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Original Article

Using Computational Video Analysis in Aging Mice to Evaluate the Effects of Chronic Monotherapy, Polypharmacy, and Deprescribing Over Time

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Abstract

Background: In clinical studies of older adults, polypharmacy (use of ≥ 5 drugs) and the Drug Burden Index (measures exposure to anticholinergic and sedative drugs) are associated with impaired physical function and frailty. We used computational video analysis of aging mice to examine the impact of medications on morphometric and gait function.

Methods: Middle-aged (12-month) male mice were administered therapeutic doses of medications in polypharmacy regimens with different Drug Burden Index scores or monotherapy with medications from the High Drug Burden Index polypharmacy regimen. At age 21 months, half of the treated animals had their medications deprescribed (discontinued). Open field videos and mouse clinical frailty index were recorded at 12, 15, 18, 21, and 24 months. After applying open-source neural networks to the videos, the gained features were analyzed to detect differences between the treatment groups and control over time using a state-space model with change point detection.

Results: We measured 49 morphometric and gait features for 278 mice. The sum of effects of constituent monotherapies did not equal the effects of polypharmacy. Consistent with clinical data, greater gait and posture changes were observed with polypharmacy regimens with increasing Drug Burden Index scores. Deprescribing effects varied between medications, consisting of reversible, irreversible, and novel changes. Different medication exposures had different effects on gait, posture, and the prediction of frailty.

Conclusion: Computational video analysis of preclinical data is a promising tool for high-throughput, sensitive detection of medication effects in aging.

Keywords: Drug Burden Index, Frailty, Geriatric medicine, Neural networks, Pharmacology

Aging is associated with multimorbidity and increased chronic medication use. Internationally, one-third to two-thirds of older adults take polypharmacy (defined as concurrent use of 5 or more medications) (1). Polypharmacy and cumulative exposure to medications with anticholinergic and sedative effects, measured using tools such as the Drug Burden Index (DBI), are associated with impaired physical and cognitive function, altered gait, increased falls, frailty, hospitalization, and mortality in older adults (2,3). Therefore, medication safety in polypharmacy is one of the priority areas targeted by the current WHO Global Patient Safety Challenge: *Medication Without Harm* (4). Presently, best practice for management of polypharmacy is comprehensive, collaborative medication review, and consideration of deprescribing (1). Deprescribing is the process of discontinuing drugs that are no longer needed and/or potentially harmful, under clinical guidance, with shared decision-making to align medication use with the patient's goals. In practice, medication optimization is limited by the lack of high-quality clinical evidence on the effects of polypharmacy, the DBI, and deprescribing on the functional outcomes that are important for older adults (5). Recently, strategies have been proposed to increase involvement of frail older people with polypharmacy in clinical trials (6), to measure frailty and functional outcomes (7), and to design deprescribing trials powered to assess not just prescribing but also clinical outcomes (8).

The current challenges and limitations in evidence derived from human studies underscore the importance of preclinical studies of polypharmacy and deprescribing. Preclinical studies enable us to build more robust evidence by conducting interventional studies that are neither ethical nor feasible to conduct in older adults (9). We conducted the first preclinical study of chronic polypharmacy and deprescribing in aging, with findings comparable to those observed in humans: chronic polypharmacy with increasing DBI increased frailty, decreased mobility, function, and activities of daily living, and deprescribing was tolerable and reversed some outcomes (10). In this study, the mouse behaviors captured through open field testing were only abstracted to simple point-extract behavioral measures of mobility (distance traveled),

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immobility time, and gait speed, and other complex behaviors were not explored. Furthermore, the analysis was very time-consuming.

Recent advancements in machine learning have enabled researchers to implement sophisticated computer vision techniques to analyze mouse behaviors more broadly. This enables objective and sensitive detection of subtle changes efficiently for high throughput screening. Kumar's laboratory modified a high-resolution network for human pose estimation to detect 12 key points of mouse anatomical location on top-down video of an open field across 62 strains of mice (11). Their research group also developed a segmentation neural network model to produce an ellipse fit of the mouse as well as a model for action detection (12). This has been used to demonstrate genetic variations in animal behavior and to validate a machine-learning-based visual frailty index (FI) for mice (13). New advances in open-field measures of gait and posture analysis now provide opportunities for measuring chronic medication effects, screening multiple combinations of medications for effects on gait and posture, and the impact of deprescribing. These studies help to fill the knowledge gaps in clinical research and provide a method to increase the clinical relevance of exposures and outcomes tested preclinically (14).

Here, we applied neural networks previously developed by Kumar's laboratory (11,13) as a tool to screen the impact of chronic monotherapy, polypharmacy with increasing DBI, and deprescribing on gait and posture in aged mice using the open field. We first demonstrated that the method can sensitively detect medication effects on gait and posture. We next compared monotherapy to polypharmacy and found the sum of effects of each monotherapy did not equal the effects of the polypharmacy regimen. We then investigated the effect of polypharmacy regimens with varying DBI. Consistent with clinical data, we observed a dose response, where polypharmacy regimens with higher DBI caused more deficits in gait and posture. We further explored the effects of deprescribing and found some changes with drug treatment were reversible, others were irreversible and deprescribing induced novel changes. Finally, applying the gait and posture data, we observed that for each treatment, different features are important for predicting frailty, demonstrating that pharmacological actions of different medications influence frailty differently.

The application of computational visual analysis of preclinical videos now offers an opportunity to screen chronic medication use, polypharmacy combinations, identify high-risk combinations, and research optimal deprescribing methods on the translatable, clinically relevant outcomes of gait and posture. This data can contribute to optimizing medication use and outcomes for older adults. The method can be more broadly applied to preclinical screening in development of drugs intended for use in older adults, modeling clinically relevant exposures and outcomes.

Method

Treatment Details

This study analyzed open-field recordings from our previously published mouse data on chronic polypharmacy, monotherapy, and deprescription (withdrawal of therapy) in aging mice (10). In brief, middle-aged (12 months) male C57BL/6J (B6) mice were randomly assigned into groups and administered control feed or feed and/or water-containing polypharmacy or monotherapy with different DBI scores (Figure 1). The polypharmacy regimens included polypharmacy zero DBI (DBI = 0; 20 mg/kg/day simvastatin, 350 mg/kg/day metoprolol, 10 mg/kg/day omeprazole, 100 mg/kg/day acetaminophen, and 5 mg/kg/day irbesartan), polypharmacy low DBI (DBI = 0.5; 20 mg/kg/day simvastatin, 350 mg/kg/day metoprolol, 10 mg/kg/day omeprazole, 100 mg/kg/day acetaminophen, and 10 mg/kg/day citalopram), and polypharmacy high DBI (DBI = 1.6; 20 mg/kg/day simvastatin, 350 mg/kg/day metoprolol, 27.2 mg/kg/day oxybutynin, 5 mg/kg/day oxycodone, and 15 mg/kg/day citalopram) and the monotherapy regimens consisted of the individual medications that comprise the polypharmacy high DBI regimen. These medication regimens were selected based on the drug classes commonly used by older Australians, having similar pharmacokinetics and pharmacodynamics between mice and humans, applying therapeutic doses tolerated by mice in previous preclinical studies of chronic oral monotherapies (10).

At age 21 months, each treatment group was re-randomized to either continue the medication or commence deprescribing, with stratification based on the 21-month mouse clinical FI scores of individual mice within each group. The deprescribing was performed gradually, with each group receiving 3 consecutive 2-week weaning regimens, during which the number and/or doses of medications were progressively reduced.

Frailty Assessment

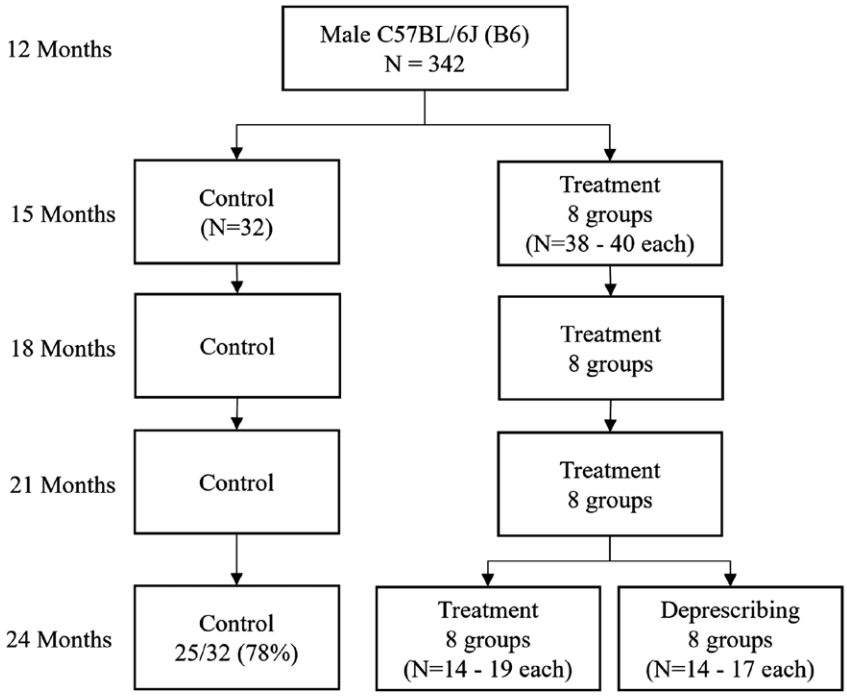
The mouse clinical FI was calculated as previously described for all mice at 12, 15, 18, 21, and 24 months (15). In brief, 31 health-related deficits were examined and graded with increasing severity, with scores 0, 0.5, and 1. The FI was derived from the sum of all deficits divided by the total number of deficits. The FI was assessed by a single trained investigator (J.M.) for all mice across all ages, and this investigator was blinded to the treatment group. Animal weights were monitored weekly.

Open-Field Testing

The activity in the open-field test was recorded as previously described (10,16,17). In brief, under red light between approximately 9 am and 12 noon, mice were acclimatized to the room for 30 min prior to testing. Each mouse was placed in the center of an open-topped, white plastic box (50 × 50 × 50 cm), and activity was recorded with a video camera over 5 min. The camera collected data at 30 frames per second with a 1280 × 720-pixel resolution.

Pose Estimation and Segmentation for Feature Engineering

After converting the MPEG files to 480 × 480-pixel monochromatic AVI files, pretrained neural networks developed by Kumar's laboratory were applied to our open-field videos to produce an ellipse fit of the mouse as well as the 12-point 2-dimensional pose estimation for each frame (11,12). Using the ellipse fit and the estimated x, y-coordinates of the points, the 47 features that include gait metrics, spinal mobility metrics, and morphometrics were generated by running the codes—with slight modifications—from the Kumar laboratory Github page (https://github.com/KumarLabJax/). All features are described in Table 1. For each feature, the median and interquartile range (IQR) were used as representative values rather than mean and standard deviation because they are more robust to outlier effects.



Monotherapy	Regimen
Citalopram	15 mg/kg/day (DBI = 0.6)
Oxycodone	5 mg/kg/day (DBI = 0.5)
Oxybutynin	27.2 mg/kg/day (DBI = 0.5)
Metoprolol	350 mg/kg/day (DBI = 0)
Simvastatin	20 mg/kg/day (DBI = 0)

Polypharmacy	Regimen (mg/kg/day)
Zero DBI (DBI = 0)	Simvastatin (20), Metoprolol (350), Omeprazole (10), Acetaminophen (100), Irbesartan (5)
Low DBI (DBI = 0.5)	Simvastatin (20),Metoprolol (350), Omeprazole (10), Acetaminophen (100), Citalopram (10)
High DBI (DBI = 1.6)	Simvastatin (20), Metoprolol (350), Oxybutynin (27.2), Oxycodone (5), Citalopram (15)

Figure 1. Study flowchart of the longitudinal aging mouse study.Treatments, their approximate Drug Burden Index (DBI) score and sample numbers are displayed.

Modeling

To evaluate the impact of medications on morphometric and gait functions following chronic monotherapy, polypharmacy, and deprescribing, we employed a state-space model with change point detection as follows (18).

System Equations:

$$\mu[t] \sim Normal(\mu[t-1], \sigma_\mu) \qquad t = 2,3,4,5$$

$$\delta_{treatment}[t] \sim Normal(\delta_{treatment}[t-1], \sigma_\delta) \qquad t = 2,3,4,5$$

$$\omega_{deprescribe} \sim Normal(\delta_{treatment}[t-1], \sigma_\delta) \qquad t = 5$$

$$\mu[t], \delta_{treatment}[t] \sim Uniform(-\infty, \infty) \qquad t = 1$$

Observational Equations:

$$Y_{control}[t] = \mu[t] + Normal(0, \sigma_Y) \qquad t = 1,2,3,4,5$$

$$Y_{treatment}[t] = \mu[t] + \delta_{treatment}[t] + Normal(0, \sigma_Y) \qquad t = 1,2,3,4,5$$

$$Y_{treatment_{dp}}[t] = \begin{cases} \mu[t] + \delta_{treatment}[t] + Normal(0, \sigma_Y) & t = 1,2,3,4 \\ \mu[t] + \omega_{deprescribe} + Normal(0, \sigma_Y) & t = 5 \end{cases}$$

The observation time points were denoted as t, where t = 1, 2, 3, 4, 5 correspond to the 12th, 15th, 18th, 21st, and 24th months, respectively. The parameters to be inferred were categorized into 3 major groups: μ, $\delta_{treatment}$, and $\omega_{\text{deprescribe}}$. The first parameter is a time-dependent baseline denoted by $\mu[t]$, which is common across the control and all 8 treatment groups. It was assumed that $\mu[t]$ at time t depends only on the previous state at time t-1 (ie, random walk process). The observed value in the control group $Y_{control}[t]$ is modeled by adding the measurement error, where $Normal(0, \sigma_Y)$ is the normal distribution with mean 0 and standard deviation σ_Y, to the baseline $\mu[t]$. The second parameter, $\delta_{treatment}[t]$, represents the deviation of each treatment group from the baseline $\mu[t]$ and changes over time. The $\delta_{treatment}$ can be different across different treatment group, enabling the model to capture the distinct effects of various treatments, such as $\delta_{HIGH\ DBI}$ or $\delta_{CITALOPRAM}$. The observed value in each treatment group $Y_{treatment}[t]$ was modeled by adding the treatment-specific deviation $\delta_{treatment}[t]$ and random measurement errors to the common baseline $\mu[t]$.

The third parameter is $\omega_{deprescribe}$ that represents the effect of deprescribing at 24 months compared to the control

Table 1. Gait and Posture Outcomes

Features	Definition	Units
Angular velocity (median, interquartile range [IQR])	The current angle of a mouse is determined by the vector connecting the mouse's base of tail to its base of neck; the first derivative of this value gives us angular velocity; for strides, angular velocity is averaged over the duration of the stride	degrees/sec
Limb duty factor (median, IQR)	The stance time of a paw (the amount of time that the paw is in contact with the ground) divided by the full stride time; duty factor is calculated for each of the hind paws and averaged	None
Temporal symmetry (median, IQR)	Temporal symmetry is calculated as $(l - r)/(l + r)$, where l is the limb duty factor of the left hind paw and r is the limb duty factor of the right hind paw	None
Lateral displacement of nose (median, IQR)	To calculate lateral displacement, we first calculate the mouse's displacement vector for a stride; we then measure the nose's perpendicular distance from this vector for each frame of a stride; we then subtract the minimum distance from the maximum and divide by the mouse's body length so that the displacement measured in larger mice will be comparable to the distance measured in smaller mice.	None
Lateral displacement of base of tail (median, IQR)	Calculated using the same approach that is applied to the nose lateral displacement, except that we are using the base of tail key point	None
Lateral displacement of tip of tail (median, IQR)	Calculated using the same approach that is applied to the nose lateral displacement, except that we are using the tip of tail key point	None
Nose lateral displacement phase offset (median, IQR)	The lateral displacement is calculated for each frame of a stride, as described for nose lateral displacement, above; we then perform a cubic spline interpolation to generate a smooth curve for displacement; then, we determine the point in time at which maximum displacement occurs; note that because of cubic interpolation, this can occur at time points between frames	Percent stride cycle
Base of tail displacement phase offset (median, IQR)	Calculated using the same approach that is applied to the nose lateral displacement phase offset, except that we are using the base of tail key point.	Percent stride cycle
Tip of tail displacement phase offset (median, IQR)	Calculated using the same approach that is applied to the nose lateral displacement phase offset, except that we are using the tip of tail key point.	Percent stride cycle
Step length (median, IQR for both paws)	Step length 1 is the distance that the right hind paw travels past the previous left hind paw strike Step length 2 is the distance that the left hind paw travels past the previous right hind paw strike	cm
Step width (median, IQR)	The averaged lateral distance separating hind paws; this is calculated as length of the shortest line segment that connects the right hind paw strike to the line that connects the left hind paw's toe-off location to its subsequent foot-strike position	cm
Stride length (median, IQR)	The full distance that the left hind paw travels for a stride, from toe-off to foot-strike	cm
Stride count	Sum of all recorded strides in video	times
Distance travelled	Sum of locomotor activity in open field	cm
Speed (median, IQR)	The speed of a mouse is determined by tracking the movement speed of the base of tail key point; stride speed is the average speed for all frames over the duration of a stride	cm/sec
aABC (median, IQR for nongait and gait periods)	The angle between the base of head point, mid-back point, and base of tail point	degrees
dAC (median, IQR for non-gait and gait periods)	The distance between base of head and base of tail, normalized by the max dAC recorded	0 - 1 (normalized)
dB (median, IQR for nongait and gait periods)	The distance between the mid-back point and the midpoint of the line AC, normalized by the max dAC recorded	0 - 1 (normalized)
Rearing count	The number of rearing bouts	times
Length of rearing bouts	Average length of rearing bouts	sec
Width (median)	Width of the ellipse fit for the mouse calculated for all frames	cm
Length (median)	Length of the ellipse fit for the mouse calculated for all frames	cm
Rear paw (median)	The distance between rear paws calculated for all frames	cm
Frailty	Clinical frailty index (n = 31 deficits)	None
Weight	Weight	g

Note: The contents of Table 1 include elements from Sheppard et al. (11) (with permission from *Cell Reports*) and additional metrics used in this manuscript.

group rather than the treatment-continuing group. Because mice within each treatment group were randomly allocated to either the continuing group or the deprescribing group at 21 months, the observed values in each treatment group $Y_{treatment}[t]$ *and* $Y_{treatment\_dp}[t]$ $(for\ t = 1, 2, 3, 4)$ are assumed to be identical. $Y_{treatment\_dp}[5]$ was modeled by adding $\omega_{deprescribe}$ to common baseline $\mu[t]$ and measurement error. This $\omega_{deprescribe}$ parameter captures the effect of the deprescribing intervention, reflecting any changes in the morphometric and gait functions that could not be attributed to the natural progression, as represented by $\mu[t]$ and $\delta_{treatment}[t]$.

These models enable inference on how the time-dependent differences between the control group and treatment groups change over time. Specifically, for continuous variables, a period during which the 95% Bayesian credible intervals (CrI) for $\delta_{treatment}[t]$ does not include 0 can be interpreted as a time with a significant difference between the control group and a particular treatment group. Similarly, if the 95% CrI for $\omega_{\text{deprescribe}}$ does not include 0, it indicates a significant difference between the corresponding deprescribing group and the control group at 24 months. When the observed values were count variables (ie, stride count and rearing count), negative binomial distribution models were applied, incorporating a log-link function to account for overdispersion.

To assess the effect of deprescribing, we operationally defined the pattern of outcomes as "reversible" if the outcomes in the continued treatment group at 24 months were statistically significantly different from those in the control group, whereas the corresponding deprescribing group did not show such a difference. In addition, outcome patterns were defined as "novel" if the outcomes at 24 months showed no significant difference between the control and continued treatment groups, but a statistically significant difference was observed between the control and deprescribing groups.

The model fitting was conducted through a Bayesian framework with Markov Chain Monte Carlo (MCMC) sampling methods to estimate the posterior distributions of the model parameters. Four MCMC chains with different starting values were run, each of which generated 5 000 posterior samples. The first 2 500 iterations from each chain were discarded as warm-up. The "adapt_delta" parameter was set to 0.99 and "max_treedepth" was set to 15 to enhance the stability and accuracy of the sampling process. Consequently, we obtained 10 000 representative samples for each parameter (2 500 per chain) in each model fitting. To verify that the sampling process had reached a stable state and was producing reliable results, convergence for the 4 chains was confirmed by ensuring that the R-hat values for all parameters were less than 1.1. The goodness-of-fit for each model was assessed using posterior predictive checks, which involved visually examining whether the observed values fell within the 95% CrI. Furthermore, the specified model was compared with an alternative model that employed a noninformative prior distribution of $\omega_{deprescribe}$ using the Watanabe-Akaike Information Criterion (WAIC).

Prediction of the Frailty Index

The predictive performance of the video-generated features against the FI was evaluated using 3 models: gradient-boosting (GB) model, random forest (RF) model, and elastic-net (EN) regression model. After excluding data collected at 24 months, the remaining data was randomly split into 2 sets: 80% for training and 20% for testing. Care was taken to ensure that repeated measurements from the same mouse were allocated entirely to either the training or the testing data, not both. The training set was used to estimate and tune the models' hyper-parameters using 10-fold cross-validation. The test set served as an independent evaluation of the models' predictive performance. Fifty different data splits were performed to allow for a comprehensive assessment of uncertainty in test set results.

The following covariates were included in the models through the stepwise method: age, weight, treatment type, and video-generated features (either all features or those selected based on permutation importance results). The models' performance was compared using mean absolute error (MAE), root mean squared error (RMSE), median absolute error (MEAE), and R^2 metrics. To investigate how feature importance for predicting the FI varies across the treatment groups, permutation importance was calculated and compared by treatment group. Data manipulation and predictive modeling were conducted using Python version 3.9 (Python Software Foundation, Wilmington, DE, USA) with sklearn. All statistical analyses were performed in R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria) with the state-space modeling carried out using RStan. Two-sided p-values <0.05 were considered statistically significant.

Results

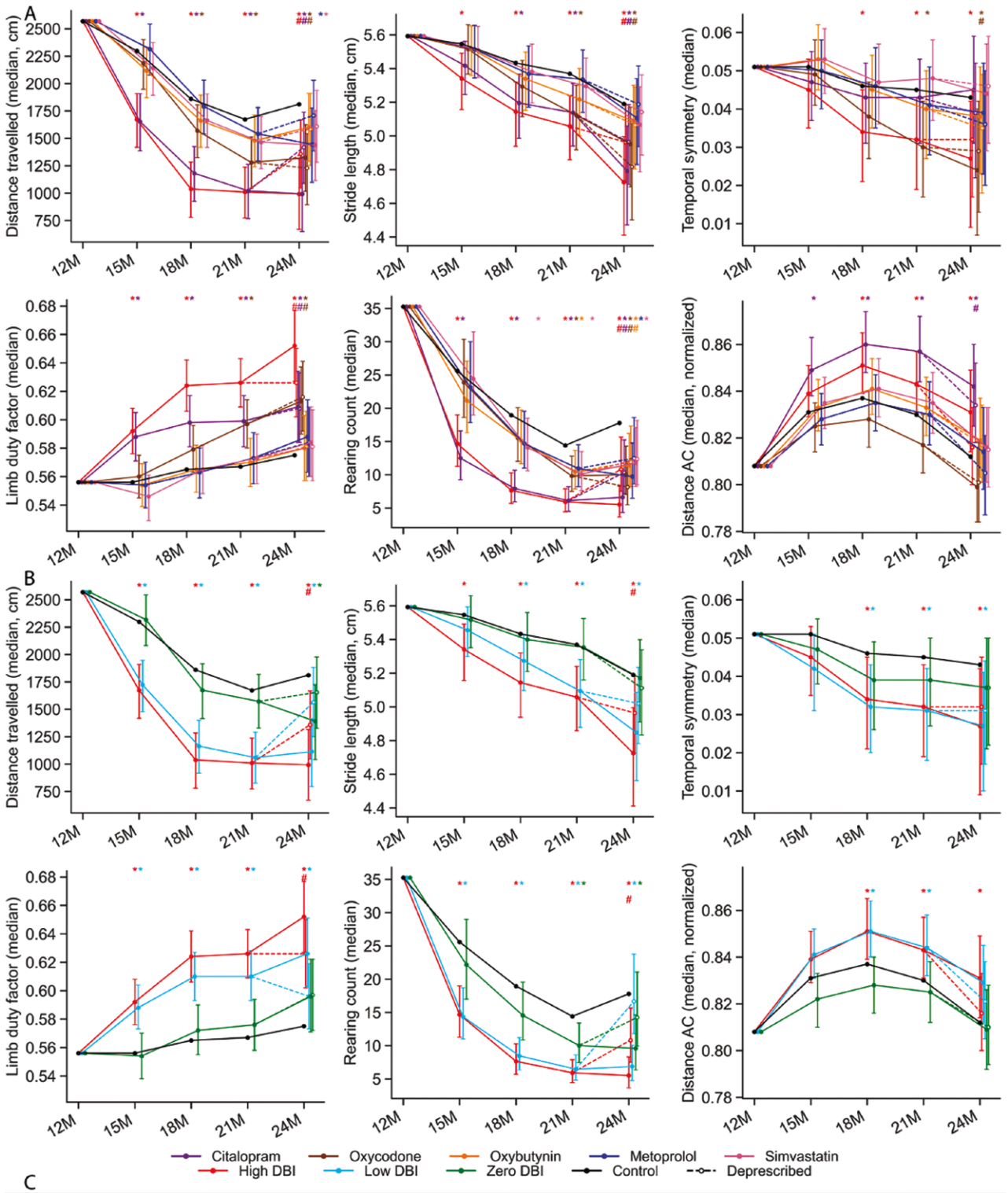
Study Overview

We applied the computation visual analysis of open field data from the first polypharmacy and deprescribing longitudinal preclinical study to investigate the effects of monotherapy, polypharmacy, and deprescribing on gait and posture in aging. Overall, 278 mice (81%), with data available at all 5 evaluation points were included in this analysis (Figure 1). We sensitively analyzed 47 video features of gait and posture, weight, and the FI (a total of 49 features). Markov Chain Monte Carlo diagnostics indicated satisfactory convergence with R-hat values below 1.1, and posterior predictive checks confirmed a good fit to the observed data. Based on WAIC, the model incorporating the normal prior distribution for $\omega_{deprescribe}$ achieved a better fit than the model with a noninformative prior. Consequently, the results derived from the better-fitting model are presented herein.

The Sum of Effects of Chronic Monotherapies Does Not Equal Polypharmacy

Firstly, we compared gait and posture differences following chronic monotherapy and polypharmacy treatments. Compared to the control group, the effect of different treatments on each feature was different in terms of the time points (ages) at which statistical differences were observed, magnitude of these differences, and the duration of the differences observed (Figure 2A, Supplementary Figure S1 and Table S1). Of the monotherapies, citalopram showed the largest number of features with statistical differences to control throughout the study period (Figure 2C). Among the medications with sedative and/or anticholinergic effects (ie, citalopram, oxybutynin, and oxycodone), the citalopram group showed statistical differences from the first measurement on treatment at age 15 months (N = 17 deficits), while oxybutynin and oxycodone showed a slowly progressive increase in the number of deficits with age until 21 months. Metoprolol and simvastatin showed small numbers of differences from control. The total sum of individual deficits observed with each of the 5 constituent monotherapies (eg, n = 20 at 15 months, excluding overlaps) did not equal the deficits observed with high DBI polypharmacy (eg, n = 28 at 15 months).

Probing further into the deficits we find a variety of medication effects on morphometric and gait parameters. Consistently, treatment groups with a DBI > 0 (citalopram, oxycodone, and high DBI polypharmacy) decreased distance traveled and speed. Citalopram increased ellipse fit length, ellipse fit width, median step width, and limb duty factor. On the other hand, oxycodone and high DBI polypharmacy decreased ellipse fit length, median temporal symmetry, and



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Treatment	Metoprolol	Simvastatin	Oxybutynin	Oxycodone	Citalopram	Total Monos	High DBI Poly	Low DBI Poly	Zero DBI Poly
15M	2	1	4	3	17	20	28	16	1
18M	2	3	7	7	23	26	36	26	5
21M	2	4	11	12	21	29	35	22	5
24M	4	8	5	11	19	25	32	19	7
DP Reversible	100% (4/4)	75% (6/8)	60% (3/5)	0% (0/11)	11% (2/19)	36% (9/25)	47% (15/32)	79% (15/19)	100% (7/7)

Figure 2. From middle age to old age (12–24 months), medications have different impact on gait and posture, while polypharmacy with increasing DBI shows dose-dependent increases in deficits. Effects of (A) individual medications and (B) polypharmacy with increasing DBI on gait and posture outcomes over time. Data are presented as time-dependent baselines ($\mu\,[t]$) + treatment effect, $\delta_{treatment}\,[t]$, (ie, the deviation of each treatment group from the baseline $\mu\,[t]$) or deprescribing effect, $\omega_{deprescribe}$, which represents the effect of deprescribing at 24 months compared to the control group, $\mu\,[5]$. The error bars show the 95% Bayesian credible intervals (CrI). The open circles with dashed lines represent the deprescribing effect, indicating the impact of deprescribing each treatment from age 21 months on gait and posture at age 24 months. "*" and "#" at the top of each graph indicate statistical

differences in the treatment effects and deprescribing effect, respectively, with the colors corresponding to the treatment groups, at the designated time point. All outcomes are defined in Table 1. Distance AC represents the distance between base of head and tail during gait. Animal numbers displayed in Figure 1. Figures showing control and individual treatment groups are shown in Supplementary Figure 1A. (C) The summary table displayed at the bottom of the figure shows the number (sum) of statistically significant different features compared to control group at the corresponding timepoint ($N = 49$ morphometric and gait features, weight and frailty). The total monotherapy ("Total Monos") was calculated by the addition of deficit numbers for each treatment group, where individual features reached statistical significance in more than 1 treatment group compared to control, duplicates were removed. Data are summarized from Supplementary Table S1. M, months of age. Poly, polypharmacy. Deprescribing reversal is displayed in the table (defined as when the outcomes in the continued treatment group at 24 months were statistically significantly different from those in the control group, whereas the corresponding deprescribing group did not show such a difference). Deprescribing (DP) Reversibility was calculated by dividing the number of reversible deficits by the number of deficits with statistical differences in the continued treatment group at 24 months. Percentage of reversibility followed in brackets by the number of reversible deficits and total deficits per treatment group.

dAC nongait (normalized distance between base of head and base of tail when not walking).

Polypharmacy With Increasing Drug Burden Index Score Increases Deficits

Next, we investigated whether this method could detect the increase in functional deficits observed with increasing DBI in older adults with polypharmacy (19). Comparing the polypharmacy groups to the control group, the number of deficits increased with the DBI score (Figure 2B, Supplementary Figure S1 and Table S1). At 15 months, the zero DBI polypharmacy group had minimal deficits ($n = 1$), similar to non-DBI contributing monotherapy groups (metoprolol and simvastatin), but the low DBI polypharmacy group ($n = 16$) and the high DBI polypharmacy group ($n = 28$) had progressively more deficits (Figure 2C). The peak number of deficits relative to control was observed at 18 months. Although the number of observed statistical differences from control for features related to measuring the median value was relatively similar between the high DBI and low DBI polypharmacy groups, for features related to the IQR a greater number of differences from control was seen in the high DBI than in the low DBI polypharmacy group (Supplementary Figure S2).

Polypharmacy regimens had some common effects and other effects that were related to increasing DBI score (Figure 3, Supplementary Figure S1). Regardless of the DBI score, polypharmacy decreased median ellipse fit length and increased median ellipse fit width of the mice. Polypharmacy regimens with increasing DBI increased weight and median limb duty factor, and decreased median base tail lateral displacement, distance traveled, median speed, stride length, and temporal symmetry. High DBI polypharmacy reduced median step length.

Most Sensitive Outcomes for Medication Screening

Several common, clinically relevant, sensitive, and/or time-dependent gait and posture features were identified (Figure 3). Of the 49 features, only rearing count at 24 months showed statistical difference in all 8 treatment groups compared to the control group (Supplementary Table S1). Specifically, at 15 months, rearing count for the high DBI polypharmacy, citalopram, and low DBI polypharmacy groups dramatically reduced to 57% (95%CrI: 0.44, 0.74), 49% (95% CrI: 0.36, 0.65), and 56% (95% CrI: 0.43, 0.73), respectively, compared to the control group (Figure 2A and B). These reductions remained until 24 months.

In addition, features that were sensitive to specific medications were identified in this study. For example, a statistical decrease in median step width was distinctively observed in the citalopram group, and poor gait balance, demonstrated by median step length2 and median temporal symmetry, was seen in the oxycodone group. Furthermore, the direction of the effects differed between the treatment groups across 14 features (cells highlighted in yellow in Supplementary Table S1).

Deprescribing Reversed Some Outcomes But Not All

We next wanted to answer the important clinical questions, "Can deprescribing reverse medication effects on gait and posture and does deprescribing have novel effects on these outcomes?." In summary, the effect of deprescribing on reversing medication effects and causing novel effects on gait and posture outcomes varied between treatment groups (Figure 2C). At 24 months, 32 features in the high DBI polypharmacy group showed a statistically significant difference compared to the control group. Among these, 15 features in the high DBI polypharmacy deprescribing group did not show a statistically significant difference to control (ie, reversibility = 47%; 15/32). The reversibility of outcomes was relatively high in the oxybutynin (60%; 3/5), metoprolol (100%; 4/4), simvastatin (75%; 6/8), zero DBI polypharmacy (100%; 7/7), and low DBI polypharmacy groups (79%; 15/19). In contrast, low reversibility was found in the citalopram (11%; 2/19) and oxycodone groups (0%; 0/11). Interestingly, some novel effects on gait and posture outcomes were found with deprescribing, including in the oxybutynin (IQR for temporal symmetry) and oxycodone (median for frailty and step length2) groups (Supplementary Table S1). Frailty status induced by oxybutynin and metoprolol showed statistically significant improvement after deprescribing. In contrast, frailty status induced by high DBI polypharmacy and oxycodone did not improve significantly after deprescribing.

Drug-Induced Frailty Status Can Be Predicted With High Accuracy

Finally, we applied the gait and posture features to predict the FI (Figure 4A, Supplementary Figure S1). As age, weight, and video-generated features were included into the prediction models, MAE and other model performance metrics improved in a stepwise manner (Figure 4B). The inclusion of the treatment group in the model with age, weight, and gait features did not improve MAE. The best model, a GB model with age, weight, and the gait features predicted the FI with an accuracy of 0.0367 ± 0.0018. Given that a mis-score of a single FI item is equivalent to a change of 0.032 in FI (ie, 1/31), this margin of error is comparable to the mis-scoring of 1 FI item, demonstrating the accuracy of the predictive model. To further determine which features contributed most to predicting frailty per treatment group, we evaluated the permutation importance of the 48 features (ie, 47 gait and posture outcomes and weight) within each treatment group using the GB model. The findings indicated that different features were important in predicting frailty, depending on the treatment groups (Figure 4C). For example, frailty in the high DBI polypharmacy group was most strongly predicted by

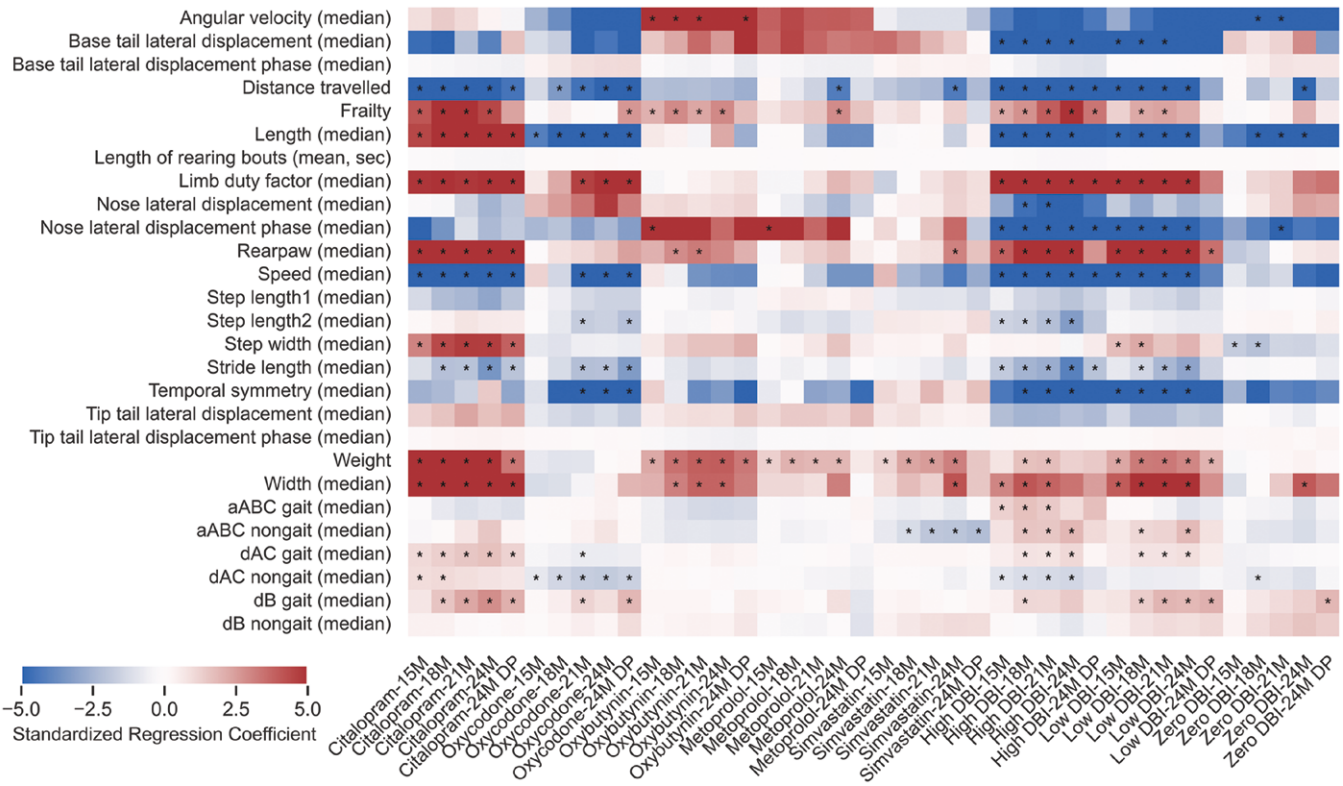

Figure 3. The sum of monotherapy deficits does not equal polypharmacy deficits. Heatmap demonstrating gait and postural changes following chronic monotherapy, polypharmacy with increasing DBI and deprescribing. Median outcomes are listed on *y* axis (left side), with treatment groups and ages on the *x* axis. Data have been standardized to allow for comparison between features with different units. The colors (blue for negative and red for positive) represent the direction and magnitude of the standardized regression coefficient. Two count valuables (ie, rearing count and stride count) are excluded from this heatmap because the other variables are continuous and were analyzed using a linear regression model, whereas the count variables required a negative binomial regression, making direct comparison of the results impossible. "*" indicates statistically significant differences. The statistical differences in these standardized data do not necessarily correspond with those in nonstandardized data seen in other figures and tables. M, months; DBI, Drug Burden Index, refers to DBI of polypharmacy regimen; DP, deprescribed.

median distance between rear paws and median step length while in the oxybutynin group the most important features were interquartile range of step length and step width, lateral displacement of tail and displacement of the mid back point; and in the simvastatin group the most important feature was weight with minor contributions from step length and width.

Discussion

In the present study, we demonstrate that recently developed neural network models (13) provide an opportunity to screen the impact of chronic monotherapy, polypharmacy with increasing DBI, and deprescribing on gait and posture, which can predict frailty. This is important preclinical screening for drug regimens intended for use in older adults. Specifically, we found that (i) the sum of individual monotherapy changes in gait and posture did not equate to the polypharmacy changes, (ii) DBI score increased changes in gait and posture in a dose-dependent manner, (iii) deprescribing reversed some outcomes but not all and resulted in some novel changes, (iv) the prediction model including age, weight, and video-generated features predicted frailty with high accuracy, and (v) different features were important in predicting frailty depending on the treatment groups, demonstrating that pharmacological actions of different medications influence frailty differently.

The differential impact on gait and posture of aged mice observed across various medication regimens is consistent with their pharmacology. Citalopram, a selective serotonin reuptake inhibitor, demonstrated the most pronounced impact on gait and posture among the monotherapies studied. Citalopram's influence on serotonergic neurotransmission in the central nervous system can lead to the observed deficits in motor function, affecting both gait and posture. The progressive increase in gait deficits with age in the oxybutynin (anticholinergic medication) and oxycodone (opioid analgesic) groups may be attributed to aging-related pharmacokinetic alterations in metabolism and excretion, leading to increased drug exposure over time, as well as increased pharmacodynamic sensitivity to these drug classes in old age. Consistent with clinical observations in older adults prescribed anticholinergic and sedatives, chronic exposures to citalopram (antidepressant) and oxycodone (analgesic) reduced gait speed (20). However, poor gait balance (based on median temporal symmetry) seen in the oxycodone group did not recover after deprescribing. This raises questions about whether the increased risk of falls observed in older adults taking opioids is reversible with deprescribing alone (21). Notably, citalopram differs from many other treatment groups by increasing variability (ie, IQR) in stride length, step length, and temporal symmetry, as well as median length of the mouse (a postural effect). Oxycodone had effects on gait that are more similar to the high DBI polypharmacy regimen. This illustrates the complexity of polypharmacy effects, which differ from and are difficult to predict from the effects of individual monotherapies. A previous study of oxycodone on gait in young adult C57BL/6 mice described a "tip toe gait" with stiff tail in mice 30–60 min after receiving a single intraperitoneal dose of

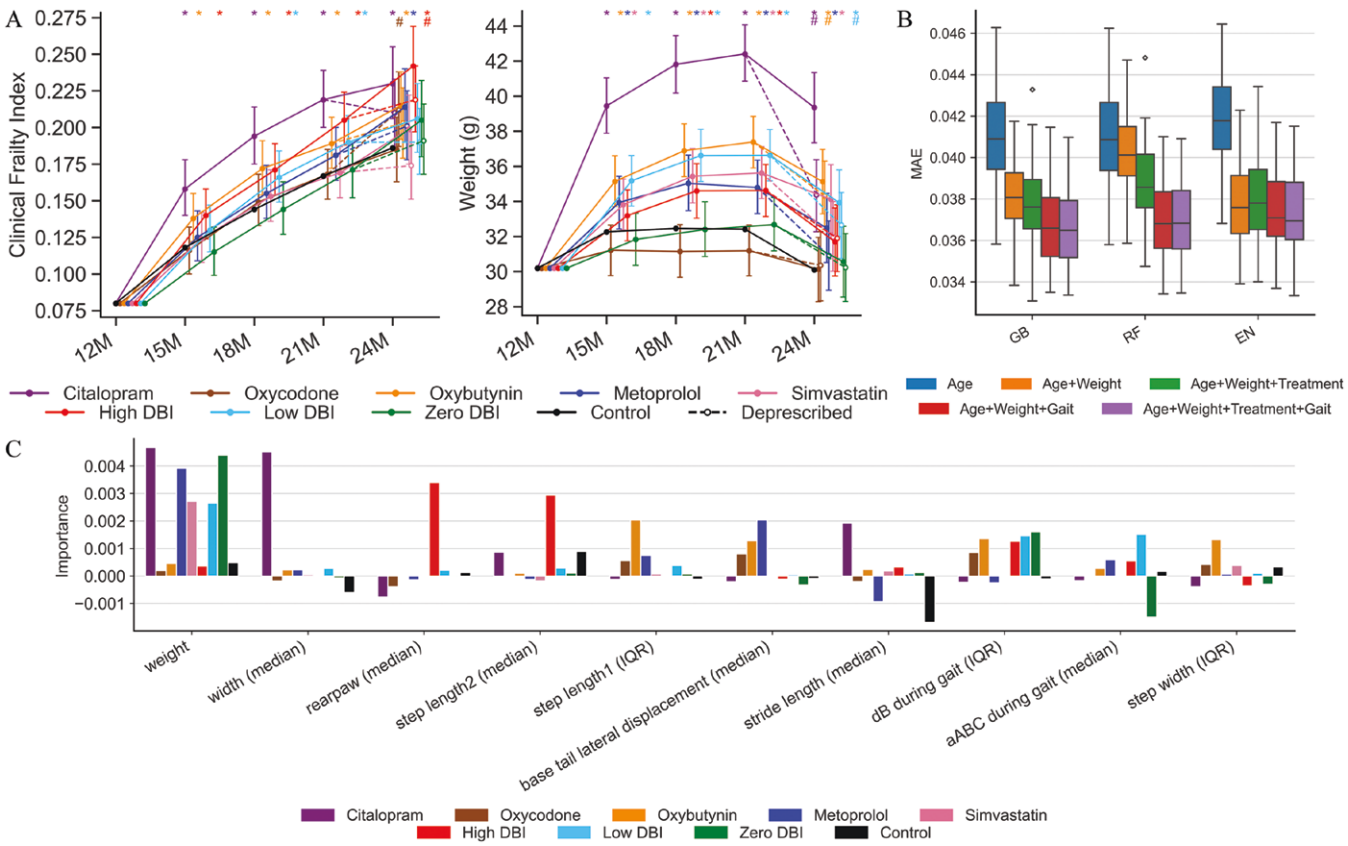

Figure 4. Impact of medication and deprescribing on morphometric and gait features, frailty, weight, and predictive models.(A) Effect of medication on the mouse clinical Frailty Index and weight. Data are presented as time-dependent baselines (μ[t]) + treatment effect, $\delta_{treatment}[t]$, (ie, the deviation of each treatment group from the baseline μ[t]) or deprescribing effect, $\omega_{deprescribe}$, which represents the effect of deprescribing at 24 months compared to the control group, μ[5]. The error bars show the 95% Bayesian credible intervals (CrI). The open circles with dashed lines represent the deprescribing effect, indicating the impact of deprescribing each treatment from age 21 months on gait and posture at age 24 months. "*" and "#" at the top of each graph indicate statistical differences in the treatment effects and deprescribing effect, respectively, with the colors corresponding to the treatment groups.(B) Mean absolute error (MAE) of predicting mouse clinical Frailty Index with the Gradient Boosting (GB), Random Forest (RF), and Elastic Net (EN) model with varying input of data (age, weight, treatment, computational video analysis of gait [n = 47 features]). (C) Top 10 predictors of the mouse clinical Frailty Index across different treatment groups, with importance derived from the GB model.

oxycodone 10 mg/kg (22). The differences to our findings support the importance of preclinical testing that reflects different doses, durations, and ages of intended drug use (9). Median stride length was shorter than control for citalopram, oxycodone, and high DBI polypharmacy treated animals, which has previously been reported in Huntington's disease mutation mice that display abnormal gait (23). The non-DBI contributing medications, metoprolol (beta-blocker) and simvastatin (HMG-CoA reductase inhibitor), showed fewer differences from the control group in gait and posture. Their relatively minor impact on gait and posture could be due to their primary pharmacological actions less directly affecting motor function in the central nervous system.

The observation that the aggregate impact of individual monotherapies does not equate to the effect of high DBI polypharmacy underscores the potential for pharmacokinetic and pharmacodynamic interactions in polypharmacy regimens. When multiple drugs are coadministered, they may interact in a synergistic or antagonistic manner. Pharmacokinetic interactions and the cumulative pharmacodynamic effects of drugs with anticholinergic, sedative, and serotonergic effects in the high DBI polypharmacy regimen (oxybutynin, oxycodone, and citalopram) may contribute to the impact on gait and posture being greater than the sum of the monotherapies.

Over the past 2 decades, many observational studies in older adults have reported poorer measures of physical function, gait, and increased falls risk with anticholinergic and sedative drug exposure measured with a wide range of tools, particularly reduced gait speed (24–28). Wouters et al. reported that among patients at a geriatric day clinic who had 22 dynamic gait parameters measured, those with a high DBI > 1 had poorer gait regularity and pace, and those with moderate (0 < DBI ≤ 1) and high DBI had poorer gait complexity (29). In our mouse model, we find that exposure to high DBI polypharmacy affected on similar functional measures, such as gait speed, symmetry, and frailty. Similarly, a cohort study from a geriatric outpatient clinic observed associations between polypharmacy and measures of gait decline, which were associated with incident falls (30). The consistency between human and mouse demonstrates an opportunity for this method to be a useful translational tool to screen for harms caused by chronic medication use, polypharmacy, and the DBI.

Clinically, it is important to understand whether deprescribing reverse chronic medication-related harm to global functions essential for independence like gait. Clinical studies have found that deprescribing is safe and does not increase hospital admission or mortality (31). However, these studies have not

demonstrated significant changes in functional outcomes (32). Our study comprehensively investigated treatment effects at different time points from middle age to old age in mice (33). We demonstrate that deprescribing can reverse some of the functional deficits induced by medications. However, not all outcomes were reversible. Importantly, we find most effects that had an early onset and lasted to age 24 months were irreversible (based on our definition). This may suggest that routine monitoring and early detection/action may increase chances of reversibility from medication harm. In addition, some novel changes were induced by the deprescribing process itself (ie, drug withdrawal effect). This suggests that while deprescribing is a promising strategy for mitigating drug-induced impairments, healthcare providers should carefully follow-up patients for drug withdrawal events and consider the need for rehabilitation after deprescribing. This study demonstrates that preclinical models can be a novel method to understand deprescribing. Further research is required to understand why some outcomes are reversible, others are irreversible, or why novel outcomes appear.

Medication use is very common in old age and affects frailty (34). Hession et al. previously demonstrated that gait and morphometric data from aging control mice could be used to predict frailty (13). The clinical translatability of this method could be improved by considering that older adults commonly take medication for multimorbidity. Applying the method to our cohort of chronic medicated animals, we found that the application of gait and posture features, along with age and weight, in the GB model achieved high accuracy in predicting the FI, with a margin of error comparable to the impact of a single mis-scored FI item. Importantly, the addition of the treatment group to the model did not improve the MAE, suggesting that the included gait and posture features, alongside age and weight, sufficiently captured the effects of treatment to predict frailty. Additionally, although we acknowledge that the results of the permutation importance are strongly affected by the correlation between features, the finding of different feature importance for predicting frailty may demonstrate that specific medications cause frailty to manifest differently. This is analogous to biologic differences identified in age-related and disease-related frailty in older adults (35).

This study is the first of its kind to measure posture and gait activity in a mouse following chronic monotherapy, polypharmacy, and deprescribing but has some limitations. Some differences between the high DBI polypharmacy group and the low DBI polypharmacy group may be partly attributed to the significant reduction in speed within the high DBI polypharmacy group, which narrows the variance of deficits. Further research will be needed to investigate this rationale by stratifying the data according to speed. While the mouse model tests exposures to chronic treatments at therapeutic doses, it does not model the indications for the treatments, which are also likely to affect on gait, posture, and frailty. Furthermore, the deprescribing model gradually withdraws all drugs, and does not test response to the clinical scenario where a person with polypharmacy has only some of their drugs stopped or dose reduced. Gait and posture in mice are not directly translatable to gait and posture in humans and the impact of the observed changes in aging mice on falls and function in older adults is not well understood. Further clinical studies of the effects of anticholinergic and sedative medications and deprescribing on gait, posture, and functional outcomes in older adults are needed.

In conclusion, we demonstrate the application of neural networks on open-field videos of mice to sensitively screen the impact of chronic monotherapy, polypharmacy and increasing DBI and deprescribing. We demonstrate the cumulative effects of medications are complex, the sum of deficits from constituting monotherapies does not equate to the deficits of polypharmacy, and deficits increase with DBI score. Deprescribing can potentially improve some outcomes but not all. Gait and posture features, alongside age and weight, sufficiently capture the treatment effects to predict frailty. Finally, medications have different feature importance for the prediction of frailty. Collectively, we demonstrate a sensitive preclinical method paired with computational analysis to detect clinically relevant effects of chronic medications and polypharmacy. This could be applied to novel and currently used medications to better predict and understand long-term medication-induced harms or benefits on gait and frailty. Further studies are needed to explore the mechanisms underlying these observations and to translate these findings into clinical practice.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None.

Data Availability

Data and code used in this study are available from the corresponding author on reasonable request.

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Author Contributions

S.N.H., K.F., and J.M. conceptualized and conceived the project. J.M. prepared the open-field data. K.F. conducted the computational visual analysis and statistical analysis with input from S.N.H. and J.M., K.F., and J.M. wrote the original draft of the paper with oversight and direction from S.N.H. All authors provided critical feedback, edited the manuscript and approved the final submission version.

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