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Effect of Ibuprofen on BrainAGE: A Randomized, Placebo-Controlled, Dose-Response Exploratory Study

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Abstract

BACKGROUND—The age of a person's brain can be estimated from structural brain images using an aggregate measure of variation in morphology across the whole brain. The brain age gap estimation (BrainAGE) score is computed as the difference between kernel-estimated brain age and chronological age. In this exploratory study, we investigated the application of the BrainAGE measure to identify potential novel effects of pharmacological agents on brain morphology.

METHODS—Twenty healthy participants (23–47 years of age) completed three structural magnetic resonance imaging scans 45 minutes after administration of placebo or 200 or 600 mg of ibuprofen in a double-blind, crossover study. An externally derived BrainAGE model from a

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sample of 480 healthy participants was used to examine the acute effect of ibuprofen on temporary neuroanatomical changes in healthy individuals.

RESULTS—The BrainAGE model produced age prediction for each participant with a mean absolute error of 6.7 years between the estimated and chronological age. The intraclass correlation coefficient for BrainAGE was 0.96. Relative to placebo, 200 and 600 mg of ibuprofen significantly decreased BrainAGE by 1.18 and 1.15 years, respectively ($p < .05$). The trained BrainAGE model identified the medial prefrontal cortex to be the strongest age predictor.

CONCLUSIONS—BrainAGE is a potentially useful construct to examine neurological effects of therapeutic drugs. Ibuprofen temporarily reduces BrainAGE by approximately 1 year, which is likely due to its acute anti-inflammatory effects.

Keywords

BrainAGE; Ibuprofen; Pharmacological manipulation; Prefrontal cortex; Structural imaging; Support vector

Ibuprofen is among the most widely used nonsteroidal anti-inflammatory drug (NSAID) in the world (1,2). The main mechanism of action of ibuprofen is the nonselective, reversible inhibition of the cyclooxygenase enzymes 1 and 2 (COX-1 and COX-2, respectively) (3). Over-the-counter use of NSAIDs is prevalent in the United States (4), but the exact mechanism of action of ibuprofen and its effects on different organ systems is still under investigation. NSAIDs have been indicated to have protective effects against neurodegenerative diseases, although the modes of action underlying these effects are not yet fully examined (5). Several meta-analyses have found a significantly lower prevalence rate of Alzheimer's disease among NSAID users (6) and there has also been some evidence that it may reduce the prevalence of Parkinson's disease (7). Beneficial cognitive effects of chronic ibuprofen administration have also been suggested (8,9). There has been some evidence that ibuprofen affects basic cell cycle progression, which may provide the molecular basis for its effects on increased longevity (10). Meanwhile, there is evidence that inflammatory processes may play an important etiological role in a subgroup of depressed individuals. For example, several meta-analyses have shown that interleukin-6, tumor necrosis factor, C-reactive protein, and interleukin-1 are elevated in the serum or plasma of depressed populations relative to healthy control subjects (11–14), while individual studies have reported alterations in the concentrations of chemokine (C-C motif) ligand 2 and interleukin-10 in mood disorders (15,16). A population study showed that ibuprofen reduced the risk of psychiatric contacts (17). Another recent meta-analysis reported lower Patient Health Questionnaire–9 score in the ibuprofen/naproxen group and Celebrex group (18). In another recent study, acetaminophen was found to affect how emotionally aroused participants reported for negative and positive stimuli (19). Thus, anti-inflammatory drugs could be useful as treatments of some forms of depression. Taken together, NSAIDs seem to have longer-term neuroprotective effects and potentially more acute effects on not only pain and inflammation, but also cognitive and emotional functioning.

To our knowledge, there have only been two neuroimaging studies of ibuprofen. In the first study, 10 healthy participants underwent a double-blind, placebo-controlled, randomized,

crossover pharmacological functional magnetic resonance imaging (MRI) study during somatosensory painful stimulation of the right median nerve. The study suggested that ibuprofen acutely attenuated the blood oxygen level–dependent response in specific pain-related brain areas (20). In a recent study, only gabapentin but not ibuprofen (600-mg single dose) suppressed the resting-state functional connectivity during central sensitization between the thalamus and secondary somatosensory cortex (21). In the current neuroimaging study of ibuprofen, we aimed to improve our understanding of ibuprofen's acute effect on the brain using a morphological measure that integrates MRI data and aging.

The ability to objectively quantify the degree of aging for different organ systems is of enormous biological importance. For example, the ability to detect deviations from normal aging, identify risk or protective behaviors, and/or identify early onset of a disorder characterized by accelerated aging could yield opportunity windows for early intervention. Franke *et al.* (22) introduced a framework for automatically and efficiently estimating the age of healthy participants from their T1-weighted MRI scans using a kernel method for regression. Other age-estimation methods have since been suggested with different imaging modalities (23,24), but the general framework is consistent: 1) extract image-related features, 2) train a machine learning model on a large normative dataset to predict age, 3) obtain predicted ages on a separate dataset of interest with the trained model, 4) compute each subject's brain age gap estimation (BrainAGE), and 5) make inferences about accelerated or delayed aging. BrainAGE, defined as the difference between the estimated age and the biological age of the individual, has been suggested to be a reliable, scanner-independent, and efficient measure of deviation from normal brain aging in healthy participants (22) and to be able to predict individual brain maturation (25). Different research groups found BrainAGE to be correlated with physical fitness (26), mortality risk in elderly participants (27), and human immunodeficiency virus status and cognitive performance (28). Others have shown that, compared with control groups, BrainAGE was higher in patients with psychiatric disorders (29), mild cognitive impairment, Alzheimer's disease (30), or diabetes (31) and much lower for long-term meditators (32). Taken together, these findings provide strong evidence that age can be estimated using features from structural brain imaging and can be meaningfully related to other age-related processes.

The primary aim of this exploratory investigation was to determine whether an acute administration of ibuprofen affects BrainAGE during its peak serum concentrations (33). This study did not examine the prespecified outcome measures. While previous epidemiological studies have focused on the long-term neuroprotective impact of NSAIDs, in this work, we focused on the acute impact on BrainAGE. During the effects of the drug, we hypothesized that the ibuprofen administration may be associated with structural characteristics of a relatively younger brain, which likely would involve more proportional gray matter volume in general but would not necessarily be localized to a specific area. Support for this hypothesis would provide the first evidence of an acute pharmacological change of BrainAGE and may help explain recent preclinical findings of beneficial cognitive effects of ibuprofen.

METHODS AND MATERIALS

We used two independent datasets to 1) establish an estimation of BrainAGE using support vector regression (SVR) (34) and 2) determine the effect of an acute dose of ibuprofen on BrainAGE. Specifically, structural imaging data from dataset 1 (training) were used to train a prediction model for age, which was then applied to dataset 2 (ibuprofen) to derive BrainAGE for evaluating the effects of ibuprofen. The dose-response crossover design helped optimize the statistical method's sensitivity to relatively small within-subject effects and to explore dose-dependent effects. A summary of the samples' characteristics in these two datasets is shown in Table 1. The image acquisition process of the two datasets is described in detail as follows.

Dataset 1 (Training)

Structural MRI data of 480 (mean age 34.5 ± 10.5 years; age range, 18–67 years; 263 women) healthy volunteers who participated in different studies at the Laureate Institute for Brain Research between 2009 and 2017 were used to develop an estimation procedure for BrainAGE as described in Franke *et al.* (22). Each volunteer was scanned in a 3T GE MR750 whole-body scanner (GE Healthcare, Malvern, PA). All images were axially acquired T1-weighted magnetization prepared rapid acquisition gradient-echo scans with a 24-cm field of view, 256×256 acquisition matrix, 8° flip angle, and $0.9375 \text{ mm} \times 0.9375 \text{ mm}$ in-plane resolution acquired with no gap between slices. Other parameters varied within the following ranges (repetition time, 5.736 to 6.292 ms; echo time, 1.896 to 2.104 ms; slice thickness, 0.9 to 1.2 mm) with either an 8-channel (General Electric, Milwaukee, WI) or a 32-channel (Nova Medical Inc., Wilmington MA) phased array coil. Supplemental Table S1 shows counts for the exact number of scans used with each set of parameters. Healthy control status was verified using the Structured Clinical Interview for DSM-IV (35) and Mini-International Neuropsychiatric Interview (36). For subjects who participated in multiple studies, only a single, randomly chosen scan was used in the training set.

Dataset 2 (Ibuprofen)

Twenty-two healthy participants ($n = 11$ men) (Supplement) between 18 and 50 years of age were recruited for a double-blind, randomized, crossover study. Two subjects withdrew for personal reasons before completion, so 20 were analyzed ($n = 10$ men). Subjects were recruited between May and October of 2015 from the community in the Tulsa, Oklahoma, area and were assessed at the Laureate Institute for Brain Research. Recruitment stopped after completing the target number of participants specified in the protocol. No adverse events were reported. Each participant completed a baseline screening assessment and was scheduled for three subsequent scanning visits. During each scanning visit, scheduled 2 weeks apart to preclude any potential carryover effects, participants received one oral pill containing placebo, 200 mg of ibuprofen, or 600 mg of ibuprofen approximately 45 minutes before scanning. Administration order was counterbalanced across participants (see Supplemental Table S2 for details), and experimenters were blinded to the medication codes. Although participants reported occasional over-the-counter NSAIDs during the study period, they never did so less than 48 hours before a scan. Each participant was also scanned in a 3T GE MR750 whole-body scanner, and scanning parameters were similar to the mode of those

used in the training set. Specifically, apart from the first three participants who had a slightly different protocol (see Supplemental Table S1 for details), the remaining scans in this study had the following parameters: repetition time/echo time = 5.992 ms/2.012 ms, field of view/slice = 240 mm × 192 mm/0.9 mm, 256 × 256 matrix, 186 axial slices. During scan days, participants completed self-report scales, behavioral tests, and a blood draw at the end of the day (see Supplemental Figure S1 for a detailed timeline). This schedule was repeated approximately 2 and 4 weeks after the first session.

Self-report Assessments

The Bond-Lader visual analog scales (BL-VAS) (37) are a set of 16 analog scales used to measure participants' feelings, and the Wong-Baker FACES Pain Rating Scale (38) is a faces scale that is widely used for pain assessment. These values were collected before and approximately 3 hours after ibuprofen administration to examine the effect of ibuprofen on acute mood and pain ratings.

Imaging Analysis

All structural images were processed using FreeSurfer (39) to segment the brain into gray matter and non-gray matter, including the cerebellum and subcortical structures. Using Analysis of Functional NeuroImages software (40) but following a procedure similar to Franke *et al.* (22), gray matter images were transformed to Montreal Neurological Institute space by affine registration, smoothed with an 8-mm full width at half maximum kernel, and downsampled to $8 \times 8 \times 8$ mm³ voxels, resulting in 3707 voxels per participant, where each voxel's value corresponds to the fraction of that voxel composed of gray matter.

Statistical Analysis

Low Variance Filtering—Ten percent of the voxels with the lowest variance across all participants in both datasets were removed from the feature set. This standard dimension reduction technique (41) allowed us to focus on the potentially more explanatory/interesting voxels and increase the analysis power without inspecting the response variable (age). After filtering, the averaged gray matter proportions between training set and ibuprofen dataset were generally comparable, except for a few slightly larger average values of the ibuprofen data as compared with those of the training set (Supplemental Figure S2).

Support Vector Machine–Based Regression—We used a 10-fold cross-validation framework on dataset 1 to tune the budget, C , and the inverse kernel width, γ , parameters in the epsilon-SVR (ϵ -SVR) with a radial kernel. SVR is the regression extension of a support vector machine, a powerful machine learning method that allows for a nonlinear kernel and helps reduce overfitting with an adjustable budget parameter in the constraint representation (42). In a support vector machine, data points that lie on the margin boundaries and on the wrong side of the classification boundary are called support vectors because they contribute to the construction of the classification hyperplane. Analogous to a support vector machine ignoring nonsupport vectors that are far away from and on the right side of the decision boundary, SVR ignores points with small residuals in its optimization by setting to zero the errors of a size less than a chosen threshold. SVR is a commonly used regression model to estimate BrainAGE, along with other methods such as Gaussian process and relevant vector

regression. However, we note that, when the parameters are tuned appropriately rather than set as default, the validation accuracy values of SVR and relevant vector regression models are comparable (22). We computed BrainAGE for participant i by taking the difference between the brain-estimated age and chronological age:

$$\text{BrainAGE}_i = \widehat{\text{age}}_{\text{brain},i} - \text{age}_{\text{chrono},i}.$$

We computed the root-mean-square error (RMSE), the mean absolute error (MAE), and the variance explained (R^2) of the cross-validated age estimates from the training dataset.

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^n \left| \text{BrainAGE}_i \right|$$

$$\text{RMSE} = \left(\frac{1}{n} \sum_{i=1}^n \text{BrainAGE}_i^2 \right)^{1/2}$$

$$R^2 = 1 - \frac{\sum_{i=1}^n \text{BrainAGE}_i^2}{\sum_{i=1}^n (\text{age}_{\text{chrono},i} - \overline{\text{age}}_{\text{chrono}})^2}$$

Even with cross-validation, we note that these values are likely an overestimate of the model's performance because they are calculated from the same training dataset. However, assessing these values provides a point of reference to compare with the cross-validated correlation, MAE, and RMSE from other models implemented in the BrainAGE literature [e.g., Gaussian process regression (27)].

Effects of Ibuprofen on BrainAGE—The learned model was then applied on the ibuprofen dataset. Because every participant in the ibuprofen study was scanned three times within a few weeks, we computed three BrainAGE scores for each participant. We further examined BrainAGE's relationship with ibuprofen dosage in a mixed effect model:

$$\text{BrainAGE} = (\beta_0 + u_i) + \beta_1(\text{ibu200mg}) + \beta_2(\text{ibu600mg}) + \varepsilon$$

where $u_i \sim N(0, \sigma_B^2)$ allows participants to have random intercept and $\varepsilon \sim N(0, \sigma_W^2)$ accounts for within-subject variation. The β parameters pertain fixed effects. Considering the small sample size, degrees-of-freedom was adjusted by the Kenward-Roger method (43). The variance of random effects could be used to estimate the intraclass correlation coefficient (ICC), which provides a means to assessing reliability of the BrainAGE values. A simple formula for the ICC is $\hat{\sigma}_B^2 / (\hat{\sigma}_B^2 + \hat{\sigma}_W^2)$, where $\hat{\sigma}$ indicates estimates of σ . This analysis will reveal the behavioral and cognitive characteristics of individuals that contribute to the amount of acceleration or deceleration of brain aging, measured directly by residual age.

Additional Analyses

To examine the effect of ibuprofen on mood and pain, we conducted a multivariate linear mixed-effects model that included all 16 BL-VAS itemized scores to examine the pre-to post-drug administration difference of the individual BL-VAS scores as well as the Wong-Baker FACES Pain Rating Scale. Specifically, the model considered ibuprofen dosage as fixed effects and random intercepts for subjects and BL-VAS items or pain rating value.

The R script for this research (R version 3.4.4; R Development Core Team, Vienna, Austria) has been made available on the GitHub repository <https://github.com/lelaboratoire/ibuBrainAGE>.

RESULTS

Trained BrainAGE Model

The low-variance filtering process removed 373 voxels from the initial 3707 voxels, so 3334 remaining voxels were entered to SVR (Supplemental Figure S3). The 10-fold cross-validation procedure led to the optimal parameters $C = 1.6245$ and $\gamma = 1.8376E-4$, which corresponded to the BrainAGE shown in Supplemental Figure S4. The trained model yielded a correlation of $r = .76$ between brain-predicted age and chronological age and explained 57% of the variance (R^2) in chronological age (Supplemental Figure S4). The MAE of prediction was 5.34 years and the RMSE was 10.5 years. The smaller amount of variance explained with comparable MAE in our tuned model compared with previous studies is likely due to our smaller age range [18–67 years compared with, for example, 18–90 years in Cole *et al.* (27)].

Contributing Brain Regions

In the regression on age, with SVR from the R package “caret” (44), we computed the importance scores of each variable (i.e., voxel locations) in the tuned model. The voxels explaining most variance in BrainAGE are located in the medial prefrontal cortex (PFC) (Figure 1). A more comprehensive view of the importance voxels in the whole brain and the score distribution are provided in Supplemental Figures S5 and S6, respectively.

Intrasubject Reliability of the BrainAGE Framework

The model trained on dataset 1 was applied to T1-weighted MRI scans of the ibuprofen study participants to estimate their brain-predicted age. At the time of scanning, the mean chronological age of these participants was 32.4 ± 6.7 years and mean brain-predicted age was 37.1 ± 4.8 years. The brain age prediction had a slightly larger MAE (6.65 years) than the previous cross-validated MAE in the training set ($t_{538} = -1.91$, $p = .06$) and showed a lower average correlation with chronological age across all subjects at each dosage ($r_{\text{average}} = .35$). These differences were likely due to the restricted age range of dataset 2 (Table 1), which might have contributed to a lower correlation of age estimate and chronological age for this population. The estimations of the BrainAGE for dataset 2 were highly reliable, with an ICC of 0.96 (95% confidence interval [CI], 0.92–0.98) for the three BrainAGE values estimated from three MRI scans of each of the 20 participants in the ibuprofen study (Figure 2).

Effect of Ibuprofen on BrainAGE: A Mixed-Effects Model

The linear mixed-effects model revealed a significant effect of ibuprofen on BrainAGE ($F_{2,38} = 5.83, p = .006$). Post hoc tests showed that compared with placebo, BrainAGE was reduced by 1.1 years when participants were given either 200 mg ($t = -3.02, p = .004$) or 600 mg ($t = -2.92, p = .006$) of ibuprofen (Figure 3). For each dosage, we estimated the Cohen's d and 95% CI interval with bootstrapping and observed a medium to large effect size (Cohen's $d_{200\text{mg-placebo}} = 0.57$ [95% CI, 0.27–0.95]; Cohen's $d_{600\text{mg-placebo}} = 0.69$ [95% CI, 0.39–1.15]). Details of the estimates and their confidence intervals are shown in Table 2. We expected individual differences in the response to ibuprofen. Therefore, while the BrainAGE of 13 of 20 individuals showed the expected dosage effect, three individuals had very similar BrainAGE across the dosages, and three had older BrainAGE after taking placebo compared with taking 200 or 600 mg of ibuprofen. Nevertheless, the mixed-effects model approach is adequately powerful in capturing the overall trend and yielding meaningful statistics while taking into account intersample variability. In addition, we conducted an analysis with gender and body mass index as well as the interaction between these covariates and the ibuprofen dose. There was a nonsignificant effect of gender on BrainAGE ($t_{17} = -1.91, p = .07$), and the effect of ibuprofen dosages on BrainAGE remained unchanged. Finally, although overweight and obese individuals have been found to have greater BrainAGE estimates (45), we found no effect of body mass index on BrainAGE ($t_{17} = -0.10, p > .5$).

Association Between Ibuprofen and Mood Change

There was no significant effect of ibuprofen on any of the 16 individual BL-VAS scores or pain rating score from pre- to postscan in the multivariate model ($F_{2,981} = 0.308, p = .735$) (see Table 2 for more details). In other words, we did not find any evidence of ibuprofen influencing the individual's physical and emotional feelings, even during its peak serum concentrations.

DISCUSSION

To the best of our knowledge, this study is the first to examine the effects of an acute pharmacological manipulation on BrainAGE, a measure that has been shown to capture deviation from normal brain aging. The BrainAGE model, after being trained on 480 healthy participants, was applied on a separate set of 20 individuals, each scanned three times within a period of 4 weeks. There were three main findings. First, the estimation of BrainAGE was highly reliable across assessments. Second, acute administration of ibuprofen significantly influenced a derived measure of brain morphometry. Specifically, 45 minutes after participants were given either 200 or 600 mg of ibuprofen, their BrainAGE was 1.1 years lower compared with placebo; in other words, their brain-predicted age was 1.1 years younger than under placebo. Third, we observed no effect of ibuprofen on subjective mood or acute pain ratings in this normative sample. Taken together, these results provide evidence for acute plasticity of BrainAGE, which could provide an important global measure of brain health.

The acute effects of ibuprofen on BrainAGE would seem to support the notion that this anti-inflammatory drug can acutely modulate the brain's morphological features. While there is evidence of ibuprofen crossing the blood-brain barrier (46,47), the mechanism underlying its acute effect on BrainAGE is unclear. The fact that the observed effect on BrainAGE occurred at both 200 and 600 mg suggests an effect specific to ibuprofen's mechanism of action, although the current study was not adequately powered to determine the optimal dosing of such an effect. These findings are consistent with a recent study that detected small but significant associations between the use of ibuprofen and better cognitive performance (48). Moreover, ibuprofen has been suggested to potentially have neuroprotective effects against neurological diseases (7). However, these studies and the current study do not elucidate the precise molecular mechanisms of ibuprofen's effects. Thus, future investigations will need to examine both the nonselective, reversible inhibition of COX-1 and COX-2 (3) and the COX-independent pathways, which affect changes in gene expression or alternative splicing and act through various cell cycle- and apoptosis-regulating pathways, including beta-catenin, nuclear factor κ B, peroxisome proliferator-activated receptor gamma, and p53 (49). Moreover, there is some recent evidence that ibuprofen allosterically affects the acid sensing ion channels, which have been implicated in neuroinflammatory processes (50). Taken together, ibuprofen's complex molecular pharmacology may affect BrainAGE via various pathways simultaneously.

BrainAGE has been proposed as a clinically-relevant neuroimaging aging biomarker, one potentially capable of determining risk of age-related deterioration and death (27), and it may prove clinically valuable in providing important prognostic information for abnormal brain maturation (25). The current study supports this notion by providing additional evidence that BrainAGE can provide quantitative estimates of an acute brain response to anti-inflammatory treatment. Derived from structural MRI scans, BrainAGE is verified to be a stable and reliable value that aggregates the multidimensional aging pattern across the whole brain of an individual. In the ibuprofen dataset, the computed ICC of 0.96 (intrasubject differences are very small relative to intersubject differences) demonstrated that the trained model was able to detect small within-subject differences but also produces consistent BrainAGE predictions across all subjects. We note that the BrainAGE framework yielded a slightly poorer fit of age in the ibuprofen dataset, potentially owing to the different distributions of age and gray matter volumes between this set and the training dataset. However, this systematic data-specific offset does not influence the result based on the robust within-subject estimates (51). Moreover, even though there is potential prediction bias due to age difference between datasets, the mixed-effects model approach minimizes this type of bias because each participant serves as her/his own control, i.e., the contrasts between placebo and ibuprofen dosages are unbiased, independent of the intercept coefficient for placebo. Furthermore, the discovery of high importance scores of voxels around the PFC in the regression with age agrees with previously found relations of aging with traditional measures of gray matter atrophy in the PFC (52–55). Moreover, these results are supported by observations of reduced numbers of connections with the medial PFC in older versus younger individuals (56). In a different study, age-related decreases in local gyrification indices were also found in the medial PFC (57). This investigation demonstrated that, in addition to providing a reliable surrogate measure for aging, the BrainAGE construct

has the potential to select meaningful brain regions that likely contribute to the aging process.

This study has several limitations. First, we did not test for clear links between BrainAGE and the molecular mechanism of ibuprofen. We also did not observe a clear dose-response relationship, which likely needs further investigation in a larger sample. Finally, we found no evidence for associations between the self-report BL-VAS scores and ibuprofen effects, although we did not expect to find such effects given the use of a healthy sample. Conducting similar investigations on a more heterogeneous set of participants, such as individuals with pain and/or inflammatory conditions, may result in different findings at the levels of symptom response and BrainAGE, and may help to reveal the molecular mechanisms for the reported effects. Further, while the contribution of the medial PFC had the largest contribution to the explained variation in our model, this effect may be part of a larger causal model involving statistical or functional interactions with other regions. Such models may help further delineate biological mechanisms that connect the medial PFC with the lateral and ventral PFC.

In summary, the current study demonstrates that the estimated difference between neuroimaging-predicted age and chronological age (BrainAGE) based on structural MRI data is sensitive to acute changes induced by oral ibuprofen. High ICC values demonstrate the strong stability of the calculated BrainAGE within each individual. The general BrainAGE model identified the medial PFC to be the strongest contributor to age prediction, consistent with earlier studies using traditional methods. We found that on average, BrainAGE reductions of 1.1 years were associated with the acute administration of ibuprofen. Although molecular mechanisms of ibuprofen need further investigation, our finding of BrainAGE's decrease during ibuprofen's acute administration provides additional evidence for anti-inflammatory drugs' immediate influence on brain's morphological features.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The principal investigator, MPP, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure 1.

Plot of the 510 most important attributes (support vector regression importance score >0.1) in the derived brain age gap estimation model: **(A)** axial view, **(B)** sagittal view, **(C)** coronal view. Red and blue indicate the most and least important voxels, respectively.

Figure 2.

Brain-predicted age vs. chronological age of participants in the ibuprofen study. The dots are connected if they belong to the same participant. Participants tended to have older brain-predicted age when given placebo (circles).

Figure 3. Effect of ibuprofen dosage on brain age gap estimation (BrainAGE). Participants who were given 200 or 600 mg of ibuprofen show a 1.1-year-younger BrainAGE.

Sample Characteristics

Table 1.

Variable	Training Dataset (<i>n</i> = 480)	Ibuprofen Dataset (<i>n</i> = 20)	Cohen's <i>d</i> or χ^2 (<i>df</i>)	<i>p</i> Value
Age, Years	34.5 ± 10.5	32.4 ± 6.7	0.274	.000301
Gender, Female/Male	263/217	10/10	0.037 (1)	.847
BMI, kg/m ²	26.5 ± 5.5	26.6 ± 5.8	0.117	.369
Ethnicity				
Hispanic or Latino	27	2	0.084 (1)	.772
Not Hispanic or Latino	437	18		
Refused/do not know	4/12	0/0		
Educational Status				
Grades 1–11	1	0	0.191	.555
12th grade (no diploma)	6	0		
Regular high school diploma	40	0		
GED or alternative credential	229	0		
Some college, no degree	142	3		
Associate's degree	51	11		
Bachelor's degree	1	2		
Master's degree	0	2		
Missing data	10	2		

Values are mean ± SD, *n/N*, or *n*. The variables of body mass index (BMI) and educational status contained missing values. The Cohen's *d* or χ^2 statistics were computed from a two-sample Kolmogorov-Smirnov or a χ^2 test along with the *p* values. However, the results should be interpreted with caution because of the small sample size of the ibuprofen dataset.

Table 2.

Fixed-Effects Results in the Mixed-Effects Linear Regressions to Analyze the Effect of Ibuprofen on BrainAGE and Mood/Pain Rating Scale

Model	Estimate	SE	df	t	Pr(> t)	95% CI
BrainAGE						
Intercept	1.703	1.507	20	1.130	0.27213	−1.315 to 4.720
ibu200	−1.184	0.392	38	−3.022	0.00448	−1.951 to −0.417
ibu600	−1.145	0.392	38	−2.923	0.00582	−1.912 to −0.378
Mood/Pain						
Intercept	0.2913	1.0206	37.8	0.285	0.777	−1.719 to 2.302
ibu200	−0.1031	1.0451	1000	−0.099	0.921	−2.151 to 1.945
ibu600	0.6528	1.0451	1000	0.625	0.532	−1.395 to 2.701

BrainAGE, brain age gap estimation; CI, confidence interval; ibu, ibuprofen.