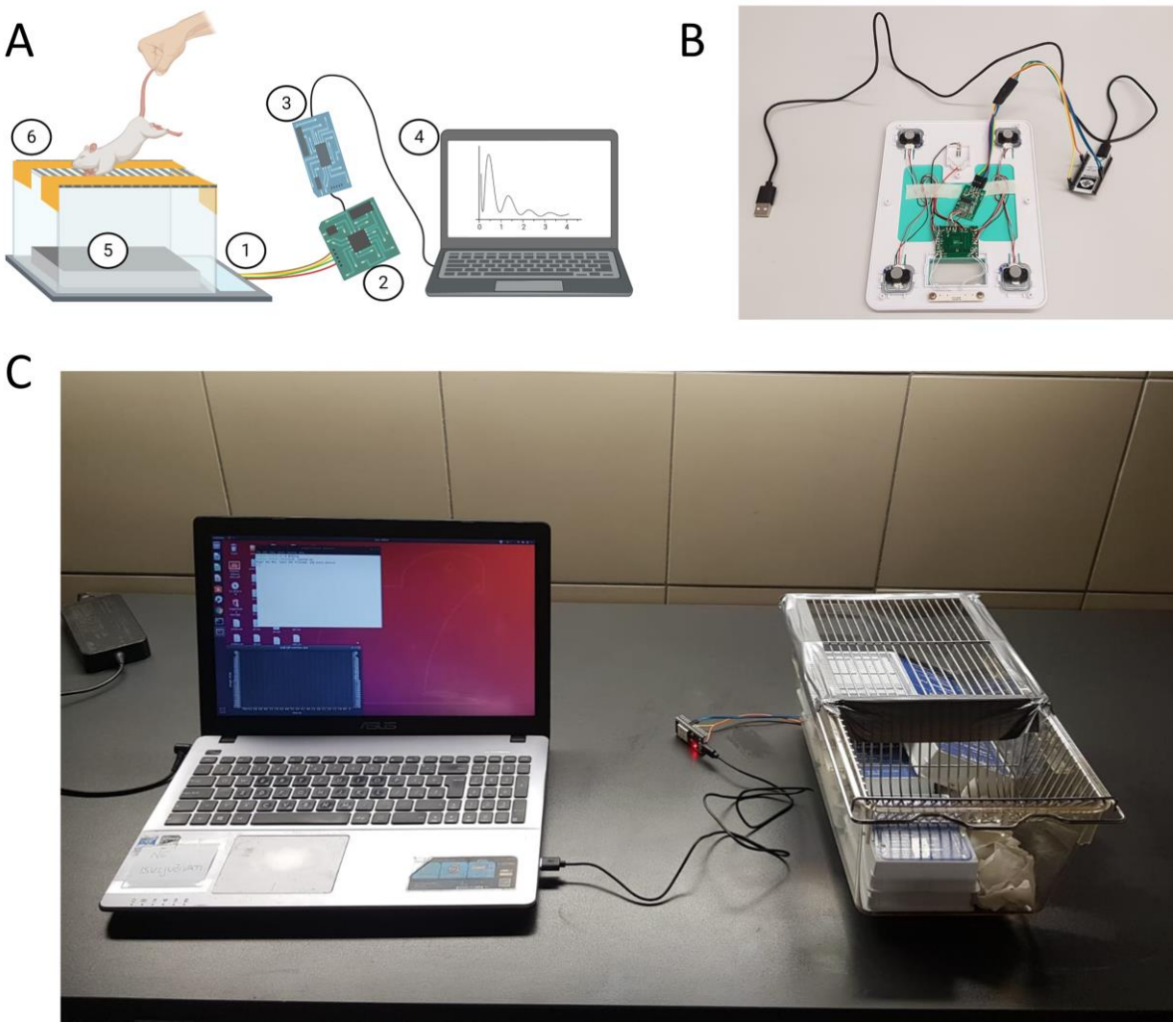


**Fig 1.** Concept of griPASTA. **A)** Schematic representation of griPASTA setup for grip strength performance measurements in rodents. A1 - PASTA: modified kitchen scale described by Virag *et al.* (Virag et al., 2020); A2 - HX711 circuit board; A3 - NodeMCU ESP-32S microcontroller; A4 - laptop; A5 - a weight stabilizing the mouse cage; A6 - Duct tape modified cage lid used as a grip platform. **B)** PASTA setup. A NodeMCU ESP-32S microcontroller interfaced with kitchen scale strain gauge load cells through the HX711 circuit board. **C)** A Photograph of griPASTA setup with a grip test platform fixed on top of the PASTA platform.

### *griPASTA Software*

In order to extract data from the platform, we used a custom Python script written for general purpose experimentation with PASTA called PASTAgp (PASTA general-purpose script), with a modified Y scale for grip testing. Once loaded, the script asks for the ID of the animal and initiates a Python PyQtGraph plugin-based real-time plot of the scale-extracted data (**Fig 1C**). When the recording is done, the script automatically saves data in a comma-separated value format in the predefined directory, using the ".pasta" extension (ID.pasta) to simplify the loading of files in the downstream analysis. The whole code is freely available under the GPLv3 copyleft license in the PASTAgp GitHub repository (davorvr, 2020).

We wrote functions in R for semi-automated detection of maximal deflection points in griPASTA data and added them to ratPASTA, an R package originally developed for the analysis of acoustic startle experiments, available on CRAN and on GitHub (https://github.com/ikodvanj/ratPASTA). Using *loadgriPASTA()* function, the user can now load griPASTA data and pass it to the *griPASTA()* function. A plot of time-series data will be displayed for each animal and the user will be prompted to declare whether the data contains artefacts. If artefacts are present, they can be removed, and maximal deflection points will be detected based on the number of deflection points and estimated time interval of each trial, provided by the user.

### *Animals*

Three months old male Wistar rats from the Department of Pharmacology, University of Zagreb School of Medicine were used in the experiment. Thirty six rats weighing 368 ± 34 g (mean ± SD) were randomly assigned to four groups (Control [CTR], 6-OHDA 6 µg [OHDA 6], 6-OHDA 6 µg w/o reboxetine, a noradrenaline transporter inhibitor [OHDA 6 w/o REB], and 6-OHDA 12 µg [OHDA 12]; n = 9 per group). The rats were housed 2-3 per cage in a room with a 12 hour light cycle (7AM/7PM). Temperature and humidity in the room were set in the range of 21-23°C and 40-70% humidity. Standardized food pellets and water were available *ad libitum*. Standard wood-chip bedding was changed twice a week.

***Drug treatment protocol***

Treatment solutions were prepared as follows: 6-OHDA was dissolved in 0.02% ascorbic acid in normal saline, and Reboxetine (Edronax, Pfizer) was dissolved in normal saline. All animals except the ones from the 6 OHDA w/o REB group received an intraperitoneal injection of reboxetine (5 mg/kg in 1ml) 40 minutes prior to induction of general anesthesia. All animals were anesthetized with ketamine/xylazine (intraperitoneal injection of 70 mg/kg and 7 mg/kg respectively), the skin was surgically opened and the skull was trepanated bilaterally with a high-speed drill. Intrastriatal administration of 6-OHDA solution (2 µl corresponding to 6 µg 6-OHDA in each hemisphere; 2 µl corresponding to 12 µg 6-OHDA in each hemisphere) or vehicle was done at stereotaxic coordinates of 0 mm posterior, ±2.8 mm lateral and 7 mm depth from pia mater relative to bregma. One animal in each group died during the induction procedure or within the following 24 hours, and two additional animals died in the group treated with the high dose of 6-OHDA within the following 2 weeks.

***Experimental protocol for grip strength assessment with griPASTA platform***

The griPASTA platform is completely open-source so both hardware and software are easily modifiable to best fit a wide variety of specific experimental purposes. In the further text, we describe the experimental protocol used in the POC study. Briefly, a modified kitchen scale (PASTA) is placed on a clean and flat surface and is connected to a computer via USB. A grip test platform is prepared by placing a weight in a clean mouse cage. In this study, a 2184L Eurostandard Type II L cage with dimensions of 365x207x140 mm was used. A cage lid, used as a grip platform in this experiment was a grid of parallel wires with a diameter of 2 mm, and a 7 mm wide gap, and a single perpendicular wire with a diameter of 2.5 mm. The precise quantification range of the kitchen scale we used (Vivax Home KS-502T (Vivax, Croatia)) was 0-5000 g so we created a platform weighing 4500g in total to maximally reduce the possibility of the platform to move during the testing procedure and to make sure that the negative force resulting from rat holding onto the cage lid while being removed by the experimenter remains in the range of maximal quantification. In the POC experiment boxes filled with old histological slides were found to be the most suitable weight. Duct tape is used to cover part of the cage lid grid the rat is not supposed to hold onto, and the whole cage is placed on the PASTA platform and additionally secured by reusable putty-like pressure-sensitive adhesive (Patafix, UHU, Germany). When the platform is ready the PASTAgp script is loaded. Once the ID of the animal is provided, a PyQtGraph real-time plot of the scale-extracted data is initiated and PASTA platform is calibrated by pressing the reset button on the microcontroller board (“EN” for NodeMCU ESP-32S). The animal is placed on the exposed part of the cage lid and gently elevated vertically

by the tail. The animal will resist being removed and negative force will be recorded by the platform. Once the trial is finished, the real-time plot is closed and the data is automatically saved in the .pasta file in the working directory.

### *Animal body weight assessment*

Animal body weight measurements were done on the day of the experiment by a standard protocol. Briefly, each animal was placed on a digital scale, and the body weight in grams was read from the screen once the animal stopped moving.

### *Data analysis and visualization*

Data analysis and visualization were conducted in the R software environment for statistical computing and graphics (R 4.0.2). Data obtained from griPASTA (.pasta) was imported into R and maximal negative deflection values (maximal force), as well as temporal values of maximal and initial deflection points for all trials, were extracted manually from interactive plots created by Plotly open-source JavaScript graphing library. Grip strength difference between groups treated with different doses of 6-OHDA and with or without reboxetine, as well as the potential effect of animal weight and experimenters' performance (inferred from the initial-to-maximal deflection time), were examined by linear mixed-effects models (LMM) with the restricted maximum likelihood (REML) estimation in the lme4 package. The variance was modeled hierarchically by fitting animal ID as a random effect in all models to account for within and between animal variance as each griPASTA trial consisted of 5±1 measurements, and group, centered time and weight variables were modeled as fixed effects. Each model was explicitly described in the results section. Dimensionality reduction was conducted by means of the principal component analysis (PCA) of the prescaled

variables in the stats package and data was visualized on a biplot with euclidean distances outlined around the group centers. Subsequently, as both grip strength and speed trial analysis yielded interesting insight, a semi-automatic function for easier analysis of data exported from griPASTA was developed and implemented in the ratPASTA package (available from CRAN and GitHub).

### *Ethics committee approval*

All experiments were conducted following the highest standards of animal welfare and only certified personnel handled animals. Animal procedures were carried out at the University of Zagreb Medical School (Zagreb, Croatia) and were in compliance with current institutional, national (The Animal Protection Act, NN102/17; NN 47/2011), and international (Directive 2010/63/EU) guidelines governing the use of experimental animals. The experiments were approved by the Ethical Committee of the University of Zagreb School of Medicine and the Croatian Ministry of Agriculture (229/2019).

## Results:

### *A pilot trial*

The initial examination of the griPASTA platform was conducted by placing a single control animal on the platform. The animal was placed on the platform, picked up by the tail and placed on the work surface next to the platform. The procedure was repeated 5 times. A single trial of 5 consecutive measurements was imported into R and visualized with ggplot2 and plotly (**Fig 2A**). An example of a single measurement from the trial is shown in **Fig 2B**. A video example of a single measurement is provided

in **Supplement 1**. As results from a single animal pilot trial suggested that griPASTA can be used for the analysis of grip strength in rodents, we moved on and conducted further validation of the instrument by means of grip strength assessment in a rat model of PD based on intrastriatal administration of 6-OHDA.

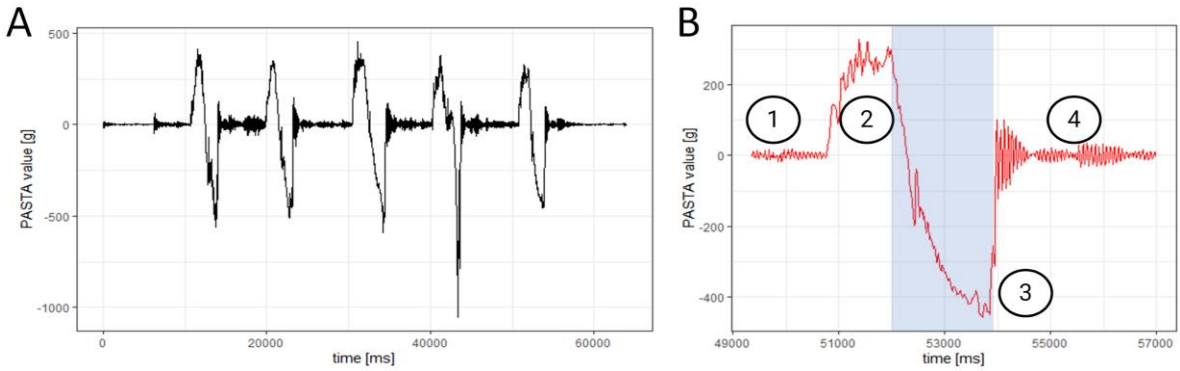


**Fig 2.** A) Raw data from a single trial with 5 consecutive measurements. B) An example of raw data from a single measurement. B1 - baseline data; B2 - positive deflection caused by the placement of the animal on top of the platform (positive deflection indicates the weight of the animal); B3 - negative deflection caused by animal holding onto the platform while being removed by the tail. Maximal negative value is the peak force the animal was able to resist; A modulus |X| of this value (|B3|) was taken as an indicator of grip strength  B4 - baseline values with noise recorded due to the placement of the animal on the table next to griPASTA. Initial-to-maximal deflection time segment, used as a proxy for trial speed, is indicated in blue.

***Grip strength in rats following intrastriatal administration of two different doses of 6-OHDA***

Grip strength assessment with griPASTA was conducted 1 month after bilateral intrastriatal administration of two different doses of 6-OHDA (6 or 12 µg per ventricle)

following reboxetine pretreatment, and an additional group (6 µg per ventricle) in which reboxetine treatment was omitted. Animals were placed on the platform in a randomized order and analyzed in the same way as described for the pilot trial with each trial consisting of 5 consequent measurements. griPASTA values of maximal negative deflection for each measurement of each trial are shown in **Fig 3A**, and griPASTA value expressed as a 5-measurement average is shown in **Fig 3B**. As evident both from individual (**Fig 3A**) and pooled measurements (**Fig 3B**), 6-OHDA-treated rats display forelimb motor rigidity in the griPASTA grip strength assessment protocol. This is in concordance with previous findings in 6-OHDA treated rats that demonstrate increased hang time in the hanging grip strength test in comparison with respective controls (Ma et al., 2014). Grip strength assessment is known to be under influence of numerous factors that are often overlooked (Maurissen et al., 2003). Some examples of such potential confounding factors are animal weight and trial speed, so we moved on to examine whether either affected the measurements obtained by the griPASTA. Animal weight was measured using a standard weighing procedure, and the possibility to obtain raw data from griPASTA platform was exploited to measure the speed of all conducted trials with initial-to-maximal deflection value corresponding times considered as appropriate approximation of trial speed. griPASTA value, animal weight and trial trial speed of each measurement were then analyzed by PCA and individual measurements in regards to their corresponding groups were displayed in a biplot (**Fig 3C**).

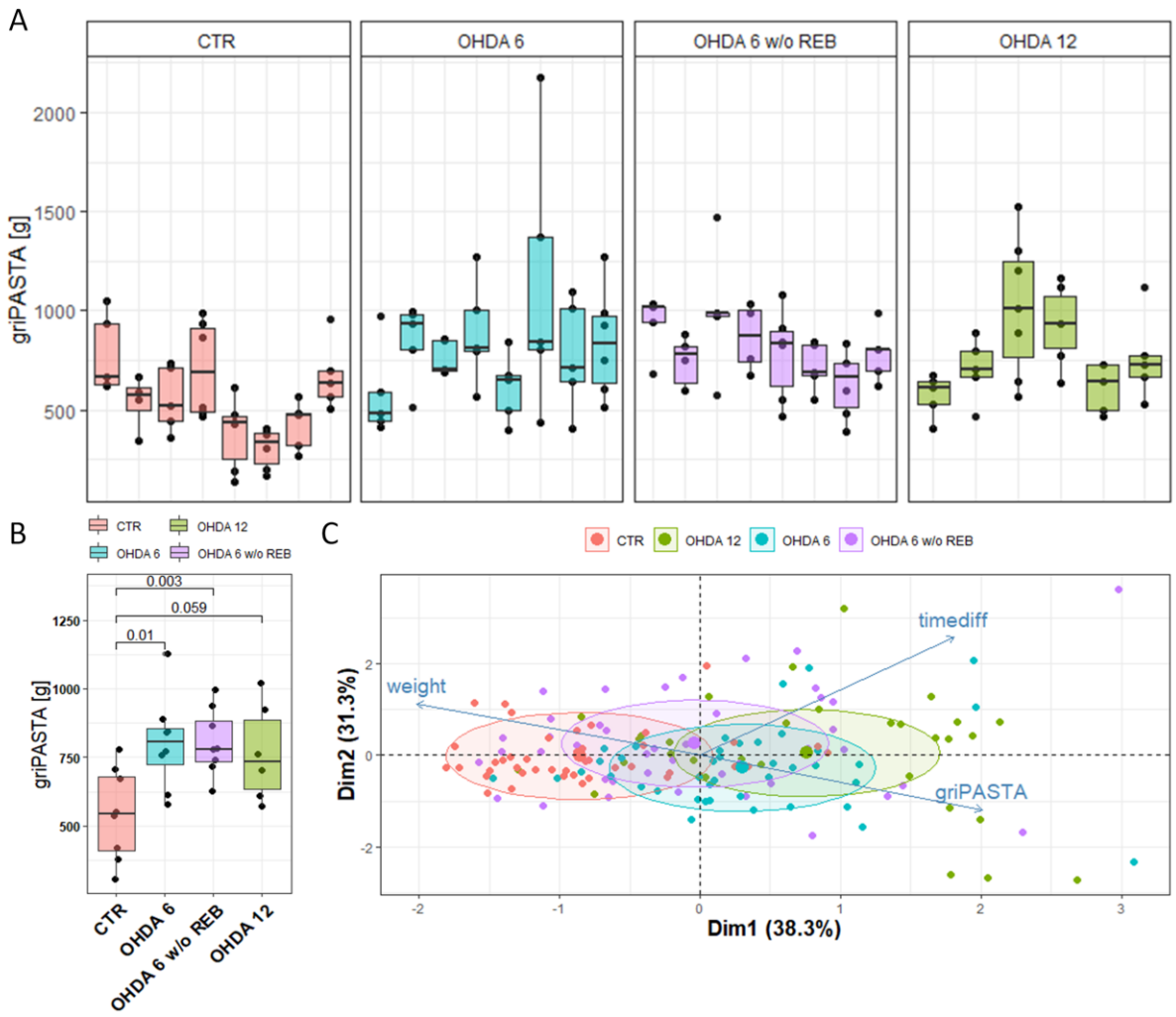


**Fig 3.** The effect of intrastriatal 6-OHDA treatment on the forelimb rigidity of rats analyzed by griPASTA grip strength assessment. **A)** Boxplot of modulus of maximal negative deflection values (Fig 2; B3) with data points corresponding to individual intra-trial measurements for each animal. **B)** Group differences displayed by a group boxplot with data points corresponding to average trial griPASTA value. Group differences were analyzed by means of a two sided Wilcoxon Rank Sum test. **C)** A biplot of first and second dimension obtained by PCA of scaled griPASTA, animal weight and trial speed data. Individual measurements from all trials are depicted and color-coded by groups. griPASTA - maximal negative deflection griPASTA value [g]; timediff - temporal difference between the point of initial and the point of maximal

negative deflection in the respective measurement [ms] ; weight - animal weight [g].

As the dimensionality reduction analysis was suggestive of the potential effects of both trial speed and animal weight on the grip strength measurements, we explored both factors by linear mixed-modeling.

***The effect of animal weight on the grip strength measured with griPASTA***

As mentioned, animal weight could be a potential confounder when measuring grip strength in rodents (Maurissen et al., 2003). In order to assess whether the weight of the animal affected grip strength measured with griPASTA in our experiment, we designed a linear mixed-model with the group, weight (used as a mean-centered variable) and group-weight interaction defined as fixed effects and ID of animals defined as a random effect to control for non-independence of data obtained from multiple measurements (5±1 in a single trial for every animal)(**Table 1; Fig 4**). The model indicated no significant effect of animal weight on the griPASTA value, and a significant difference between groups of 6-OHDA-treated rats in comparison with vehicle-treated controls in this particular experiment.

**Table 1.** Linear mixed-model 1 used to examine the potential effect of the animal weight on the griPASTA performance.

| **griPASTA LMM1:** | | | |
|---|---|---|---|
| **griPASTA ~ group + centeredweight + group*centeredweight + (1\|ID)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | 502.12 | 375.31 – 628.93 | **<0.001** |
| group [OHDA 12] | 254.64 | 65.89 – 443.39 | **0.008** |
| group [OHDA 6] | 342.12 | 165.27 – 518.97 | **<0.001** |
| group [OHDA 6 w/o REB] | 335.89 | 158.73 – 513.05 | **<0.001** |
| centeredweight | 2.23 | -1.35 – 5.82 | 0.223 |
| group [OHDA 12] * centeredweight | -2.76 | -6.89 – 1.36 | 0.189 |
| group [OHDA 6] * centeredweight | 0.51 | -4.68 – 5.69 | 0.849 |
| group [OHDA 6 w/o REB] * centeredweight | -3.69 | -8.00 – 0.62 | 0.093 |
| **Random Effects** | | | |
| $\sigma^2$ | 53769.13 | | |
| $\tau_{00\ ID}$ | 14568.78 | | |
| ICC | 0.21 | | |
| $N_{ID}$ | 30 | | |
| Observations | 158 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.203 / 0.373 | | |

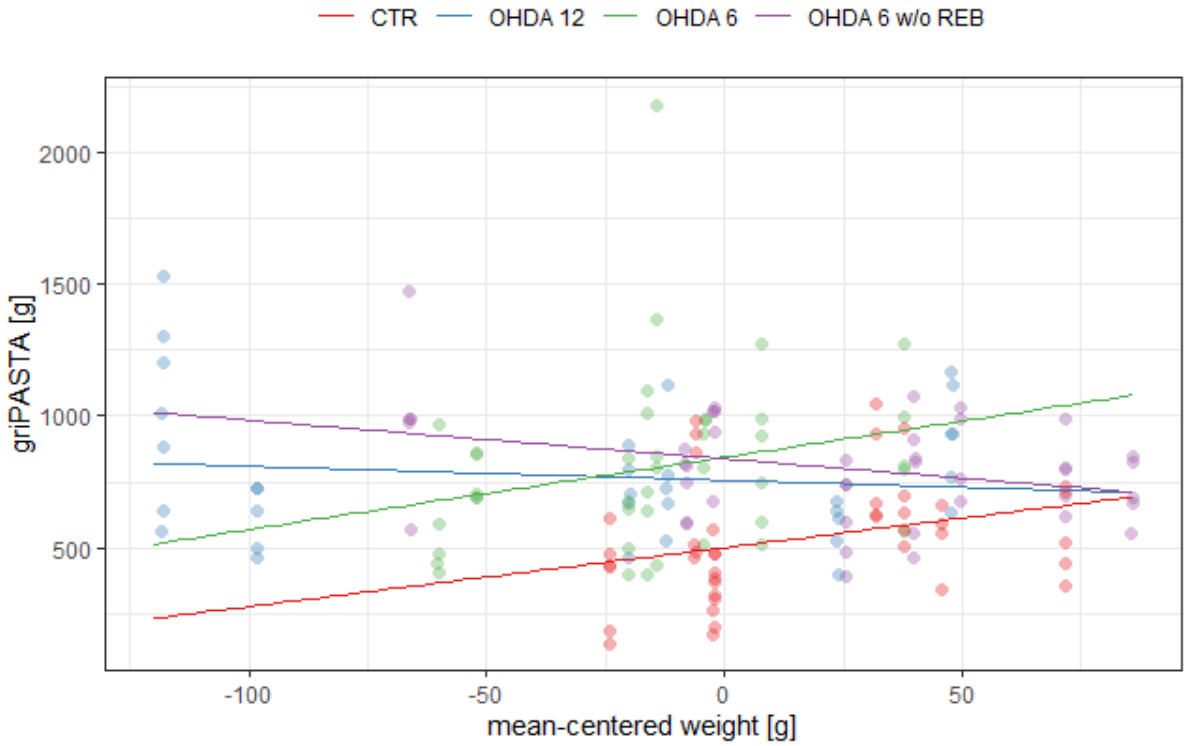


**Fig 4.** A visual representation of the griPASTA LMM 1.

***The effect of initial-to-maximal deflection time on the grip strength measured with griPASTA***

A possible effect of trial speed was also analyzed as it has been discussed as a possible confounder in the context of grip strength assessment in rodents (Maurissen et al., 2003; Takeshita et al., 2017). Here, initial-to-maximal deflection value corresponding times (shown in blue in **Fig 2B**) were used as a proxy for trial speed, as this value indicated how fast the animal was pulled. A total of 158 trials was analyzed (mean = 1398.89; median = 1298.00; SD = 630.31). A relationship between mean-centered time values and griPASTA values was first analyzed by a linear regression with the results suggestive of no effect of trial speed on the griPASTA values in this experiment (**Fig 5**). A linear mixed model was designed to assess

whether the effect will remain insignificant when group, trial speed, and group-trial speed interaction are defined as fixed effects and animal ID defined as a random effect to control for non-independence of data obtained from individual animals. Interestingly, a small effect was observed suggesting association of shorter trials and lower grip strength in controls when compared to OHDA 12 and OHDA 6 w/o REB groups (**Table 2; Fig 6**).

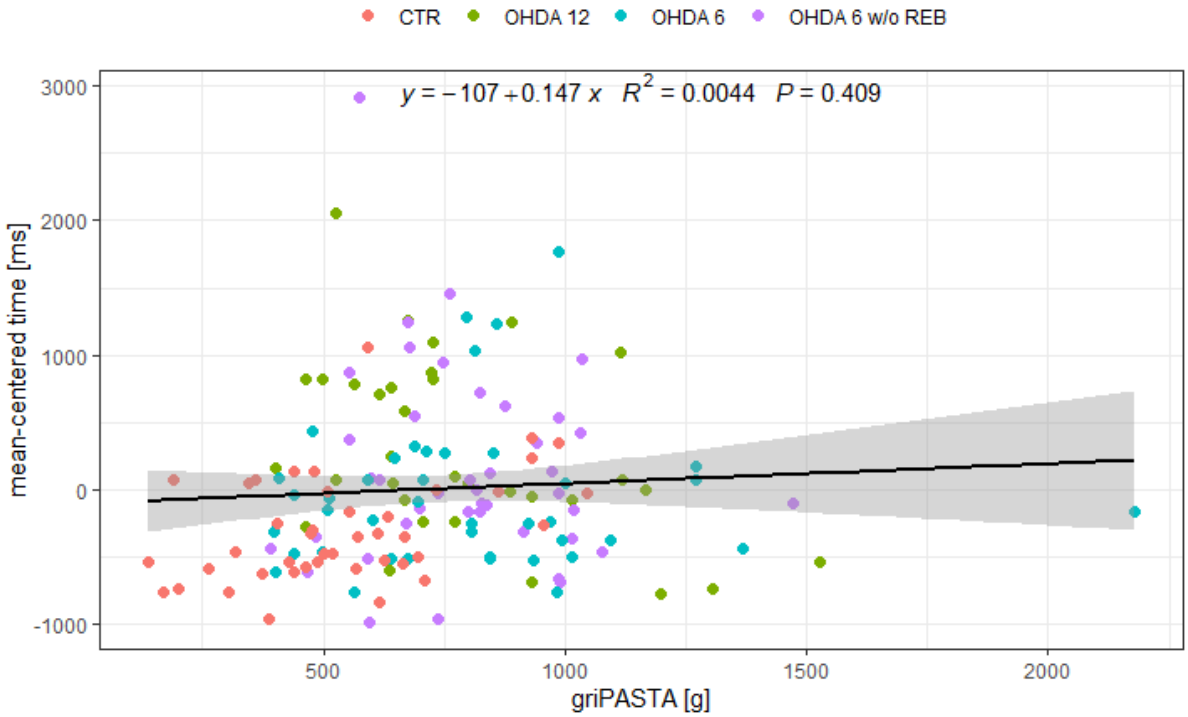


**Fig 5.** Relationship between griPASTA values [g] and mean-centered values of initial-to-maximal deflection value corresponding times [ms] color-coded by groups.

**Table 2.** Linear mixed-model 2 used to examine the potential effect of trial speed on the griPASTA performance.

| **griPASTA LMM2:** | | | |
|---|---|---|---|
| **griPASTA ~ group + centeredtime + group*centeredtime + (1\|ID)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | 596.73 | 475.76 – 717.71 | **<0.001** |
| group [OHDA 12] | 208.90 | 31.88 – 385.92 | **0.021** |
| group [OHDA 6] | 208.86 | 47.02 – 370.69 | **0.011** |
| group [OHDA 6 w/o REB] | 215.80 | 53.66 – 377.94 | **0.009** |
| centeredtime | 0.19 | -0.01 – 0.38 | 0.058 |
| group [OHDA 12] * centeredtime | -0.29 | -0.53 – -0.06 | **0.016** |
| group [OHDA 6] * centeredtime | -0.14 | -0.38 – 0.10 | 0.248 |
| group [OHDA 6 w/o REB] * centeredtime | -0.26 | -0.48 – -0.03 | **0.023** |
| **Random Effects** | | | |
| $\sigma^2$ | 53201.51 | | |
| $\tau_{00\ ID}$ | 13557.61 | | |
| ICC | 0.20 | | |
| $N_{ID}$ | 30 | | |
| Observations | 158 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.196 / 0.359 | | |

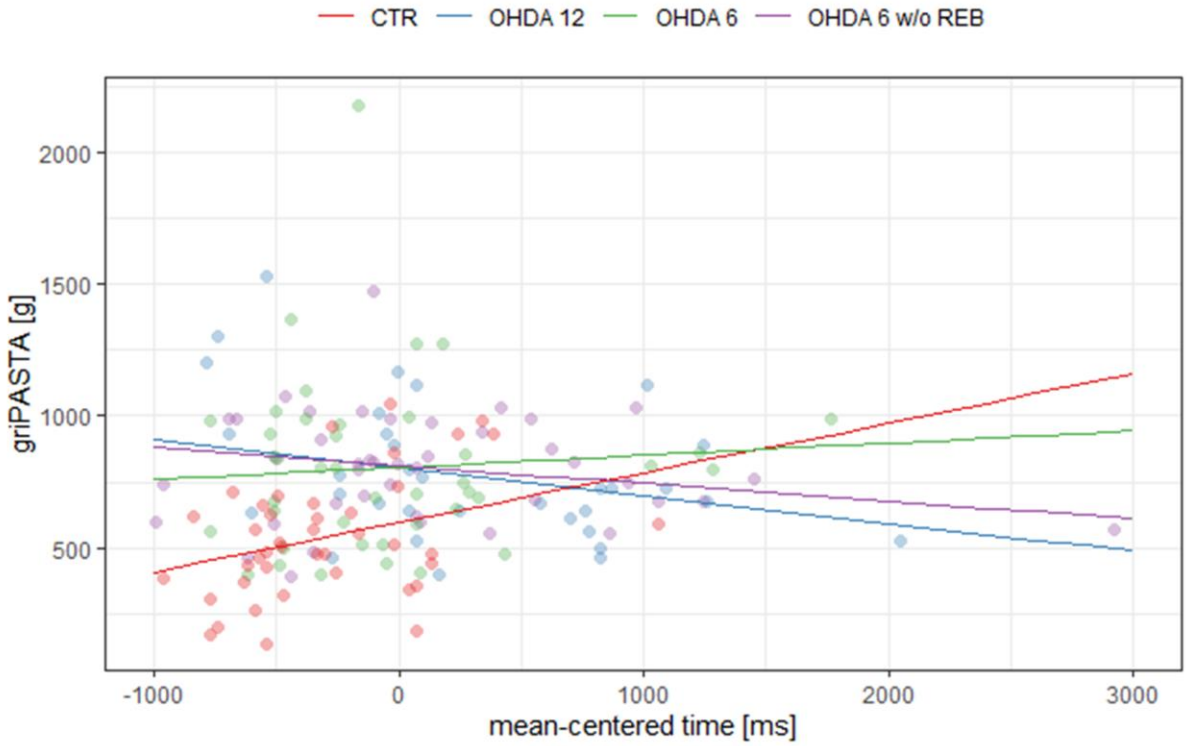


**Fig 6.** A visual representation of the griPASTA LMM 2 with group, centered values of initial-to-maximal deflection value corresponding times and interaction of the two factors being defined as fixed effects and animal ID defined as a random effect. Results are suggestive of a different trend in controls when compared with OHDA 12 and OHDA 6 w/o REB groups.

***Development of the semi-automatic analysis algorithm and implementation in the ratPASTA R package***

Finally, as both grip strength and speed trial analysis yielded interesting insight, a semi-automatic function for easier analysis of data exported from griPASTA was developed and implemented in the ratPASTA package (available from CRAN and GitHub). Briefly, four functions were introduced: *loadgriPASTA, errors, findPeaks,* and *griPASTA* function. The *loadgriPASTA* function imports and handles .pasta data. The

function *errors* is an artifact identifier and processor that calls the interactive plotly-type plot with time values depicted on the x, and griPASTA value on the y axis. Once the user specifies the coordinates of an artifact, the function removes an artifact, returns the processed plot, and asks whether other artifacts are present. The loop continues until there are no artifacts that would introduce noise in the analysis. The function *findPeaks* takes the *errors*-processed data and asks the user for the number of peaks and the estimated length of each trial in seconds. Once the information is provided, the function scans the griPASTA data and returns values of peaks corresponding to maximal negative deflection times (taken as a proxy for grip strength). Both *errors* and *findPeaks* are subfunctions used by the main griPASTA function. griPASTA is essentially a wizard function that takes the griPASTA data, and exploits *errors* and *findPeaks* functions to guide the user through the analysis process and helps with automation of the manual data extraction from the plot. An example of semi-automatic extraction of grip strength from a single animal trial is shown in **Fig 7**.

A

```
> griPASTA(animal_1)
New experiment.
Are there any artifacts present?
1: Yes
2: No
Selection: Yes

Enter starting x coordinate of the
artifact (seconds):34

Enter ending x coordinate of the
artifact (seconds):42

Are there any artifacts present?
1: Yes
2: No

Selection: No

Enter number of peaks:4

Enter estimated timespan of each
peak (seconds):5

time value animal
2 15720 -640.64 animal_1
3 23175 -725.11 animal_1
4 30631 -723.93 animal_1
5 47253 -497.02 animal_1
```

B

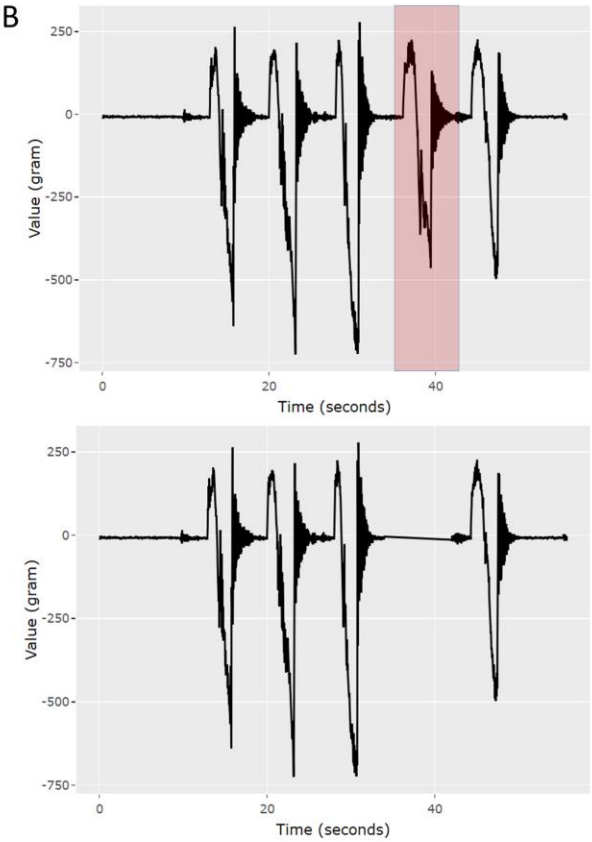


**Fig 7.** An example of the *griPASTA*-assisted grip strength data extraction from the raw griPASTA-obtained data in R. **A)** An example of the user-griPASTA wizard interaction from the R console. **B)** A plotly-type interactive plot called by the *griPASTA* function. For the demonstrative purposes, the fourth experimental measurement (highlighted in red) from the trial was used as an artifact due to the fact that the animal didn't hold onto the grip properly. The trial was marked as an artifact and removed by the *griPASTA* wizard using the *errors* function. Rest of the peaks were automatically extracted by the *findPeaks* and reported to the user in the console (A).

## Discussion

Custom made instruments have been used by different groups over the years to obtain continuous level grip strength data. In 1979, Meyer *et al.* proposed an affordable, rapid

and efficient method for forelimb and hindlimb grip strength assessment in rats and mice based on a mechanical strain gauge (Meyer et al., 1979). In the following years, the proposed method was modified and used for toxicological research by different groups. For example, Mattsson *et al.* used a custom-built device based on a horizontal coarse wire screen attached to a mechanical strain gauge with a clutch which retains the position of the maximal reading in their toxicological analysis of neuropathic consequences of repeated dermal exposure of 2,4-dichlorophenoxyacetic acid (Mattsson, 1986). Development and increased availability of electronics led to increased use of electronic components in custom-made instruments based on the original proposal by Meyer *et al.* (Meyer et al., 1979). In an important study on the factors affecting grip strength testing by Maurissen et al., a custom-built electronic version of the original grip strength apparatus was used (Maurissen et al., 2003). Here, the authors built a device based on the Chatillon DFA series digital force gauge (DFA-R-ND with F31197 10 kg load cell (AMETEK, USA)) connected to a 7 $mm^2$ wire grid with corner support and compared the performance of the custom-built apparatus to the commercial grip strength measurement system 1027DSx (Columbus Instruments, USA). Although custom made instruments for grip strength assessment in rodents have been described in the literature, open-source solutions that would enable researchers to easily implement simple devices into their laboratory practice, use the flexibility of the freely available hardware and software to best adapt the system for their specific experimental goals, are scarce. One example of a custom-built instrument is a bilateral grip strength assessment device designed by Kivitz and Lake (Kivitz and Lake, 2017), and used for grip analysis in a rat model of post-traumatic elbow contracture (Reiter et al., 2019). The importance of modularity of the grip strength performance system is further corroborated in the study by Maurissen *et al.*

(Maurissen et al., 2003). Here, the researchers bought a commercial instrument, but also built their own, with main motivation for such effort being the concern that sampling rate and platform support, both fixed and unmodifiable in the commercial setup, might significantly affect the outcome of the grip strength measurements (Maurissen et al., 2003).

We describe a simple, low cost and robust system for quantitative assessment of grip strength performance in rodents based on an open-source hacked digital kitchen scale multimodal platform called PASTA (Virag et al., 2020). The idea behind griPASTA is to record the force exerted by an animal resisting experimenter who is trying to remove it by the tail from the grip test platform placed on top of the PASTA system. The platform records the force with high temporal resolution and stores the unprocessed data, enabling flexibility in the process of subsequent data analysis. Consequently, griPASTA platform is able to provide more information on animal grip strength performance in comparison with most of the expensive commercial products used for this purpose. Furthermore, in contrast to commercial equipment that is usually relatively static in terms of design, griPASTA is characterized by simple and modular hardware based on readily available equipment in the animal research setting. In further text, we briefly discuss advantages of griPASTA open-source hardware and software in the context of grip strength assessment in rodents, with a special focus on trial speed and grip strength testing angle, as both factors have been recognized in the literature as potential confounders in grip strength testing, and commercial platforms offer limited solutions for their control.

***Trial speed***

Commercial instruments for rodent grip strength assessment standardly measure maximal force the animal is able to resist while being removed by the tail from the test platform. Maximal force the animal is able to resist corresponds to the maximal negative deflection value in the griPASTA plot (**Fig 2B-3**). In other words, in the griPASTA plot, the greater the negative deflection, the stronger the grip of the animal. However, griPASTA provides additional information closely related to this particular metric that might prove to be invaluable in the specific experimental context. For example, approximations of trial speed can be calculated from raw data provided by griPASTA. As briefly discussed in the results section, trial speed has been considered as a potential confounding factor that might influence grip strength assessment in rodents (Maurissen et al., 2003; Takeshita et al., 2017). However, potential influence of trial speed on the grip strength performance is hard to assess, let alone quantify. Maurissen et al. (2003) examined whether the grip strength performance was dependent on the speed of the trial in their work focused on factors affecting the grip strength testing. Here, the authors classified trials as either “slow”, “medium” of “fast”, with “medium” arbitrarily defined as the speed at which grip trials are performed under “normal” testing conditions (Maurissen et al., 2003). The authors concluded that the trial speed didn’t exert a significant effect on the grip performance in their study on healthy animals, but data from slow trials demonstrated less variability (coefficient of variation (CV) = 0.14) in comparison with data from medium or fast trials (CV = 0.21 and 0.23 respectively). Interestingly, slower trials also demonstrated the highest CV in terms of trial duration indicating it was more challenging for the experimenter to control the actual duration of the trial when slower movement speed was used (Maurissen et al., 2003). Nevertheless, a potential confounding effect of trial speed should not

automatically be disregarded, as indicated by our POC study in 6-OHDA-treated rats, where the interaction effect of trial speed and group variable was observed (**Table 2., Fig 6.**). In this experiment, although no clear main effect of trial speed (inferred from initial-to-maximal deflection time) was observed (**Fig. 5**), a confounding potential, masked by the noise introduced by pooled analysis of animals from different groups, was uncovered by LMM2 (**Table 2., Fig 6.**). Data from the experiment with 6-OHDA-treated rats suggests trial speed should be taken into account during grip strength assessment as it might differently affect grip strength in animals with and without underlying motor system-related pathology. However, it should be taken into account that average trial duration in 6-OHDA POC experiment was 1398.89 ms (SD=630.31), and Maurissen et al. reported an average duration of “slow” trials of 690 ms in their study with SD approximated by WebPlotDigitizer being around 273 ms (Maurissen et al., 2003). Additionally, the real difference in trial duration is hard to assess due to the fact that a different approach was used for trial speed approximation by us and Maurissen et al. (Maurissen et al., 2003). Apart from the study by Maurissen et al., we are not aware of any other reports considering a potential influence of trial speed in the context of grip strength assessment in rodents, probably due to the fact that commercial solutions were not designed to take potential trial speed interference into account, and custom-made solutions are seldomly used and rarely provide trial duration information. Consequently, apart from the fact that trial speed might be an important factor to take into account in animal experiments where grip strength is measured, the real effect of trial speed on the grip strength performance in rodents remains to be explored. Availability of open-source solutions that provide raw trial data such as the griPASTA platform, might improve the overall quality and reproducibility of grip strength experiments in rodents as they offer tools for better understanding of

different factors affecting grip strength assessment with trial speed being one obvious example.

***Trial angle***

Standard rodent grip strength assessment protocols are based on horizontal systems where the animal is pulled backwards by the experimenter after it grasps the grip strength meter platform (Aartsma-Rus & van Putten, 2014; Cabe et al., 1978). Nevertheless, the angle at which the animal is pulled away from the platform has been recognized as a factor affecting the overall grip strength assessment (Maurissen et al., 2003; Takeshita et al., 2017). Consequently, Takeshita *et al.* proposed a simple modification of the standard protocol based on vertical rotation of the platform to improve the reliability and consistency of the conventional method (Takeshita et al., 2017). Vertical rotation of the platform ensures the animals are maximally motivated to keep grasping the bar while being pulled. Takeshita et al. compared the modified vertical test with the conventional protocol in a cohort of 16 week old male and female C57BL6 mice. Modified test demonstrated lower inter-trial and inter-day variability, when compared to the conventional protocol. Interestingly, the reported effect was more pronounced in males, and inter-day variability was significantly lower in females than in males in the conventional protocol (Takeshita et al., 2017). Likewise, although modified protocol demonstrated lower overall variability in young animals, the inter-day variability was lower for the conventional test in comparison with modified test when old (two years of age) mice were tested (Takeshita et al., 2017). In the case of grip strength comparison between male and female mice, and young and old, a modified protocol was found to be more reliable (Takeshita et al., 2017). Platform angle is another parameter that affects the measurements, as it determines the way the

animal is going to grab the platform while being pulled. For this reason, platform angle is usually flexible in the commercial setups and can be set at a desirable angle in the predefined range (eg. https://www.sandiegoinstruments.com/wp-content/uploads/2018/08/Grip-Strength-data-sheet.pdf). One-size-fits-all principle cannot be applied to the grip strength assessment. Consequently, maximal modularity of the design of a grip strength apparatus is important as it enables the researchers to adapt the instrument for a specific experimental purpose. A fully open-source hardware and software ensures maximal flexibility of the griPASTA platform. A griPASTA setup used in the POC experiment exploited a horizontal grip platform made from a rodent cage lid (**Fig 1C**). More specifically, we found the 2184L Eurostandard Type II L cage with dimensions of 365x207x140 mm to be the best fit as the grid arrangement was optimal for the forelimb grip in rats and enabled a reduction in grip variability between subjects once the duct tape was placed on the lateral side of the cage lid. Besides, the platform and the load cell angle of the device can be modified in various ways. A horizontal pull preferred by some laboratories can be measured by either constructing a holder for the apparatus, or introducing a simple pulley (**Fig 8A**). Furthermore, a vertical setup, as proposed by Takeshita *et al.*, can be created simply by placing the griPASTA on the holder (**Fig 8B**) (Takeshita et al., 2017). Additionally, griPASTA can be connected to a cheap and widely available load cell fixed permanently in the vertical setup (**Fig 8C**), and the instrument can be plugged in either a kitchen scale-based platform or in the vertically positioned strain gauge depending on the requirements of a specific experiment.

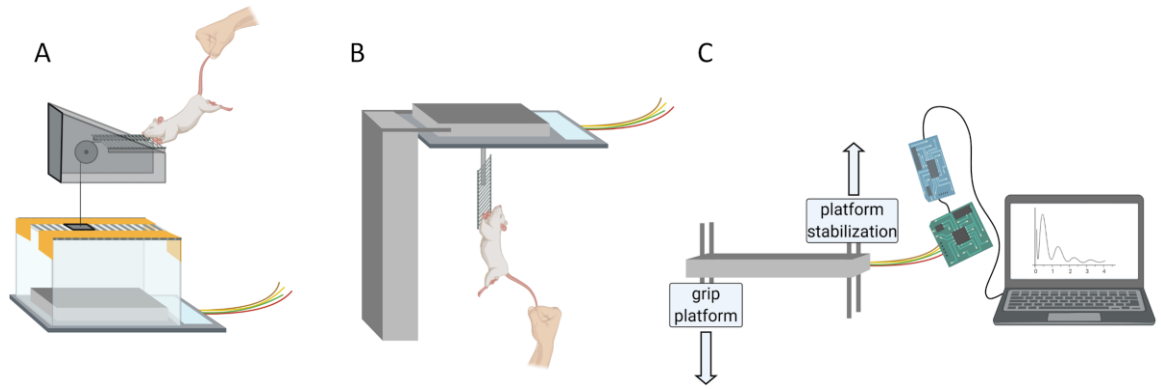


**Fig 8.** A schematic representation of modular design of griPASTA adapted for different grip strength measurement strategies. A) A variant of the griPASTA grip strength assessment with pulley-based force transduction to ensure the horizontal pull. B) A vertical griPASTA setup, as proposed by Takeshita *et al.*, with a custom holder used as a griPASTA platform to align the load cell displacement vector with the pulling motion, and ensure maximal motivation of the animal to resist being removed from the platform. C) For easier implementation of the vertical setup, introduction of an additional load cell is proposed, so the experimenter can simply plug in griPASTA system either in a standard kitchen scale-based setup, or in a single load cell-based vertical setup with minimal modifications.

***Grip strength as an indicator of muscular rigidity in rat models of Parkinson's disease***

For further validation of the griPASTA setup, we conducted grip strength analysis in the rat model of PD as previous reports indicated that administration of 6-OHDA might be followed by development of muscular rigidity in rats. Alongside akinesia, bradykinesia, dystonia, resting tremor and gait abnormalities, muscular rigidity, has been considered an important symptom of PD, and important feature of successful animal models of PD (Meredith & Kang, 2006; Potashkin et al., 2010). Muscular

rigidity, characterized by an increased resistance to passive movement, has been proposed to emerge as a result of potentiation of long-latency electromyographic components related to the stretch reflex and to co-activation of antagonistic muscles (Wolfarth et al., 1996). Among other animal models of PD, it has been suggested that 6-OHDA treated rats successfully mimic Parkinson-like muscular rigidity (Cenci et al., 2002; Wolfarth et al., 1996). Wolfarth *et al.* reported that bilateral administration of 1ul of 6.5 µg/µl 6-OHDA into substantia nigra induces muscle rigidity reflected both by mechanomyographic and movement-induced reflex electromyographic activity (Wolfarth et al., 1996), and similar results have been reported following intrastriatal administration of 4 µg of 6-OHDA (Klockgether et al., 1987). Quantification of rodent muscular rigidity has been considered a daunting and challenging task and a number of different assays have been proposed over the years (Meredith & Kang, 2006). For example, a forced lateral movement test has been used for quantification of muscular rigidity. Here, the experimenter places one hand along the side of the rat and gently pushes the animal laterally for 90 cm at the approximate speed of 20 cm/s on a stainless-steel surface and records the number of steps or distal forelimb adjustments (Lindner et al., 1999). Others have used a grasping test as a quantitative tool for assessment of rigidity as it has been shown that a prolonged grasping response is correlated with direct measures of muscle rigidity (Jolicoeur et al., 1991). For example, Sotnikova *et al.* reported increased rigidity in dopamine transporter knockout mouse model of PD measured by the grasping test (Ma et al., 2014; Sotnikova et al., 2005), and Ma *et al.* reported increased rigidity by the same method in medial forebrain bundle 6-OHDA-treated rats (Ma et al., 2014). Being closely related to the grasping test, some authors proposed a grip strength test might also reflect rigidity in the animal models of PD. Interestingly, following a unilateral administration of 6-OHDA into the

nigrostriatal bundle, a marked increase in the grip strength on the contralateral side was observed (Dunnett et al., 1998). The original findings by Dunnett *et al.* were replicated, however, in a later study by Jeyasingham *et al.*, an increase in grip strength was also observed ipsilaterally following unilateral administration of 6-OHDA into the nigrostriatal bundle (Jeyasingham et al., 2001). Although the mechanism of the increased grip strength remains to be elucidated, it has been proposed that it reflects mechanisms closely related to those mediating nigrostriatal degeneration-induced rigidity in human PD (Dunnett et al., 1998). As some studies reported that the grasping test indicates increased muscular rigidity in different rodent models of PD (Ma et al., 2014; Sotnikova et al., 2005), and earlier reports suggested that bilateral 6-OHDA lesions induced active resistance to physical displacement (Dunnett et al., 1998), in the POC experiment, we exploited griPASTA system to assess muscular rigidity following bilateral intrastriatal 6-OHDA administration. We considered previous reports of increased grasping test performance as a potential indicator of bilateral rigidity and hypothesized that, although bilateral grip strength measurements are seldom reported in this context, quantitative nature and sensitivity of griPASTA might reveal a difference in rigidity between control and PD rats. Furthermore, previous reports suggested that intrastriatal lesions induce much less pronounced muscular rigidity in comparison with nigrostriatal lesions (Jeyasingham et al., 2001). Nevertheless, we noticed some motor rigidity while handling rats following the induction of intrastriatal lesions, so we were motivated to explore whether the perceived differences would be detectable with a quantitative griPASTA grip strength assessment protocol. Interestingly, our results suggested that the dose of 6 µg of 6-OHDA per ventricle, administered with or without reboxetine pretreatment induced a pronounced muscular rigidity after bilateral intrastriatal administration of the toxin when compared to the

control group (**Fig 3A** and **Fig 3B**). Increased muscle grip strength was also observed in the group treated with 12 µg of 6-OHDA per ventricle, and no significant difference in muscular rigidity was observed between different groups that received the toxin (**Fig 3A** and **Fig 3B**). The result of our POC study in the rat model of PD indicates that a significant muscular rigidity that develops in the 6-OHDA rat model of PD, usually examined by means of the grasping test might also be assessed by the quantitative grip strength test. Additionally, taking into account the limitations of the standard grasping test, such as the effect of body weight, and problem of its linearity, as well as the choice of the cut-off time and subsequent statistical problems related to the ceiling effects (Liu & Wang, 2020), the grip strength test should be explored as an alternative solution for quantification of muscular rigidity in this context. The latter is especially true considering that open-source low cost solutions such as griPASTA enable quantification without sophisticated and expensive research equipment. Finally, our results confirm previous findings of muscular rigidity as a pathological hallmark of 6-OHDA-lesioned rats and encourage further investigation of this often marginalized symptom in animal PD research.

The griPASTA project is fully open-source and is described in concordance with the proposed principles of open-source bioinstrumentation (Booeshaghi et al., 2019), gaining considerable attention in recent years (Maia Chagas, 2018; Pearce, 2012, 2014; White et al., 2019). As already emphasized by others, apart from significant economic advantages of open-source lab equipment (Dolgin, 2018), the main strength of open-source bioinstrumentation stems from modularity, flexibility and the active engagement of the scientific community to implement and further improve freely available hardware and software solutions (Booeshaghi et al., 2019). In this context,

we consider griPASTA to be a work in progress, and a project we remain actively engaged with, and use this opportunity to invite our colleague scientists to offer their opinions and suggestions and become actively involved in its further development.

## Conclusion

griPASTA is a simple open-source PASTA-based (Virag et al., 2020) solution for grip strength assessment in rodents that can be made on the cheap using readily available utensils. The whole setup consists of a hacked kitchen scale connected to a computer and a mouse cage used as a grip platform. Apart from the cost, griPASTA provides several advantages over the commercially available solutions as the platform provides raw data which makes it compatible with different data analysis strategies, and allows the researcher to control for potential confounding factors such as trial speed. Additionally, griPASTA open-source hardware and design are maximally flexible which makes the platform highly customizable, so with minimal modifications both platform and load cell angle can be easily adapted. Finally, our POC experiment suggests that griPASTA provides a good insight into motor dysfunction and rigidity in the 6-OHDA rat model of Parkinson's disease. Considering the observed differences, robustness, low cost and simple design of griPASTA platform, we believe that the proposed quantitative grip strength assessment protocol should be further explored in different animal models as it might provide important additional information on motor dysfunction in a rapid and economical way.

## Data and code availability statement

A general-purpose python script for extraction of data from the PASTA platform (PASTAgp) is available on GitHub at https://github.com/davorvr/pasta-gp

Data from the POC experimental trial (ID.pasta) and a video example of a single measurement trial are available on GitHub at https://github.com/janhomolak/gripasta

ratPASTA is available on CRAN and on GitHub (https://github.com/ikodvanj/ratPASTA).


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## Conflict of interest

None.

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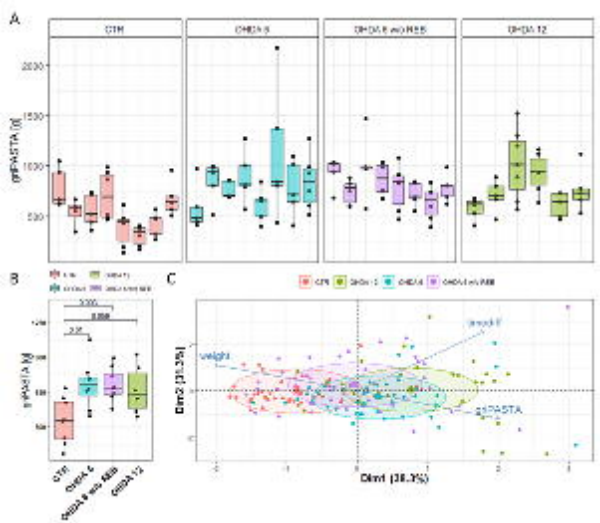
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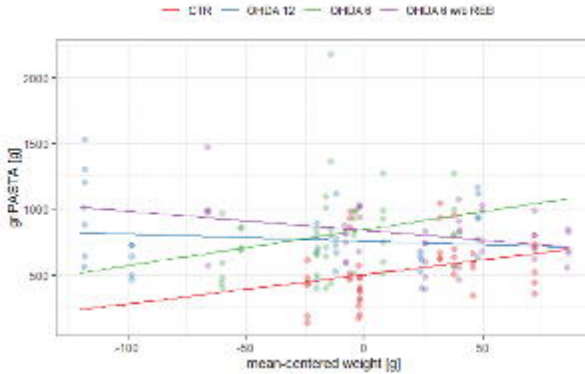
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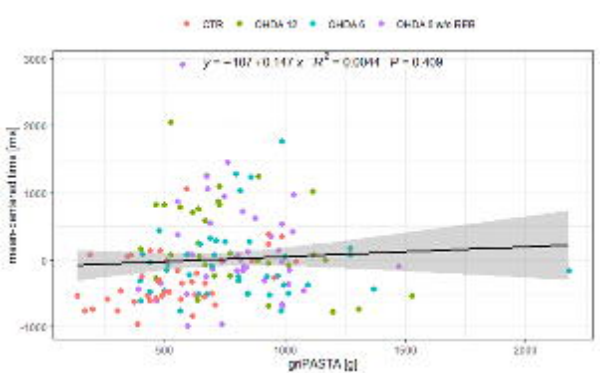
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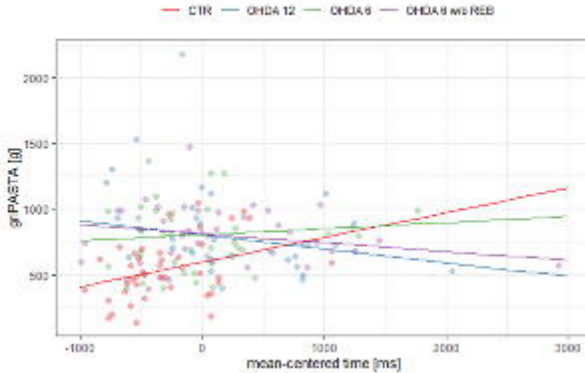
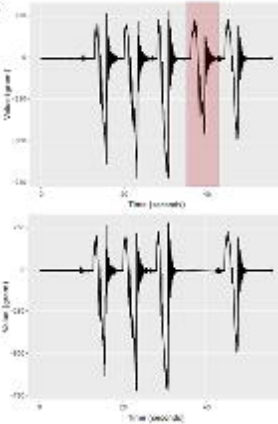

CTR
OHDA 12
OHDA 6
OHDA 6 w/o REB
gr PASTA [g]
2000
1500
1000
500
-1000
0
1000
2000
3000
mean-centered time [ms]

A

```
> griPASTA(animal_1)
New experiment...
Are there any artifacts present?
1: Yes
2: No
Selection: Yes

Enter starting x coordinate of the
artifact (seconds):34

Enter ending x coordinate of the
artifact (seconds):42

Are there any artifacts present?
1: Yes
2: No

Selection: No

Enter number of peaks:4

Enter estimated timespan of each
peak (seconds):5

time value animal
2 15720 -640.64 animal_1
3 23175 -725.11 animal_1
4 30631 -723.93 animal_1
5 47253 -497.02 animal_1
```

B



A
B
C

**griPASTA LMMI:**

**griPASTA ~ group + centeredweight + group*centeredweight + (1|ID)**

| *Predictors* | *Estimates* | *CI* | *p* |
|---|---|---|---|
| (Intercept) | 502.12 | 375.31 – 628.93 | **<0.001** |
| group [OHDA 12] | 254.64 | 65.89 – 443.39 | **0.008** |
| group [OHDA 6] | 342.12 | 165.27 – 518.97 | **<0.001** |
| group [OHDA 6 w/o REB] | 335.89 | 158.73 – 513.05 | **<0.001** |
| centeredweight | 2.23 | -1.35 – 5.82 | 0.223 |
| group [OHDA 12] * centeredweight | -2.76 | -6.89 – 1.36 | 0.189 |
| group [OHDA 6] * centeredweight | 0.51 | -4.68 – 5.69 | 0.849 |
| group [OHDA 6 w/o REB] * centeredweight | -3.69 | -8.00 – 0.62 | 0.093 |
| **Random Effects** | | | |
| $\sigma^2$ | 53769.13 | | |
| $\tau_{00\ ID}$ | 14568.78 | | |
| ICC | 0.21 | | |
| $N_{ID}$ | 30 | | |
| Observations | 158 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.203 / 0.373 | | |

**griPASTA LMM2:**

**griPASTA ~ group + centeredtime + group*centeredtime + (1|ID)**

| *Predictors* | *Estimates* | *CI* | *p* |
|---|---|---|---|
| (Intercept) | 596.73 | 475.76 – 717.71 | **<0.001** |
| group [OHDA 12] | 208.90 | 31.88 – 385.92 | **0.021** |
| group [OHDA 6] | 208.86 | 47.02 – 370.69 | **0.011** |
| group [OHDA 6 w/o REB] | 215.80 | 53.66 – 377.94 | **0.009** |
| centeredtime | 0.19 | -0.01 – 0.38 | 0.058 |
| group [OHDA 12] * centeredtime | -0.29 | -0.53 – -0.06 | **0.016** |
| group [OHDA 6] * centeredtime | -0.14 | -0.38 – 0.10 | 0.248 |
| group [OHDA 6 w/o REB] * centeredtime | -0.26 | -0.48 – -0.03 | **0.023** |
| **Random Effects** | | | |
| $\sigma^2$ | 53201.51 | | |
| $\tau_{00\ ID}$ | 13557.61 | | |
| ICC | 0.20 | | |
| $N_{ID}$ | 30 | | |
| Observations | 158 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.196 / 0.359 | | |