Basal Forebrain Degeneration and Cortisol as Biomarkers Mediating Alzheimer's Disease Pathology: A Machine Learning Approach

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Abstract

The impact of Alzheimer's Disease (AD) on today's society and healthcare is unprecedented. As a larger portion of today's population enters an age for which AD becomes a health concern, there is growing support among health practitioners to prevent the disease's progression and development. Early identification of the disease may serve as a critical step towards combating the disease, allowing earlier interventions in the disease process to foster healthy aging. The focus of such interventions includes alleviating risk factors of AD, two of which include cortisol and degeneration in the basal forebrain. Importantly, increased levels of cortisol and reduced volume in the basal forebrain are attributed to higher risks of AD. In the present study, we make use of machine learning and the Alzheimer's Disease Neuroimaging Initiative (ADNI) database to characterize individuals with AD by using data from cortisol levels and basal forebrain degeneration. This allowed us to test whether cortisol and basal forebrain degeneration were predictively valuable for AD diagnosis. Our data partially supported our prediction—the machine learning classifier yielded significantly above chance classification accuracy for basal forebrain degeneration, but the classification accuracy for cortisol was not significantly above chance. Consequently, our results indicate that basal forebrain degeneration might serve as a diagnostically useful biomarker for AD, while cortisol's role in AD characterization necessitates further investigation.

Introduction

Alzheimer's Disease (AD) is one of the most common forms of dementia facing patient populations today. Characterized by progressive and often irreversible cognitive decline, AD is a serious public health issue that preferentially affects elderly populations. AD pathology involves a progression of events as individuals age. This progression may involve an intermediate case of pathology, mild cognitive impairment (MCI), which is distinct from healthy individuals and those with moderate AD. MCI patients exhibit memory differences when compared to healthy patients, while differing from mild AD patients in cognitive faculties other than memory **(**Petersen et al., 1999). The need to address Alzheimer's Disease has become particularly relevant in recent years, with 1996 United Nations projections indicating that from 2000 to 2050, a significant increase in the proportion of the population above 65 years of age is to be expected (Katzman & Fox, 1999). This increase emphasizes the need to work towards identifying therapeutic strategies against AD. Due to the progressive nature of this neurodegenerative disease, identifying AD at early stages may be critical for preventing its progression and combating the pathology. Often times, AD is only recognized after the disease has irreversibly progressed, making successful patient outcomes less likely. Early diagnosis may allow for interventions to be implemented in order to potentially maintain cognitive function at high levels in individuals with Alzheimer's Disease (Mueller et al., 2005). Early diagnosis could afford the possibility of disease treatment before AD irreversibly progresses, improving patient outcomes significantly.

Early diagnosis depends on recognizing biomarkers of interest that may be targeted with preclinical interventions in order to improve future outcomes for AD patients. Recent research has revealed that cortisol, a hormone that induces physiological responses to stress, may play a role in AD pathogenesis on account of its interaction with traditionally relevant AD markers. For example, rodent studies have established links between cortisol, cognitive impairment, and physiological anomalies (e.g. amyloid beta plaque and tau tangle formation). Researchers found that exposing amyloid beta-infused rats to chronic stress resulted in a greater significance in cognitive defects for short term memory, suggesting that stress may exacerbate cognitive decline in AD-pathological models (Srivareerat, Tran, Alzoubi, & Alkadhi, 2009). An additional study has also shown that glucocorticoid presence elevated amyloid beta presence in older mice and

localized tau abnormally. Based on the study, glucocorticoids seem to increase levels of these two crucial physiological markers of AD pathogenesis (Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006). Studies in human participants also point out a potential role cortisol may play in AD pathology by showing that cortisol levels can be significantly higher in AD vs. control or MCI individuals (Popp et al., 2009). Indeed, research utilizing urinary free cortisol levels suggests that "elevated cortisol may affect age-related cognitive and brain changes and possibly facilitate the development of AD" (Ennis et al., 2017).

AD pathogenesis is also believed to be related to basal forebrain degeneration—which may interact with cortisol to contribute to a downstream deficiency of the neurotransmitter acetylcholine in memory systems within the brain. Basal forebrain projections target brain areas populated by glucocorticoid receptors (e.g. the medial temporal lobe memory system), indicating that basal forebrain degeneration may interact with cortisol levels in AD pathology (Paul, Jeon, Bizon & Han, 2015; Helm, Han, & Gallagher, 2002). Importantly, it has also been demonstrated that basal forebrain degeneration predicts deterioration in the medial temporal brain areas such as the entorhinal cortex (Schmitz et al., 2016). Finally, one study has pointed to the idea that a loss of basal forebrain connectivity may lead to increased cortisol levels that damage the brain in an AD-associated manner. The study specifically related age-associated degeneration of the basal forebrain to hypothalamic–pituitary–adrenal axis dysfunction in such a way that can lead to the development of AD (Paul, Jeon, Bizon & Han, 2015).

Fully cognizant of how important early diagnosis is in successfully treating AD, the Alzheimer's Disease Neuroimaging Initiative (ADNI) constitutes an extensive program for integrating data regarding AD biomarkers from a community of researchers. This unprecedented program is designed not only to help diagnose AD and MCI, but also to provide insight into AD pathology and progression (Mueller et al., 2005). ADNI serves as a useful tool for developing AD diagnoses because its wealth of biomarker data adds power to statistically relevant conclusions drawn from the database.

In this study, we use the machine learning technique known as multivariate pattern analysis (MVPA) in order to classify AD individuals from cognitive normal (CN) individuals based on cortisol and basal forebrain degeneration. These predictors were independently used to evaluate how well the machine learning algorithm was capable of distinguishing between AD

and CN patients. This approach will allow for powerful insight into the predictive value of cortisol and basal forebrain degeneration in AD pathology and can help validate the importance of both biomarkers in identifying Alzheimer's Disease.

Literature Review

Recent demographic trends illustrate that the current global population is composed of a growing proportion of elderly individuals. In fact, the proportion of our population above the age of 65 is expected to increase significantly from the year 2000 to 2050, according to United Nations predictions (Katzman & Fox, 1999). This alarming increase in the cohort of aging individuals presents a corresponding increase in the number of potential age-associated neurological deficits. In particular, AD preferentially affects individuals as they age. With an anticipated increase in the aging demographic, the need to address this issue has become salient in recent years.

AD is a neurodegenerative disorder characterized by a progressive decline in the number of neurons in the aging brain. This neurological disorder is exacerbated with time, and late-stage AD patients experience substantial decline in cognitive faculties, including memory. The physical and emotional toll of AD on both patients and their loved ones underscores why interventions to treat this disorder are particularly necessary. Current problems with treating AD include diagnosing and identifying the disease too late, at a point at which the disease has irreversibly progressed. To treat AD, early diagnosis has been noted as an important avenue through which early interventions can be applied to preserve patient function for longer periods of time (Khachaturian, 1985). Consequently, present scientific literature aims to answer the question of what biomarkers, metrics of interest that are indicative of AD, are especially useful in identifying the disease at an early, asymptomatic stage. Importantly, identifying these biomarkers also helps clarify the disease process by which AD leads to progressive, irreversible neurodegeneration.

AD has traditionally been characterized by two pathophysiological processes and one common symptom. The pathophysiological processes associated with AD include the presence of amyloid beta and tau tangles, while the common symptom of this disorder is cognitive deficits (including significant memory loss). These three metrics all serve as the traditional markers by which AD is identified and by which AD may progress. As a result, tracking predictors of these markers has been explored as a way to achieve earlier diagnoses of Alzheimer's Disease. While these traditional markers characterize AD progression, the search for biomarkers that may be

detected preclinically has enormous implications for treating this debilitating disease and promoting healthy aging.

In pursuit of biomarkers to help clarify AD progression and help diagnose AD at early stages, cortisol and basal forebrain degeneration represent promising new biomarkers at the forefront of study. Cortisol is a hormone released by the adrenal cortex in response to stressful situations. Cortisol is known to induce a variety of changes to put the body in a state of "fight-or-flight," in which humans are better equipped to handle stressors in the environment. Multiple regions of the body express receptors to cortisol, and the brain is no exception. One particular area of the brain that is implicated in memory function, the hippocampus, is known to express extensive receptors for cortisol (McEwen & Sapolsky, 1995). In particular, studies have shown that prolonged exposure to cortisol can reduce hippocampal volume, a characteristic associated with neurodegenerative diseases such as AD (Kim, Pellman, & Kim, 2015). In fact, one study specifically concluded that increased cortisol measures could lead to the development of AD by inducing age-associated cognitive and neurological changes (Ennis et al., 2017). Alternatively, literature also proposes the idea that insufficient glucocorticoid signaling may lead to AD pathology: reduced cortisol signaling could allow pro-inflammatory processes to exacerbate brain damage and lead to the development of AD (Raison & Miller, 2003). Regardless, these findings, coupled with other literature findings, has motivated further research into cortisol as a potential pathophysiological property of AD.

Researchers have found that exposure of chronic stress to amyloid beta-infused rats resulted in more cognitive defects in short term memory, indicating that stress may cause cognitive decline in AD models (Srivareerat, Tran, Alzoubi, & Alkadhi, 2009). Additionally, glucocorticoids such as cortisol increased amyloid beta load in older mice and abnormally localized tau proteins. This finding reveals a potential relationship between cortisol and AD pathogenesis in the context of physiological biomarkers of AD (Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006). Finally, the loss of basal forebrain connectivity can increase cortisol levels due to dysfunction in the hypothalamic-pituitary-axis. In doing so, individuals may then be more predisposed to develop AD in the future due to cognitive impairment (Paul, Jeon, Bizon & Han, 2015).

Studies have also linked degeneration in the basal forebrain to the development of AD. The nucleus basalis of Meynert, a specific density of neurons within the basal forebrain, has been shown to undergo degeneration within Alzheimer's patients, helping explain cholinergic insufficiency in these patients (Whitehouse et al., 1982). Studies also demonstrate a link between the aggregation of neocortical amyloid beta, a pathological biomarker of AD, and basal forebrain degeneration (Kerbler et al., 2015).

As discussed, a number of studies have now explored the relationship of cortisol and basal forebrain degeneration in the characterization of Alzheimer's Disease pathology. In the present study, we make unique use of robust machine learning techniques (multivariate pattern analysis) to analyze how cortisol as well as basal forebrain degeneration can distinguish individuals who are cognitively normal from individuals who exhibit AD pathology. By using our current research approach, we hope to validate both cortisol and basal forebrain degeneration as biomarkers to be studied for a role in the characterization and development of AD.

Methodology

ADNI Database

The data obtained for this study came from a database known as the Alzheimer's Disease Neuroimaging Initiative, ADNI (http://adni.loni.usc.edu/). Dr. Michael W. Weiner, Principle Investigator of ADNI, started this program in 2004 with the desire to establish an integrative database that was focused on accumulating a diverse set of data to assist in the early identification of Alzheimer's Disease. Since its inception in 2004, ADNI data have allowed for a multitude of studies to explore the relationship between unique biomarkers and the development of Alzheimer's Disease. The patients within the ADNI database were obtained from various sites within the United States and Canada. Before obtaining biomarker data, each patient provided written informed consent. More information regarding the database can be found at the ADNI website (http://adni.loni.usc.edu/).

ADNI Sample Data

The majority of this study's data came from one spreadsheet that contained important data tables consolidated into one central location (a spreadsheet referred to as ADNIMERGE). Examples of biomarkers in this dataset included whole brain volume, age, ApoE4 genotype, and various other biomarkers. Cortisol biomarkers came from a separate spreadsheet, "Biomarkers Consortium Plasma Proteomics Project RBM." Basal forebrain volume measurements were obtained from study participants who had structural MRI images taken of their brains. Not all of the study participants on the ANDIMERGE spreadsheet had cortisol values or structural MRI images. Based on the relevant data (cortisol and basal forebrain volume) that were present within the ADNIMERGE spreadsheet, a cortisol sample (n = 163) and basal forebrain sample (n = 94) were obtained for data analysis. Of the cortisol sample, 122 individuals had Alzheimer's Disease (AD) while 41 were cognitively normal (CN). Of the basal forebrain sample, 41 individuals had AD while 53 were CN.

Cortisol Measurements

To obtain the cortisol measures, participants fasted overnight and provided blood samples the following day. The blood samples were centrifuged at room temperature and at 1500 RCF. The plasma from the blood samples was obtained and sent to the UPenn Bio-marker Core laboratory.

There, the plasma samples were stored at −80°C. When ready for testing, cortisol measures, along with other measurements, were calculated using guidelines by Rules-Based Medicine, Inc. (see http://www.rulesbasedmedicine.com for more information) (Toledo et al., 2013). "Biomarkers Consortium Plasma Proteomics Project RBM multiplex data" provided a spreadsheet within the ADNI database in which these cortisol measures were recorded for use in our study.

Basal Forebrain Volumes

In order to obtain basal forebrain volume measurements, structural MRI images for the relevant participants were first collected. Inclusion criteria in order to obtain basal forebrain volumes referred to participants within the ADNIMERGE spreadsheet for whom there was a T1 MRI image with 1.2 mm slice thickness. After obtaining the images, we used a procedure similar to that of Schmitz et al. 2016 in order to create a mask for the basal forebrain and extract volume measurements. Similar to Schmitz et al. 2016, this study chose to focus on the nucleus basalis of Meynert on account of the fact that this structure has been linked to neuronal cell loss in Alzheimer's Disease (Arendt, Bigil, Arendt, & Tennstedt, 1983). Ch4, a cholinergic cell group in the basal forebrain, resembles the nucleus basalis of Meynert, and we focus on brain volume in this structure as a potential biomarker for AD (Mesulam, Mufson, Levey, & Wainer, 1983). To obtain volume measurements from the Ch4 region, we used DARTEL to perform voxel based morphometry (VBM). In this process, all downloaded T1 MRI images were normalized into standard Montreal Neurological Institute (MNI) space. Each voxel in these structural images was assigned a metric (VBM parameter) based on the extent to which the voxel was warped to fit the standard MNI space template, and these metrics serve as a relative measure of brain volume. After normalizing the MRI images to MNI space, a mask was created for the Ch4 region using the SPM Anatomy Toolbox. This mask specified which voxel measurements corresponded to the Ch4 region, and the VBM parameters within the mask were extracted as measurements of raw relative basal forebrain volume. To standardize basal forebrain volume based on each subject's brain size, the VBM parameters were divided by total brain volume measurements provided by the ADNIMERGE dataset. At the conclusion of this process, basal forebrain volume measures were subtracted at various MRI visits (depending on available data) to derive a measure of change in basal forebrain volume.

Data Analysis: MVPA

After obtaining biomarker data on plasma cortisol level and change in basal forebrain volume, we used multivariate pattern analysis (MVPA) to determine the predictive validity of each biomarker in AD or CN classification. Figure 1 refers to a convenient image that explains the intuition behind the MVPA machine learning model (Pereira, Mitchell, & Botvinick, 2009). Measures of cortisol and basal forebrain change were independently fed into the MVPA algorithm in order to derive measures of classification accuracy. Cortisol measures were provided to the MVPA algorithm to obtain a classification accuracy to evaluate how well the model could distinguish between AD and CN individuals on the basis of variation in these measures. Thereafter, basal forebrain changes were provided to the MVPA algorithm to obtain a classification accuracy by which the model could identify AD and CN individuals from the variation in these measures. To obtain classification accuracy, the algorithm first partitioned the data set (cortisol, basal forebrain change) into a testing set and training set. The algorithm then associates variation in the data with a particular label (AD or CN). Finally, the algorithm calculates a classification accuracy based on how well it correctly identified the individuals in the testing set with the appropriate AD or CN label. Thus, classification accuracy serves as a measure of predictive validity for AD identification based on the biomarkers on which it is trained.

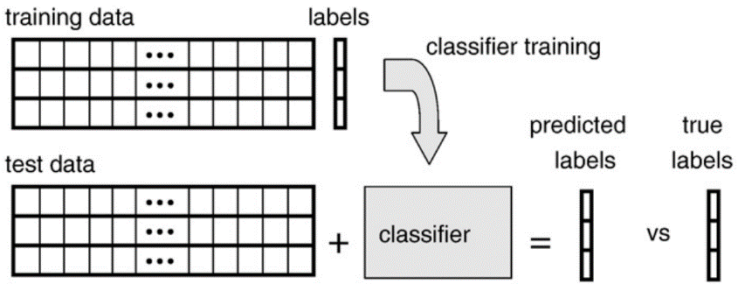

Figure 1. MVPA Classification. This image depicts the general mechanism by which MVPA operates. A set of data (biomarker data in the case of this study) is fed into the classifier, along with each of the respective labels (AD or CN). The classifier partitions the data set into training data and testing data. The classifier "learns" to associate variation in the data to a particular label then "tests" itself on the testing data set. The classification accuracy is then calculated based on how well the classifier accurately labels the testing data (Pereira, Mitchell, & Botvinick, 2009).

Data Analysis: Establishing Significance

In order to determine whether the MVPA algorithm's classification accuracy was significantly above chance, an empirical p-value was determined. To establish the p-value, an MVPA scrambling analysis was run. In the scrambling analysis, the data labels of the training set (AD or CN) were scrambled in such a way that a label was randomly assigned to each individual's data profile. Thus, an individual who was truly labeled "AD" might be randomly assigned a "CN" label as a consequence of the scrambling. In doing so, the MVPA algorithm is unable to consistently associate variation in the data to a particular data label, and it effectively "guesses" whether an individual is AD or CN when testing itself on the testing data set. As part of the scrambled analysis, 100 independent iterations were run. To establish a p-value, the number of instances a particular scrambling iteration yielded a classification higher than the unscrambled analysis was divided by the total number of iterations for the scrambling analysis (100). Statistical significance was established as $p < 0.05$.

Results

Experimental Validation of MVPA

Before evaluating the predictive potential of cortisol and basal forebrain degeneration in Alzheimer's Disease identification, an experimental validation of MVPA was required. Experimental validation occurred by running an MVPA model using traditional markers of Alzheimer's Disease to determine whether the model was capable of successfully distinguishing between AD and CN individuals, thereby replicating the sample's diagnostic results. In order to do this, two traditional markers of Alzheimer's Disease were utilized: neuropsychological scores and brain volumes. Two separate MVPA analyses were run, one analysis containing neuropsychological scores and another analysis containing brain volumes (Figure 2).

In the MVPA analysis containing neuropsychological scores, data collected from three cognitive assessments were input for the classifier to use to distinguish AD and CN patients: CDR-SB, ADAS 11, and ADAS 13 (Samtani, Raghavan, Novak, Nandy, & Narayan, 2014; Kueper, Speechley, & Montero-Odasso, 2018). The MVPA classifier trained on data from these three cognitive assessments was able to distinguish between AD and CN with 99.01% accuracy (Figure 2).

In the MVPA analysis containing brain volumes, volume measurements from the entorhinal cortex and hippocampus were used as inputs for the algorithm. These areas have been characteristically associated with Alzheimer's Disease based on previous research (Du et al., 2001). The MVPA classifier trained on brain volume data was able to distinguish between AD and CN subjects with 85.20% accuracy (Figure 2).

The high classification accuracy (99.01%, 85.20%) by the MVPA algorithm when trained on neuropsychological scores and brain volumes indicates the validity of using this approach for our experimental study. These tests were deliberately circular and were designed to demonstrate that the algorithm could use variation in neuropsychological scores and brain volumes to replicate the diagnoses assigned to the subjects in the first place. As a result, the high classification accuracy demonstrates the ability of the MVPA approach to correctly distinguish AD and CN individuals based on traditional Alzheimer's Disease markers. Thus, the use of MVPA to explore the more

unique biomarkers that were the focus of this study (cortisol, basal forebrain degeneration) was appropriate.

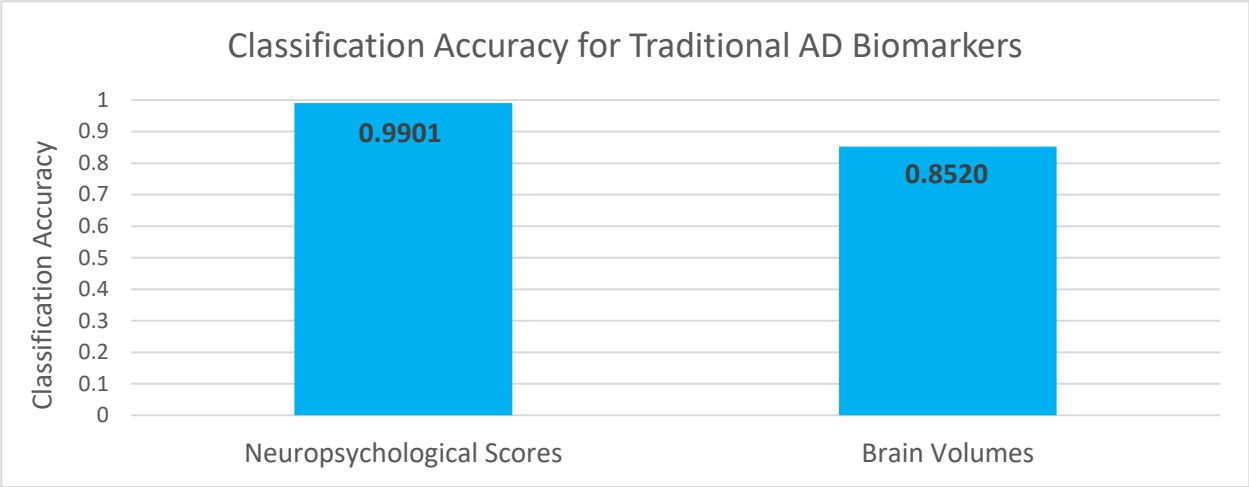

Figure 2. Classification Accuracy of Traditional AD Biomarkers. This graph indicates the MVPA classification accuracy of Alzheimer's (AD) and healthy (CN) patients on the basis of two traditional markers of Alzheimer's Disease: neuropsychological scores and brain volumes. Neuropsychological scores were obtained from the subjects' performance on cognitive assessments as measured by CDR-SB, ADAS 11, and ADAS 13. The brain volumes used in the MVPA analysis include volume measurements gathered from the entorhinal cortex and hippocampus. MVPA classification accuracy when trained on neuropsychological scores was 99.01%, while MVPA classification accuracy when trained on brain volumes was 85.20%.

MVPA Cortisol Analysis

To test the predictive validity of cortisol in distinguishing between AD and CN patients, a MVPA classification algorithm using cortisol as the only feature was run. Through this approach, the extent to which the classifier is capable of accurately identifying AD and CN subjects on the basis of variation in cortisol values could be investigated.

The MVPA analysis revealed that the algorithm could distinguish between AD and CN subjects with 55.24% accuracy. Scrambling analysis revealed that the classification accuracy considered "chance" was 49.89%. While the 55.24% classification accuracy was greater than the "chance"

classification of 49.89%, the data were not above chance at a statistically significant level (p = 0.14) (Figure 3).

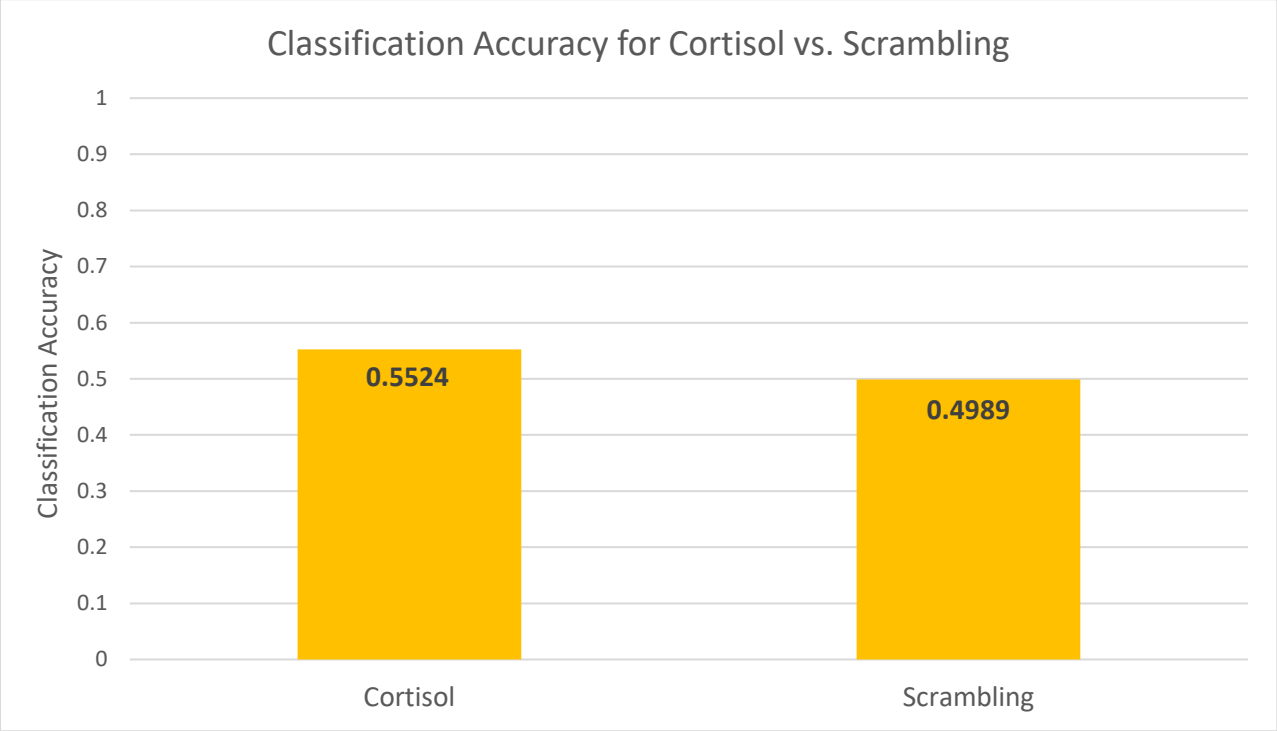

Figure 3. Classification Accuracy of Cortisol and Scrambled Analyses. This graph indicates the MVPA classification accuracy of Alzheimer's (AD) and healthy (CN) patients on the basis of cortisol, a physiological biomarker of stress. The MVPA analysis indicated 55.24% classification accuracy for cortisol. The scrambling analysis of cortisol established chance at 49.89%. While the classification accuracy is above chance, the results were not significantly above chance (p = 0.14).

MVPA Basal Forebrain Degeneration Analysis

To evaluate the strength of the predictive nature of basal forebrain degeneration in identifying AD and CN individuals, an MVPA classification algorithm using change in basal forebrain volume over time (from repeated MRI visits) was run. Using this approach, the success with which the classifier can correctly distinguish AD and CN subjects based on change in basal forebrain volume could be analyzed.

The MVPA analysis for basal forebrain change showed that the algorithm was able to identify AD and CN subjects with 68.09% accuracy. Scrambling analysis for basal forebrain change demonstrated that "chance" was 49.88%. The data show that the 68.09% MVPA classification accuracy based on basal forebrain change is greater than chance at a statistically significant level ($p < 0.005$) (Figure 4). The directionality of the relationship between basal forebrain change and AD vs. CN classification was also confirmed: negative values (indicating basal forebrain degeneration) were diagnostic of the "AD" label.

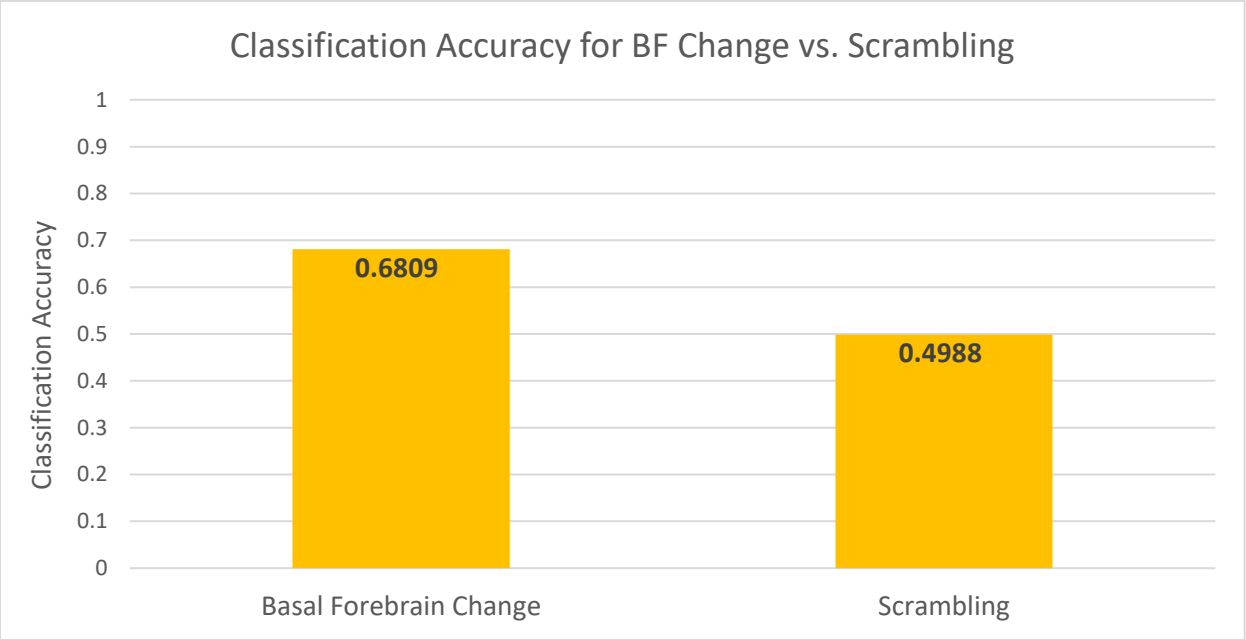

Figure 4. Classification Accuracy of Basal Forebrain (BF) Change and Scrambled Analyses. This graph indicates the MVPA classification accuracy of Alzheimer's (AD) and healthy (CN) patients on the basis of basal forebrain change. MVPA classification accuracy for basal forebrain change was 68.09%, while scrambling (chance) performance was 49.88%. The difference in basal forebrain change and scrambling analysis was statistically significant ($p < 0.005$).

Confusion Matrix Analyses

Following an analysis of the MVPA classification accuracy, confusion matrices for cortisol and basal forebrain change were also analyzed. Confusion matrices offer unique insight, helping characterize the diagnostic choices made by the MVPA classifier. The confusion matrices delve

deeper into what the classification accuracy entails for each category (AD or CN), and the information that comes from these analyses reveals more about the ability of these biomarkers to distinguish between healthy and pathological forms of aging. Based on the data provided to the MVPA classifier (cortisol or basal forebrain change), the confusion matrix analyses provide information about how often the classifier correctly classified AD individuals, correctly classified CN individuals, misclassified AD individuals as CN individuals, and misclassified CN individuals as AD individuals.

The confusion matrix for cortisol reveals that, based on the variation in cortisol levels, the classifier correctly recognized AD patients only 50.99% of the time. In contrast, the classifier correctly classified CN patients at a much higher rate, at 67.88% of the time. Furthermore, the classifier was more likely to call an AD patient CN (49.01%) than a CN patient AD (32.12%) (Table 1).

Table 1. Confusion matrix, expressed as percentages (%), for cortisol MVPA classification.

	AD Guess	CN Guess
AD True	50.99	49.01
CN True	32.12	67.88

The confusion matrix for basal forebrain change reveals a similar pattern to that of cortisol. Based on variation in basal forebrain change, the classifier correctly classified CN patients (77.36%) more often than it correctly classified AD patients (56.10%). In addition, AD patients were more likely to be misidentified as CN patients (43.90%) than CN patients were to be misidentified as AD patients (22.64%) (Table 2).

Table 2. Confusion matrix, expressed as percentages (%), for basal forebrain change MVPA classification

	AD Guess	CN Guess
AD True	56.10	43.90
CN True	22.64	77.36

Discussion

This study's approach involved independently testing whether cortisol and basal forebrain degeneration were capable of serving as indicators of Alzheimer's Disease by analyzing the classification accuracy of each biomarker. Importantly, the results have revealed that basal forebrain degeneration classification accuracy for Alzheimer's Disease identification is significantly above chance ($p < 0.005$). While classification accuracy for basal forebrain degeneration (68.09%) is not as high as traditional markers of AD (neuropsychological scores, 99.01%; entorhinal cortex and hippocampal volume, 85.20%), the above chance classification accuracy for this biomarker points to its potential benefit as a biomarker that can help play a role in the identification of AD. In addition, the directionality of this relationship was found to be consistent with relevant literature that suggests that reduction in basal forebrain volume is indicative of Alzheimer's Disease development (Schmitz et al., 2016; Paul, Jeon, Bizon & Han, 2015). The findings from our study advocate not only for more research to be conducted on how basal forebrain degeneration is specifically implicated in AD pathogenesis, but also for how diagnostic practice within medicine may be able to utilize basal forebrain degeneration to provide additionally relevant evidence to help inform diagnoses of Alzheimer's Disease and healthy aging. In particular, because our study has demonstrated that basal forebrain degeneration is informative for AD pathological characterization, the results also help validate AD therapeutic initiatives focused on addressing basal forebrain degeneration.

While basal forebrain degeneration MVPA classification revealed above chance accuracy, cortisol MVPA classification failed to reveal above chance classification accuracy (55.24%, $p = 0.14$). This result was surprising on account of the variety of research published establishing the relevance of cortisol in AD pathogenesis (Ennis et al., 2017; Popp et al., 2009; Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006). However, it is important to note that the 55.24% classification accuracy was still trending above chance, and the lack of statistical significance could be attributed to limitations within the study. In particular, the cortisol data utilized in this analysis came from plasma measurements, rather than from cerebrospinal fluid (CSF). Because CSF is more closely associated with the central nervous system than plasma, CSF cortisol measures could be a more accurate representation of the cortisol profile of each subject (Popp et al., 2009). Consequently, an MVPA analysis that utilizes CSF cortisol as opposed to plasma

cortisol may provide a higher (potentially significant) classification accuracy, thereby adding more value to the diagnostic role cortisol can play in AD identification. Fundamentally, the results from the cortisol MVPA analysis call for more research to be done in order to evaluate the extent to which cortisol may serve as a predictor of Alzheimer's Disease. In doing so, the manner in which cortisol and life-long stress may contribute to AD pathogenesis and pathological identification can become better clarified.

While the MVPA analysis of cortisol was not significantly above chance ($p = 0.14$), the 55.24% classification accuracy still warranted further exploration. As such, the confusion matrix for cortisol MVPA classification provided important insight into how the classifier performed when using cortisol to discriminate between AD and CN subjects. The most glaring observation of this analysis was the lack of symmetry within the confusion matrix: AD patients were correctly classified 50.99% of the time, while CN patients were correctly classified 67.88% of the time. When observing this dissymmetry, it became apparent that there was more to the cortisol biomarker of Alzheimer's Disease. Based on the confusion matrix analysis, cortisol variability in healthy (CN) patients was much better characterized for the classifier than cortisol variability in AD patients; this is evidenced by the fact that CN patients had a higher chance of being correctly classified (67.88%) than AD patients (50.99%). Similarly, MVPA analysis of basal forebrain degeneration revealed that CN patients also had a higher chance of being correctly classified (77.36%) than AD patients (56.10%). These data highlight an important finding: cortisol and basal forebrain degeneration variability in individuals who age healthily is much less variable than that of individuals who age pathologically (those who have AD). This finding could hold clinical relevance, as measures of cortisol and basal forebrain degeneration might be able to help physicians discern whether a given patient is likely aging in a healthy manner.

Furthermore, the classifier's success in associating cortisol and basal forebrain degeneration variability in CN patients, while failing to successfully associate cortisol and basal forebrain degeneration variability in AD patients, is particularly interesting. This lack of symmetry helps advise future directions for the project. In particular, exploring the underlying influences that could explain why the MVPA cortisol classifier and MVPA basal forebrain degeneration classifier correctly characterizes some AD subjects, while incorrectly classifying other subjects,

is a logical next step given these data. For example, a multitude of factors including age, brain volume, and pathophysiological characteristics of AD could help explain why the MVPA cortisol classifier and MVPA basal forebrain degeneration classifier correctly classify some individuals, while incorrectly classifying other individuals (that is, why they exhibit AD despite having normal levels on these specific measures). The conclusions that come of these retroactive analyses have the potential to more specifically investigate the role cortisol and basal forebrain degeneration might play in the characterization of Alzheimer's Disease, helping further inform the set of events by which aging populations develop Alzheimer's Disease.

Finally, the present study focused on the predictive validity of cortisol and basal forebrain degeneration for CN and AD individuals. It is important not to neglect mild cognitive impairment (MCI), a diagnostic state that might serve as an intermediate step in the AD pathological process (Petersen et al., 1999). Because ADNI data are available for patients with MCI, an experimental approach that utilizes a similar framework to the present study but rather substitutes CN vs. AD analyses for MCI analyses (CN vs. MCI and MCI vs. CN) constitutes an important future direction. The use of an MCI MVPA analysis in the context of cortisol and basal forebrain degeneration can help analyze the time course of when these biomarkers play a role in AD pathology. Consequently, a mechanistic sequence of events detailing the role that cortisol and basal forebrain degeneration in AD progression can arise from an analysis that uses MCI patients. Additionally, the results arising from such an analysis can help advise when diagnostic approaches using cortisol and basal forebrain degeneration may yield the most benefit. Results of MCI analyses may also help inform when therapeutic interventions targeting cortisol and basal forebrain degeneration may be particularly useful during AD pathogenesis.

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