Title: More running causes more ocular dominance plasticity in mouse primary visual cortex: new gated running wheel setup allows to quantify individual running behaviour of group-housed mice.

Abbreviated title: More running, more ocular dominance plasticity

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Abstract

Environmental enrichment boosts neuronal plasticity of standard-cage raised (SC) mice. We previously showed that wheel running during 7 days of monocular deprivation (MD) can restore ocular dominance (OD)-plasticity in the primary visual cortex (V1) of adult SC-mice beyond postnatal day (P) 110 (P160). It remained – however – unknown whether individual wheel running parameters boost OD-plasticity differently and whether running for just 4 days would also boost OD-plasticity in younger mice aged 50-100 days (P70).

To this end, we designed a gated running wheel setup (gRW) attached to the home cage, and tracked individual running wheel activity of group-housed mice. Mouse vision was checked by optometry and OD-plasticity in V1 was visualized using intrinsic signal optical imaging. As hypothesized, wheel running during MD boosted OD-plasticity in both P160 and P70 mice: after 4d/7d of MD (P70/P160), the OD-index, comparing ipsi- and contralateral eye induced V1-activation, dropped from 0.25±0.04 without MD to 0.12±0.03 with MD in P70 (t-test $p<0.01$), and from 0.24±0.04 to 0.05±0.04 in P160 mice (t-test $p<0.01$). Quantitative comparison of running parameters yielded that average running distance, running time and speed were similar in P70 and P160 mice, but P70 mice ran more bouts (P70/P160: 19.5±3.3/17.0±3.3 bouts/d, t-test $p<0.001$) of shorter durations (P70/P160: 2.9±0.5/5.0±0.5 min, t-test $p<0.05$). Most notably, individual running parameters correlated with individual OD-indices after MD: mice running longer distances, for longer time, at higher velocity and with longer bouts displayed a stronger OD-shift, i.e. animals showed more experience-dependent V1-plasticity (Pearson $r^2=0.42$, $p=0.022$).

Significance statement

The link between individual choices and brain physiology has become a rising topic in systems neuroscience. With our newly developed gated running wheel setup, we observed a striking correlation between individual running activity, and experience-dependent plasticity in mouse primary visual cortex. More running caused more plasticity: *running speed, running distance, total running time, number of running bouts and bout duration all significantly correlated with a measure of visual cortical plasticity, the ocular dominance index.* Thus, our observations add to the growing body of evidence that individual behavioural choices can strongly affect individual brain plasticity.

Introduction

The link between individual choices and brain physiology has become a rising topic in systems neuroscience, and reflects the growing importance to study individuals, rather than populations by giving meaning to variability in biological data. While the natural environment of humans and mice is complex, that of laboratory rodents has been simplified and standardised in the past decades to an extent that deprives the animals of essential behaviours (Löwel et al., 2017). Furthermore, standard cage rearing (SC) leaves minimal room for the expression of individual traits and both behavioural and physiological inter-individual variability is further reduced by using inbred strains lacking genetic variability. Recently, a number of laboratories has started to systematically investigate the impact of less deprived rearing conditions on brain wiring and plasticity, demonstrating that raising animals in a so-called “enriched environment” elicits remarkable effects on the brain across molecular, anatomical, and functional levels when compared to animals raised in an SC environment (Sale et al., 2007; Fabel et al., 2009; Baroncelli et al., 2010; Di Garbo et al., 2011; Greifzu et al., 2014; Kalogeraki et al., 2014; Löwel et al., 2017; Stryker and Löwel, 2018; Bogado Lopes et al., 2023).

Both environmental enrichment and voluntary wheel running boost ocular dominance (OD) plasticity in mouse primary visual cortex (V1) (Greifzu et al., 2014; Kalogeraki et al., 2014). OD-plasticity is typically induced after monocular deprivation (MD), which shifts V1-activation strength towards the open eye (Cang et al., 2005; Gordon and Stryker, 1996). During the critical period of visual system development of SC mice, a 4-day MD is sufficient to induce an OD-shift in V1 intrinsic signal optical imaging responses to drifting bars, which is quantified using an OD-index (Cang et al., 2005). The required MD duration to induce OD-plasticity in SC-animals increases with age: While SC-mice between postnatal day 50 to 90 (P70) require 7 days of MD for significant changes in OD-index (Lehmann and Löwel, 2008; Sato and Stryker, 2008), this reaches ~7 weeks in mice older than postnatal day 110 (Hosang et al., 2018). Importantly, providing a running wheel to P160 SC-mice was sufficient to re-enable OD-plasticity after just one week of MD (Kalogeraki et al., 2014). While it is clearly established that running increases OD-plasticity in P160 mice (Kalogeraki et al., 2014; Kaneko and Stryker, 2014) the consequences of behavioural inter-individual variability of wheel running on experience-dependent V1-plasticity has not yet been studied. Moreover, it is unknown whether younger animals would also benefit from short-term running during MD.

The aim of this study was therefore to analyse the effects of individual running wheel activity on OD-plasticity in group housed P70 and P160 mice. As tracking individual wheel running activity of group housed mice is still challenging (Mayr et al., 2020; Reuser et al., 2022), we designed a custom-built gated running wheel (gRW) setup that allowed individual tracking of mice implanted with an RFID sensor while using a running wheel accessible from their home cage. We quantified various parameters of running activity and tested whether they correlated with the OD-index of individual P70 and P160 mice. The OD-index compares V1-activation via the ipsi- and contralateral eye and thus quantifies the magnitude of OD-shift (Cang et al., 2005). As hypothesized, gRW housing boosted OD-plasticity in both P70 and P160 animals. Average running distance in the gRW setup was similar to published values of group housed mice with running wheels provided directly in the home-cage (Kalogeraki et al., 2014; Plenz and Kanold, 2021). Importantly, our newly designed experimental gRW setup allowed us to correlate the macro (running distance, time and speed) and micro (bout number and duration) architecture of running wheel activity of individual mice with their individual OD-index: our data

clearly show a strong correlation between running wheel parameters of individual mice and their OD-index quantifying OD-plasticity after MD that was absent in no MD control animals: mice who ran more and longer distances displayed stronger OD-shifts.

Methods:

Animals and rearing conditions

Female C57Bl6/J mice were group-housed on a 12 h light dark cycle and lived in standard cages (SC) until the day an MD was performed. Mice were provided with ad libitum food and water. Two age groups were used for this study: P70 mice (age range P46-86) and P160 mice (age range P127-225). All experimental procedures complied with the National Institutes of Health guidelines for the use of laboratory animals and were approved by the local government of lower saxony, Germany (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit).

Monocular deprivation and implantation of RFID tags

To ensure that experimental mice had regular vision before induction of OD-plasticity, we tested the spatial frequency threshold (SFT) of the optomotor reflex of both eyes before MD (Prusky et al. 2004; Lehmann and Löwel, 2008). SFT values were within published values and differed by less than 0.02 cyc/deg between the two eyes in all mice. The right eye was deprived for 4 days (d) (P70) or 7 d (P160) according to published protocols (Gordon and Stryker, 1996; Cang et al, 2005; Greifzu et al., 2014). The eyes of the animals were examined daily to ensure that eyes remained healthy and the MD-eye stayed closed. Optomotor reflexes of the open eye were tested daily after MD including the day of intrinsic signal optical imaging.
Cylindrical glass-covered radio frequency identification devices (RFID; length: 12.5 mm; diameter: 1.93 mm, Sparkfun Electronics, Colorado, USA) were implanted during the MD or noMD (control without MD) surgery to minimize mouse discomfort. First, a patch of fur was shaved in the nape of the animal's neck using small surgical scissors, then a local anaesthetic gel (Lidocaine, xylocaine gel 2 %, Aspen Pharma Trading Limited, Ireland) was applied to the exposed skin. A small incision was made and the chip carefully placed subcutaneously in the scruff to make it difficult for the mice to pick at and thereby reducing the risk of an infection. The incision was sealed with a simple interrupted stitch (suture material: 7-0 Perma-Hand silk, Ethicon, 8.0mm diameter). After MD/RFID implantation and complete recovery from anaesthesia, mice were moved to a gRW setup.

Optical imaging of intrinsic signals and visual stimuli.

Surgery. Surgical preparations for optical imaging were performed as published (Cang et al., 2005; Greifzu et al., 2014). Briefly, mice were box-anaesthetised with 2% halothane in O_2 and N_2O (1:1) and injected with atropine (5mg/kg, s.c.; Franz Köhler Chemie), dexamethasone (8mg/kg s.c.; Merck), and chlorprothixene (8mg/kg, i.m.; Sigma-Aldrich). After placing animals in a stereotaxic frame, anaesthesia was maintained with 0.8% halothane in a 1:1 mixture of O_2 and N_2O.

Data acquisition and visual stimulation (Kalatsky and Stryker, 2003; Cang et al, 2005). Mouse V1 responses were recorded through the skull using the "Fourier" imaging method of Kalatsky and Stryker (2003) and optimised for the assessment of OD-plasticity (Cang et al., 2005). V1-signals were visualised with a CCD camera (Dalsa 1M30) using a 135x50 mm tandem lens configuration (Nikon) with red illumination light (610±10 nm). Active brain regions absorb more red light and appear darker in the images. Frames were acquired at a rate of 30 Hz, temporally binned to 7.5 Hz, and stored as 512x512-pixel images after spatial binning of the camera image. Visual stimuli were presented on a 60 Hz refresh rate monitor (21 inches; Accuvue HM-4921-D, Hitachi) positioned 25 cm from the eyes. Stimuli consisted of white drifting horizontal bars (2° wide) as described previously (Greifzu et al., 2014). The amplitude component of the optical signal represents the intensity of neuronal activation (expressed as fractional change in reflectance times 10^{-4}) and was used to calculate an ocular dominance (OD) index. At least three maps per animal were averaged to compute the OD-index as (C+I)/(C-I), with C and I representing the response magnitudes of each pixel to visual stimulation of the contralateral (C) and ipsilateral (I) eye.

Gated running wheel setup (gRW)

In order to assess individual running wheel (RW) behaviour, we developed an open source gated running wheel cage, which served as the home cage for the duration of the experiment. It consisted of a standard size rat cage (43cm x 27cm) connected to a separate gRW-compartment, equipped with the same RW as used in the enrichment cages in Greifzu et al., 2014 (Figure 1A). Wheel diameter is 12.5 cm, so that the calculated RW-circumference was 39.3 cm. RW-turns were registered using a hall sensor for detecting motion of two magnets attached at opposing sites of the RW. The mice needed to pass through a seesaw in order to reach the RW-compartment. Entering would flip the seesaw blocking other mice from entering (see video 1 and 2). To ensure the seesaw remained closed while a mouse was inside the RW-compartment, the seesaw was actively blocked from flipping back. When activity was detected by beam break sensors outside the RW-compartment, an electromagnet was activated to hold the seesaw closed. Attempts of mice to exit the RW-compartment were detected using beam break sensors inside the RW-compartment, which would deactivate the seesaw magnet. Seesaw position was registered through a roller switch.
Mice were chipped with radio frequency identification devices (RFID). The RFID-tags transmitted the encrypted individual ID to a USB-driven reading device (ID-20LA SparkFun Electronics, Colorado, USA) at 125 kHz via a custom-made RFID coil. Thus, each time a mouse passed underneath the RFID coil into the gRW compartment, RFID and time stamps were saved. All sensors were connected to the GPIO pins of a raspberry pi 4B+. In addition, a raspberry pi noir camera was used to record movement inside the RW-compartment at 40 Hz; camera data were used to confirm sensor data and to extract RW-turns when the Hall sensor failed. All data was recorded using custom written python scripts running on the raspberry pi. A 3D .pdf file illustrating dimensions of cage components and electronic assembly is provided upon request.

Hardware essentials

Item	Catalog No.	Company
Raspberry pi 4B+ 4GB, 32	RP-4B-4GB	Raspberry Pi Foundation,

GB SD		Cambridge, England
Raspberry Pi Noir Camera 2.0 8MP	RB-CAMERAV2IR	Raspberry Pi Foundation, Cambridge, England
Raspberry Pi® RB-LCD-7 Display-Modul 17.8 cm (7 Zoll) 800 x 480 Pixel	RB-LCD-7	Raspberry Pi Foundation, Cambridge, England
RFID reader	ID-20LA	SparkFun Electronics, Colorado, USA
RFID implants	SEN-09416	Sparkfun Electronics, Colorado, USA
roller switch for seesaw	190.072.013	Marquardt, Germany
phototransistor for beam break sensors	BPV11F	Vishay, Germany
reed contact for Hall sensor	MS-213-3	PIC Proximity Instrument Controls GmbH, Germany

Analysis of gated wheel running

In order to assign each RW-turn to individual mice, we first defined running bouts as running at a speed above 0.1 Hz, then the most recently detected RFID was assigned to all wheel turns within the respective running bout. When more than one RFID was detected since the last gate flip, this indicated that more than one mouse had entered the gRW compartment. This occasionally happened, due to two mice squeezing through the gate simultaneously, or due to failure of the magnetic seesaw lock. In this case, the RW-turns were labelled as ambiguous. Mice with ambiguous RW-turns of more than 9% were excluded from the RW-analysis, but included for analysis of OD-plasticity in Fig. 2. For the remaining mice, on average, we quantified 2.7±0.8% ambiguous RW-turns for P70 mice (n=10 mice) and 1.7±0.5% ambiguous RW-turns for P160 mice (n=20 mice).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES

Statistical analysis

Inter-group comparisons between two groups were done by Student's t-test, for 3 or more groups, we used one or two-way analysis of variance (ANOVA), as detailed. In analyses in which a within-subject factor was present (i.e. eye), ANOVA with repeated measurements was performed. Post hoc multiple comparison tests were corrected by applying Sidak correction to p-values. The levels of significance were set as *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Data are represented as means ± standard error of mean (s.e.m.).

Software accessibility

Any software and code used for running the gRW setup and for processing obtained data are available upon request.

Results

The gated running wheel setup allows for detailed readout of individual mouse running behaviour.

Given the importance of social interactions for the well-being of mice, we specifically designed a gated running wheel setup (gRW) so that we could track individual running activity of group-housed mice in a home cage setting. The gRW setup consists of standard rat cage connected to a separate compartment with a running wheel for voluntary physical exercise (Figure 1A). Upon entering the gRW-compartment, mice are individually registered using an implanted RFID sensor allowing to allocate running bouts to individual mice (Figure 1C) and to correlate individual running behaviors with individual V1-plasticity parameters assessed using intrinsic signal optical imaging. In order to avoid conflict between group housed males, we limited our experiments to groups of two to six female mice belonging to two age groups, termed P70 and P160, whose gRW activity was tracked for 4 or 7 days respectively (Figure 1) and occurred predominantly during night time (Figure 1C). As typically only single mice entered the RW compartment the activity of individual mice could be well separated highlighting interindividual variability for example in running bout length and speeds achieved by mice (Figure 1C,E). P160 gRW mice ran on average 3307±763 RW turns per day, corresponding to 1.3±0.3 km/d (Figure 1B). These values are similar to previously published data showing 3991±445 RW turns/day, corresponding to 1.6±0.2 km/d for seven days of RW enrichment in group housed P160 C57Bl/6J mice using identical running wheels (Kalogeraki et al., 2014). The total average distance individual mice ran per day was highly variable and ranged from 0 to 3.7 km/d in P70, and from 0 to 3.5 km/d in P160 mice. Cumulative running distances consistently increased across days (Figure 1D), reaching 6.7±1.2 km after 4 days in P70 animals and 8.2±1.8 km after 7 days in P160 animals. We could not identify significant differences in the trajectories of cumulative wheel running between the age groups: linear regression lines fitted to cumulative running distances of each age group revealed neither difference in slopes ($F=1.21$, $p=0.272$) nor in intercepts ($F=3.54$, $p=0.062$) of the fitted curves.

We next compared several wheel running parameters between between P70 and P160 mice (Figure 1E). Mice of both age groups reached similar average daily running distances of 1.7±0.3 km/d and 1.3±0.3 km/d ($p=0.31$), corresponding to daily running times of 1.8±0.3 h/d and 1.5±0.3 h/d for P70 and P160 age groups, respectively ($p=0.37$). The average running speed of P70 and P160 was 18±2 cm/s and 18±1 cm/s, again not significantly different ($p=0.84$). However, P160 mice showed longer average running bouts of 5.0±0.5 min, compared to the 2.9±0.5 min of P70 mice ($p=0.009$). P70 mice also ran 19.5±3.3 bouts/d, and thus more than P160 mice, which only ran 17.0±3.3 bouts/d ($p=0.01$). Together, these data demonstrate that voluntary physical exercise in group-housed mice shows huge interindividual differences in various parameters, that may influence cortical plasticity, and that our new gRW-setup is ideally suited to quantify these parameters of individual running activity of animals at different ages.

gRW re-enables OD-plasticity in P70 and P160 mice

It was previously shown that 4 days of MD in P70 mice and 7 days of MD in P160 is not sufficient to induce OD-plasticity in standard cage (SC) raised rodents measured using intrinsic signal optical imaging (Lehmann and Löwel, 2008; Sato and Stryker, 2008). Here, we tested whether running in the gRW setup might also boost OD-plasticity in adult mice as previously shown for standard running wheels after 7 days of MD (Kalogeraki et al., 2014). gRW-housing started immediately after MD, and V1-activity maps were visualized after 4 (P70) or 7 days MD (P160), respectively. We recorded intrinsic signal optical imaging responses of binocular V1 to horizontal drifting bars (Cang et al., 2005). Confirming previous imaging data in mice without MD (Lehmann and Löwel, 2008; Sato and Stryker, 2008), visual stimulation of the contralateral eye induces a stronger V1-activation compared to ipsilateral eye stimulation, visible as the darker patches in the V1-activity maps (Figure 2A,E), and reflected in the V1-activation strength (Figure 2C,G): V1 was dominated by input from the contralateral eye (P70 contra/ipsi: 2.4±0.2/1.5±0.0.2, n=9, p<0.0001; P160: 2.0±0.2/1.3±0.0.1, n=10, p=0.0003). In contrast, in gRW mice of both age groups, MD induced a clear OD-shift: after MD, both eyes activated V1 more equally strong, i.e. V1-activity patches induced by ipsi- and contralateral eye stimulation show more similar grey values. Quantification of V1-activity (Fig. 2C,G) and the OD-index, comparing ipsi- and contralateral eye induced V1-activation (Fig. 2B,F) confirms the gRW boosted OD-shifts: while in noMD mice, the OD-index was positive, indicating contralateral eye dominance, after MD, the OD-index was lower compared to the noMD control groups (P70 noMD/MD: 0.25±0.04/0.12±0.03, n=9/11, t-test p-value 0.007; P160 noMD/MD: 0.24±0.04/0.05±0.04, n=10/9, t-test p-value 0.003). Comparing V1-activation values thus shows that in P70 animals, contralateral eye dominance was diminished after MD (P70 contra/ipsi: 2.6±0.03/2.2±0.3, n=11, p=0.016). In P160 animals after MD, the contralateral eye no longer activated V1 more strongly than the ipsilateral eye (MD P160 contra/ipsi: 1.7±0.2/1.5±0.0.2, n=9; p=0.7). Furthermore, V1-activation values in P70 mice indicate that the MD-induced OD-shifts do not result from a reduction of V1-activation through the previously deprived eye, like observed in juvenile OD-plasticity (Sato and Stryker, 2008), but rather from an increase in V1-activation through the open eye (Fig. 2C), as previously shown for OD-plasticity in young enriched mice (Kalogeraki et al., 2017).

Together our data highlight that even short-term gRW enrichment is able to boost OD-plasticity in group-housed mice of both age groups.

Optomotor results in gRW mice

To test animals' basic visual abilities and to confirm the effectiveness of MD, we used the virtual reality optomotor system to measure the spatial frequency threshold (SFT) of the optomotor reflex before and after MD (Prusky et al., 2004): vertical drifting gratings of various spatial frequencies evoke small horizontal head movements following the stimulus. MD typically leads to an enhancement of optomotor reflex thresholds through the open eye. As expected, and confirming effective MD, in P70 mice, 4 days of MD led to an increase of the SFT from 0.38±0.00 to 0.43±0.01 cyc/deg, while SFT remained stable in noMD mice (P70 day 0/4: 0.38±0.00/0.39±0.00cyc/deg; n(noMD/MD)=9/10; 2-way ANOVA for effect of MD: p=0.002; Figure 2D). Similarly, in P160 mice, 7 days of MD led to a significant increase of the SFT from 0.39±0.00 (P160, day 0) to 0.51±0.00 cyc/deg (n=10/9; 2-way ANOVA for effect of MD p< 0.0001; Figure 2H), while values remained stable in noMD mice (P 160, day 0/7): 0.39±0.00/0.39±0.00 cyc/deg).

gRW running wheel parameters correlate with ocular dominance index

In order to investigate how individual behavioural choices feedback to shape brain plasticity, we tested whether individual running wheel parameters were correlated with individual OD-indices which quantify the magnitude of OD-plasticity in both age groups. For this we performed a principal component analysis (PCA) to reduce the dimensions of *average* running wheel parameters (daily running distance, daily running time, average daily running speed, average daily bout number, average daily bout duration) to the first principal component (explaining 96 % of interindividual variability in wheel running of pooled P70 and P160 data) as a measure of overall gRW activity. Mice showing less than median gRW activity (median gRW activity = 2.3) had weaker or absent OD-plasticity than mice showing higher than median gRW activity: pooling data from P70/P160 (Figure 3B), the OD-index of mice with high gRW activity was -0.03±0.02 compared to 0.12±0.04 for low gRW activity (n=6/6, p=0.007).
Confirming this result, inter-individual variability in gRW activity of P160 mice explained 67% in variability of the OD-index (r^2=0.67, p=0.013). Correlation analyses in P70 mice closely followed the same distribution as data from P160 mice such that the pooled correlations were also significant (r^2=0.48, p=0.012, Figure 3B). Since we could obtain only 4 wheel-parameter/OD-index pairs for P70 mice we refrained from testing correlations in this dataset separately.
In addition, we also observed striking correlations of individual running wheel parameters with individual OD-index (Figure 3C). In P160 mice, bout duration, speed, bout no, running distance and running time explained close to or above 60% (e.g. $r^2 \geq 0.60$) of the interindividual variability in OD-index (P160, n=9; bout duration: r^2=0.69, p=0.0102; speed: r^2=0.63, p=0.0175; bout no: r^2=0.58, p=0.0271; running distance: r^2=0.60, p=0.0243; running time r^2=0.62, p=0.0192). Correlation analyses in P70 mice again closely followed the same distribution such that the pooled correlations were significant for all quantified parameters except bout number (bout duration: r^2=0.61, p=0.0025; speed: r^2=0.45, p=0.0163; bout no: r^2=0.20, p=0.14; running distance: r^2=0.42, p=0.0219; running time r^2=0.45, p=0.0150). Importantly, no significant correlation between running wheel parameters and OD-index was found in the no MD control group (p>0.05 for all correlations).
Previous experimental evidence has demonstrated that running wheel activity correlates with cortical gamma activity in V1 (Niell & Stryker, 2010), and that MD is associated with increased gamma activity in V1 for several hours after MD, at least in juvenile mice aged 24-27 days (Quast et al., 2023). Hence, we wondered whether running wheel activity was correlated more strongly with the OD-index on a specific day after MD. For this we used PCA for dimensionality reduction of running wheel parameters measured for *each day separately* and correlated the first principal component (explaining 90 % of variability of running wheel parameters across days and animals) with the OD-index: gRW activity significantly correlated with the OD-index only on days 6 and 7 after MD (day6/7: r^2=0.52/0.53, p=0.045/0.041), while this relationship was not significant on days 1-5 (p>0.05 for all correlations). Nevertheless, gRW activity on the first day still contributed to variability in OD-index, since averaging gRW activity of increasing numbers of days, already produced significant correlations when only data from days 1 and 2 was included (p>0.05 for day 1 alone, p<0.05 for data from >1 days).
Overall these data document that even short-term voluntary physical exercise strongly boosts experience-dependent changes in V1-activation and highlight that more running leads to stronger OD-plasticity in mouse V1. Since interindividual variability in running behaviour is

very high, individual behavioural choices and their influence on brain physiology and plasticity should be more strongly considered in future studies.

Discussion

Using our newly developed gated running wheel setup (gRW) that allows to track individual running activity in group-housed mice, we observed a striking correlation between individual running parameters and experience-dependent plasticity in mouse primary visual cortex. More running caused more plasticity: *running speed, running distance, total running time, number of running bouts and bout duration all significantly correlated with a measure of visual cortical plasticity, the ocular dominance index.* Thus, our observations add to the growing body of evidence that individual behavioural choices can strongly affect individual brain plasticity.
Confirming previous studies with conventional running wheels (Kalogeraki et al. 2014), 7 days of gRW-running was sufficient to boost OD-plasticity in P160 mice. Likewise, running for only 4 days also restored OD-plasticity in P70 mice. While OD-shifts obtained in P160 were similar to previously published data after 7 days of RW-enrichment, OD-shifts were smaller compared to animals experiencing lifelong enrichment (Kalogeraki et al. 2017). Similarly, the magnitude of OD-shifts for P70 mice was smaller compared to lifelong environmental enrichment (Kalogeraki et al. 2017). Thus, while short-term running can restore OD-plasticity to adult mice more enrichment, like adding a maze, for longer periods of time, can induce stronger experience-dependent V1-activity changes. Alternatively, extension of MD-duration can also boost OD-plasticity in adult SC-mice (Hosang et al., 2018), indicating that there is a trade-off between age and MD duration in SC-raised animals and that longer MD times can be shortened by specific environmental interventions, as shown here.

Using C57Bl/6J inbred mice housed in the gRW-setup allowed to test how individual behavioural trajectories affect brain plasticity with minimal influence of genetics on the observed phenotype. In line with the model of Kempermann (2019), we observed a linear relationship between running parameters and OD-plasticity quantified by the OD-index: individual running performance predicted up to 67% of phenotypic variability between mice. Thus, individual behavioural choices exert a strong influence on experience-dependent plasticity. Interestingly, bout duration was the best predictor of individual OD-index than bout number, running speed, running distance and running time, as suggested by the obtained p-values. Both high running speed and bout duration require high physical fitness and might be associated with better sensory-motor coordination for efficient running that may improve with practice (De Bono et al., 2006), suggesting that pre-training could also benefit OD-plasticity by enabling more efficient running.

Wheel running comprises a rewarding behaviour that even mice in the wild pursue voluntarily (Sherwin, 1998). Multiple factors affecting wheel running have been identified, including mouse strain, sex, group housing, social hierarchy, age, circadian rhythms (Kopp, 2001; De Bono et al., 2006; Basterfield et al., 2009; Bains et al., 2018; Balog et al., 2019; Plenz and Kanold, 2021). In addition, variability in the activity of specific neural networks has been linked to mouse wheel activity (Rhodes et al., 2003): lateral hypothalamic orexin/hypocretin and GAD65 networks have emerged as drivers of locomotion initiation (Kosse et al., 2017; Karnani et al., 2020), suggesting that inter-individual differences in network activity might explain variability in wheel running. Importantly, social hierarchy has been shown to affect both wheel

running and OD-plasticity in male mice, with dominant males exhibiting stronger OD-shifts compared to subordinate males (Balog et al., 2019). While social hierarchies are less prominent in female mice (Williamson et al., 2019) used here, this may have contributed to variability of OD-index and wheel running in our data.

Mechanisms underlying V1-activity changes differ between juvenile and adult mice: In juveniles, OD-plasticity is initially mediated through depression of V1-responses through the previously closed (contralateral) eye, while adult mice show OD-shifts initiated by increased open (ipsilateral) eye V1-responses (Lehmann and Löwel, 2008; Sato and Stryker, 2008). While we have not examined this in detail, our P70 data suggest that gRW-enrichment enables OD-plasticity mainly through potentiation of ipsi-eye V1-responses, without contra-eye V1-response depression. Our data are thus in line with published data showing that, both short-term running and life-long environmental enrichment restore the *adult* form of OD-plasticity but can*not* reinstate juvenile-like OD-plasticity (Kalogeraki et al 2017).

Together, our newly developed gRW-setup allowing to correlate individual mouse running behaviour with individual measures of experience-dependent plasticity has demonstrated a striking correlation between mouse running activity and OD-plasticity, highlighting the importance of individual behavioural tracking for explaining experimental data. Thus, our observations add to the growing body of evidence that individual behavioural choices can strongly affect individual brain plasticity, and thus should be analysed more carefully in future studies.

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Figure legends

Figure 1. Gated running wheel setup allows to track individual mouse running wheel activity.
A. Standard-sized rat cage (left, 43 cm x 27 cm) with an additional compartment (right) containing beam break sensors (1,3), a seesaw (2), RFID sensor (4), hall sensor (5), magnet (6), and a running wheel (7). All the sensors are connected to a raspberry pi 4B+ (8). (9) Illustrates the size of the implanted RFID.
B. Comparison of average daily running activity of gRW housed mice (orange) with published data from Kalogeraki et al. 2014 (black).
C. Example of quantified gRW-activity of two co-housed mice illustrating allocation of running bouts to individual mice (blue/black line for P70, orange/black line for P160) for 3 consecutive days and nights (grey background) (top) and 90 minutes during night time (bottom) for P70 (left) and P160 mice (right), with detected running bouts highlighted in gray. Note that animals predominantly run during night time.

D. Cumulative running trajectories of individual P70/P160 (left/right) mice housed in gRW enrichment for 4/7 days, document a large range of individual running behaviour.
E. Comparison of running wheel parameters in individual P70 (blue dots) and P160 mice (orange squares); *p < 0.05, **p < 0.01 in t-test. Note the huge interindividual variability.

Figure 2. gRW enrichment boosts ocular dominance plasticity in group-housed P70 (A,B,C,D) and P160 (E,F,G,H) mice.
A,E. Intrinsic signal optical imaging examples of grey-scale coded activity maps after visual stimulation of the contralateral and ipsilateral eye in binocular V1 of C57Bl/6J gRW housed mice after 4 days (P70; A-D) or 7 days (P160, E-H) of monocular deprivation (MD) and without MD (no MD). The magnitude of V1-activation is expressed as the fractional change in reflectance $x10^{-4}$ and indicated in the top right. Warm colors in the color-coded OD-maps indicate contralateral eye dominance, colder colors indicate an OD-shift towards to ipsilateral eye. Histograms in A,E represent distribution of pixel wise OD-scores. Scale bar: 1mm.
B,F. Quantification of OD-index. Symbols represent values of individual mice, means are marked by horizontal lines.
C,G. V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye (filled circle indicates MD eye). Note that MD did not reduce V1-activation through the deprived eye in C.
D,H. Spatial frequency threshold of the optomotor reflex for noMD and MD mice, plotted vs. days (x-axis). Note that thresholds increase after MD, indicating successfully induced MD (*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 in t-test or 2-way ANOVA with Sidak's post hoc test).

Figure 3. Individual running wheel (RW) activity correlates with ocular dominance (OD) plasticity, quantified via the OD-index after monocular deprivation (MD) in P160 mice.
A. Example intrinsic signal optical imaging recordings from noMD and MD animals, with low (gRW activity <2.3) and high (gRW activity >2.4) RW performance (gRW activity was defined as PCA1 of RW parameters displayed in C, and displayed in B). Data displayed as in figure 2A,D. Note that OD-plasticity after MD is only boosted when mice run sufficiently in the gRW setup.
B. The OD-index of individual P70 /P160 mice after MD (blue/orange squares) is correlated with their individual gRW activity. Lines represent linear regression of P160 mice (orange) or pooled data from P70/P160 groups (black). Pearson r-values and p-values for P160 and pooled P70/P160 in figure.
C. The OD-index of individual mice P160 after MD is correlated with all quantified running wheel parameter. Data is displayed as in B. For the P160 mice, Pearson correlation between OD-index and individual wheel running activity was significant for all tested running wheel parameters. Pearson correlation for pooled P70/P110 data was significant for all wheel parameters except bout no.

Video 1. Video footage of a mouse entering the gated running wheel compartment via flipping a seesaw.
Video 2. Video footage of a mouse leaving the gated running wheel compartment via flipping a seesaw. Note that its cage mate is blocked from entering the compartment.
Model 1. 3D model of gated running wheel components
Figure S1. Electronic circuit of gated running wheel setup

Figure 1 Gated running wheel setup allows for detailed readout of wheel running behavior in group housed mice

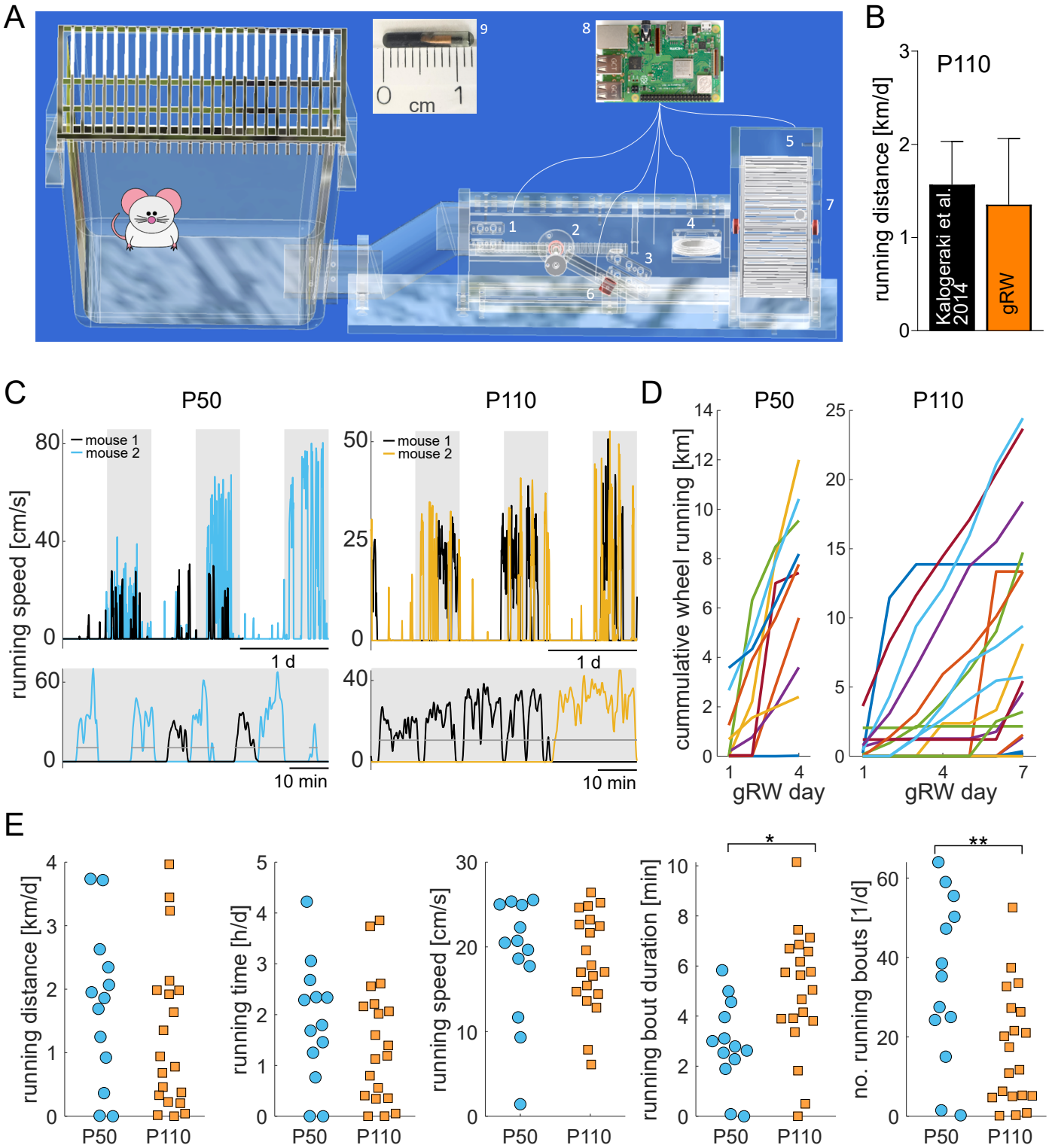

Figure 2 Gated running wheel housing promotes ODP in group housed mice

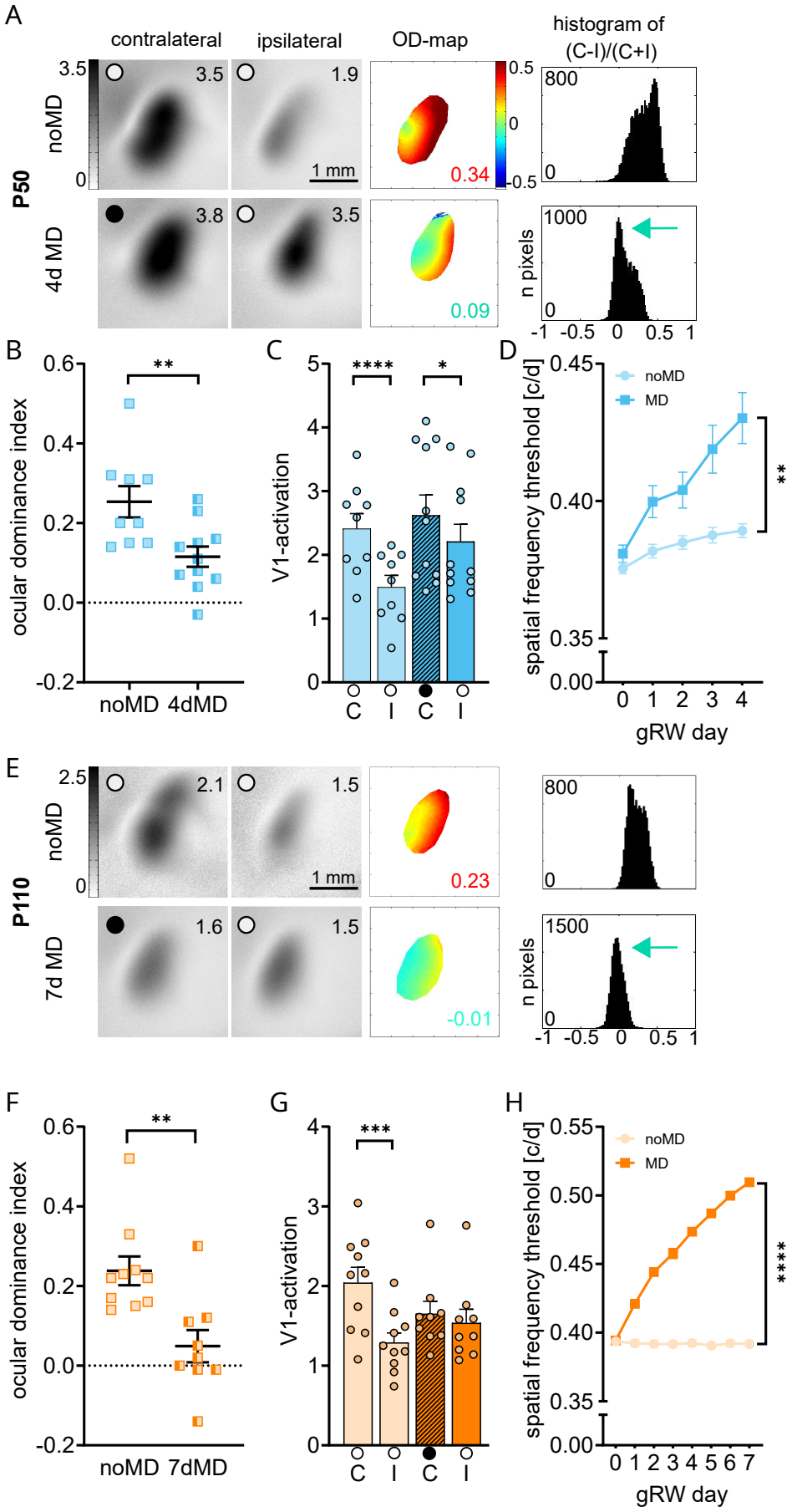
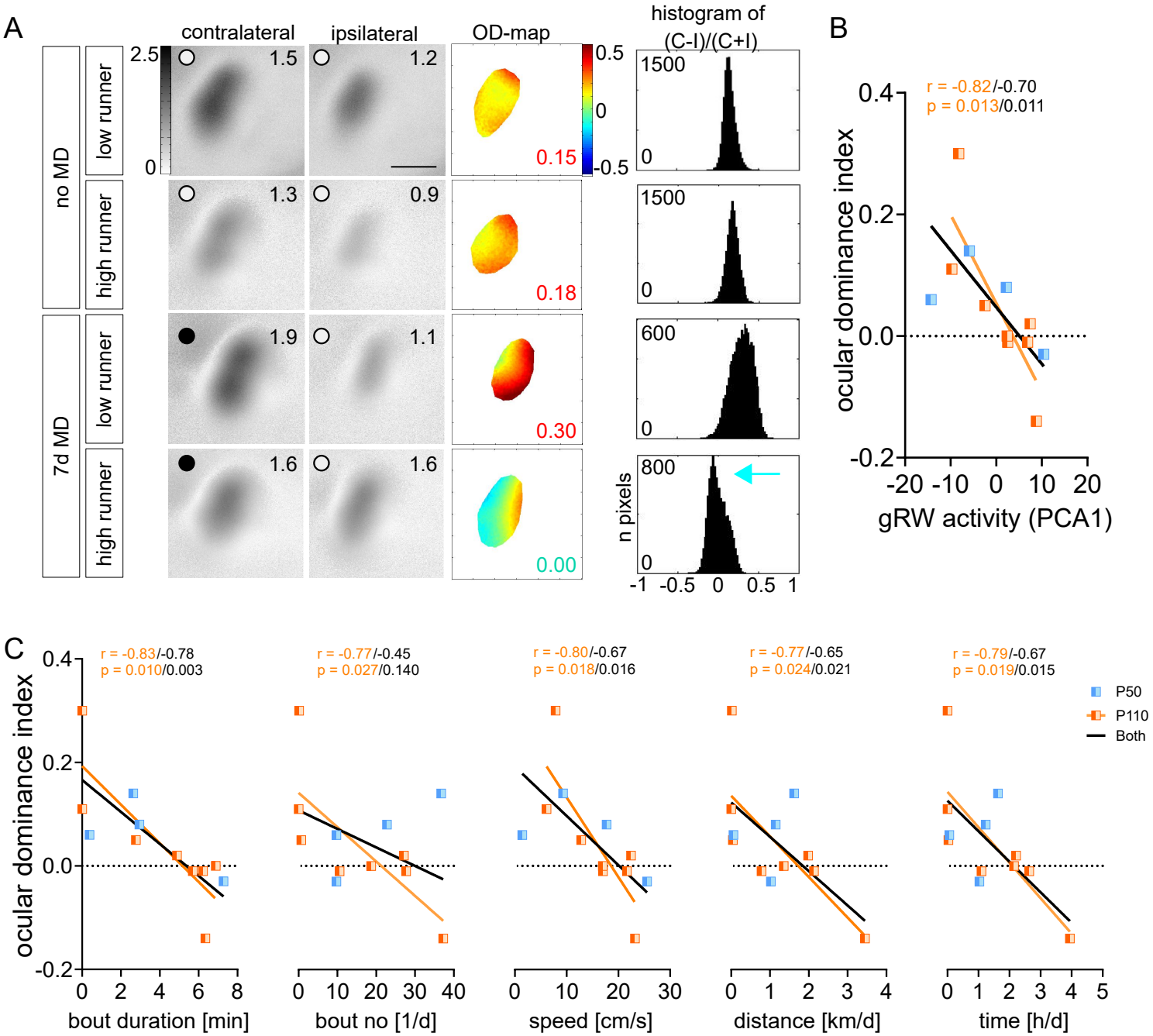
Figure 3 Running wheel activity correlates with ODI in P110 animals