Periaqueductal Gray Activation and Pain Responses in Alzheimer's Disease

by

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This is dedicated to my mother, who I lost far too soon, but whose unconditional love and incredible support throughout my life prepared me for this endeavor.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

Alzheimer's disease (AD)
American Nurses Association (ANA)
American Society for Pain Management Nursing (ASPMN)
Analysis of Functional NeuroImages (AFNI)
Anterior cingulate cortex (ACC)
Blood oxygenation level-dependent (BOLD)
Department of Health and Human Services (HHS)
Descending pain modulatory system (DPMS)
Dorsal horn (DH)
Dorsolateral prefrontal cortex (DLPFC)
Enkephalin (ENK)
Family-wise error (FWE)
Full-width half maximum (FWHM)
Functional Magnetic Resonance Imaging (fMRI)
Gamma-aminobutyric acid (GABA)
General Linear Modeling (GLM)
Health and Human Services (HHS)
International Consortium for Brain Mapping (ICBM)
International Association for the Study of Pain (IASP)
MARSeille Boîte À Région d'Intérêt (MarsBaR)
Medoc Pathway Pain and Sensory Evaluation System (Medoc)
Mini-Mental State Exam (MMSE)
Monroe and Cowan labs (MCL)
Montreal Neurological Institute (MNI)
National Alzheimer's Project Act (NAPA)
National Institute on Aging (NIA)
National Institutes of Health (NIH)
Neurofibrillary tangles (NFTs)
Neurologic pain signature (NPS)
Nociceptor (NOC)
Pain Assessment in Impaired Cognition scale (PAIC15)
Pain Assessment in Advanced Dementia Scale (PAINAD)
Periaqueductal gray (PAG)
Positron emission tomography (PET)
Primary Investigator (PI)
Region of interest (ROI)
Resting-state functional connectivity (RSFC)

Robust weighted least squares estimation (rWLS)
Rostral ventromedial medulla (RVM)
Secondary somatosensory cortex (S2)
Small volume correction (SVC)
Spinomesencephalic tract (SMT)
Statistical Parametric Mapping (SPM)
United States (US)
Visual analogue scale (VAS)
Ventrolateral PAG (vlPAG)
Wake Forest University PickAtlas (WFU PickAtlas)

CHAPTER 1

INTRODUCTION

Pain processing is altered in Alzheimer's disease (AD)[1-4] but the neural basis is not well understood, which may be a reason pain is underrecognized and undertreated in this condition.[5-9] There are 6.2 million individuals with AD in the United States (US)[10] and over 50 million with dementia worldwide.[11] Of concern is that 50-75% of people with AD and related dementias experience pain regularly.[12,13] Preliminary neuroimaging studies indicate that major brain regions involved in pain processing continue to demonstrate activity in response to painful stimuli in AD,[1-3] however, this activity is altered from that of healthy older adults and it is unclear how this activity maps onto the pain experience. Neuropathological changes that occur during the disease process of AD[1,2,4,14-17] may cause this altered pain experience.[1-4]

Pain is mediated by multiple brain regions often described within the medial and lateral pain networks as well as the less understood rostral pain network.[4] Within the pain processing brain regions is a subset known as the descending pain modulatory system (DPMS), of which the periaqueductal gray (PAG) is an essential component.[18-21] The PAG operates in response to painful stimulation and modulates pain by transmitting information to and from higher brain structures[22-24] and through the actions of opioids. Stimulation of the PAG typically causes inhibition of nociceptive signals that cause pain,[19,25] likely because the PAG is a primary site of endogenous opioid release,[22,26] and is significantly involved in the pain-relieving effects of exogenous opioid analgesics.[26-29] The PAG is damaged during the disease process of AD,[14-17] and consequently there may be an amplified pain experience in people with AD compared to

healthy older adults. An amplified pain experience may result from an impaired ability to mount a sufficient endogenous opioid response to pain or mediate the effects of exogenously administered opioid analgesics because of damage to the PAG in AD. Therefore, it is critical to investigate the function of the PAG in people with AD.

Functional Magnetic Resonance Imaging (fMRI) is a powerful but noninvasive way[30] to investigate neurobiological mechanisms in the brain in response to pain[31-34] and is the most common type of imaging used for pain as it offers better resolution of images than other methods.[35] Thus far, only three published fMRI studies have investigated pain processing and self-reported pain in mild to moderate AD,[1-3] and none focused on the PAG or definitively outlined the pain experience. Psychophysical results from these fMRI studies as well as other non-imaging studies demonstrate that people with AD detect pain stimuli at similar[36-41] or greater intensities[1-3,42,43] than controls and their pain threshold,[36-38,41,42] tolerance,[36-38] and habituation[38,41] are also similar to that of controls. Pain unpleasantness is the same[1-3,36,39,40,43-45] or worse than controls.[1,38,41,45-49] In neuroimaging of cognitively intact participants, PAG activation[50,51] scales with pain intensity in that greater pain corresponds to greater activation. Greater brain activation has been found in AD in general[4] and also with corresponding reports of greater pain unpleasantness in AD.[1]

An analysis of blood oxygenation level-dependent (BOLD) activation from fMRI brain images captured during a heat-induced pain paradigm can offer insight into the functional activity of the PAG in AD and permits a comparison of this activity to healthy age- and sex-matched controls. This valuable information can improve our understanding of the pain experience in AD, which may then lead to improved pain assessment and management.

Aim 1: To determine between-group differences of heat-induced pain responses in participants with AD and healthy age- and sex-matched controls. Hypothesis 1a: Participants with AD will report detecting mild and moderate pain at higher temperatures than controls. Hypothesis 1b: Participants with AD will rate mild and moderate pain as just as unpleasant or more unpleasant than controls.

Aim 2: To determine between-group differences in PAG activation in response to a heat-induced pain paradigm in participants with AD and healthy age- and sex-matched controls. Hypothesis 2: There will be increased brain activation during heat-induced pain in the PAG in participants with AD compared to controls.

Advancements in understanding the neurophysiology of pain in AD must be made in order to improve pain assessment and management, and thereby reduce undertreatment of pain and patient suffering in AD.[52] The problem of pain in people with AD is significant not only because of the suffering of the individual, their loved ones, and hardships to their healthcare team, but also because of the high prevalence and costs of the disease. Data from this study will provide preliminary evidence about the function of the PAG in people with AD, thus advancing our understanding of pain neurophysiology.

Significance

This study is in line with the Department of Health and Human Services' (HHS) National Alzheimer's Project Act (NAPA) goals to enhance the quality and efficiency of care of people with AD, and reduce financial burdens of AD.[53] NAPA also states that the care of individuals with AD should be modified and tailored depending on their physical, cognitive, emotional, and behavioral needs as well as co-occurring conditions.[53] A co-occurring condition of pain reduces

the quality and efficiency of care and modifies physical, cognitive, emotional, and behavioral needs, as well as increases financial burdens.[5,12,54,55]

Despite estimates of the substantial prevalence of pain,[12,13,56] people with AD report pain less frequently and receive minimal to no pain medication, even when there is a known condition that causes pain, such as cancer.[5,6] Underrecognized or untreated pain sanctions continued suffering of this vulnerable population which is unethical[57] and, legally, could constitute elder abuse.[58,59] Pain in this population increases: the use of inappropriate treatments (e.g. antipsychotic medications),[60,61] functional loss,[5] risk for agitation and psychosis,[12] depression,[54] and significant caregiver stress/emotional burden.[54,59] Pain in these individuals also: worsens cognitive impairment,[5,54] impairs social interactions and appetite,[12] decreases immune function and ability to recover,[5,12,54] compromises sleep[5,12] and exacerbates co-morbidities and overall morbidity risk.[12,54] Pain contributes to an overall decreased quality of life,[5,57,60] lower life satisfaction,[62] and higher costs to the health care system.[54]

AD is one of the costliest diseases to society, ranking slightly higher in expenditures than heart disease and cancer.[63] These costs will continue to rise because the prevalence of AD is rapidly increasing with a new diagnosis every 65 seconds that is expected to increase to every 33 seconds by mid-century.[64] The 2021 estimates for healthcare and long-term care for people with AD in the US is $355 billion.[10] Costs are expected to reach over $1.1 trillion per year by 2050.[10,65] Additionally, unpaid caregivers provided $256.7 billion worth in care in 2020,[10] resulting in combined overall yearly costs of approximately $612 billion in the US. In general, chronic pain in the US costs at least $635 billion annually for medical treatments and lost productivity.[66,67] Individuals with both AD and pain are likely to incur even higher costs than

either condition on its own[54] because pain worsens impairment[5] and co-morbidities[12,54] which increases costs.[55]

Problems with Pain Assessment in AD

People with AD may not be able to communicate their pain verbally[19] or behaviorally which increases the likelihood of underrecognized or untreated pain.[4] Numerous tools exist to measure pain but none are ideal for AD, and the challenge of measuring pain increases when cognitive impairment and pain co-exist.[32,52,68] The inability of many individuals with AD to effectively communicate, coupled with controversy about behavioral expressions of pain, and accurate measurement thereof, make self-reported and/or observational pain scales ineffective in many cases.[69,70] Compounding these problems, a recent review found that nurses were not adequately educated in pain recognition or use of pain assessment tools for people with dementia.[71]

Registered nurses, nurse practitioners, and other clinicians are frontline caregivers who often assess and treat pain.[57] In their position statement, the American Nurses Association (ANA) use McCaffery's pain definition of "whatever the experiencing person says it is, existing whenever he says it does." [72 (para. 2)] The American Society for Pain Management Nursing (ASPMN) support this ANA position statement.[73] Yet, self-report is often ineffective for non-communicative individuals and those unable to understand a pain scale because of memory/ cognitive impairment.[32,52,74-76] Recently, the International Association for the Study of Pain (IASP) revised their definition of pain as: "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." [77 (p. 1977)] The previous definition included "or described in terms of such damage" [77 (p. 1979)] which led to the assumption that a verbal description was needed.[78] The IASP also includes six notes to add

context to their new definition, one of which states that a person may experience pain even if there is an inability to communicate.[77] This revised definition displays progress in understanding and assessing pain.

The ability of an individual with AD to comprehend a scale like the widely-used visual analogue scale (VAS) sgnificantly declines as AD worsens.[79] In a study including participants with severe AD, none were able to rate their pain reliably with a simliar pain scale.[46] Two other studies found that the relability of pain reports worsened with, and was statistically significantly correlated with, lower Mini-Mental State Exam (MMSE) scores.[45,49] For those unable to communicate or adequately self-report their pain, observational pain scales, such as the Pain Assessment in Advanced Dementia Scale (PAINAD)[80] are recommended.[76,81] Recently a new tool, the Pain Assessment in Impaired Cognition scale (PAIC15), was developed in efforts to combine the best of the many existing scales into one improved scale.[82] While more testing of the scale is needed, the PAIC15 may represent current best practices. However, if a pain behavior is absent[4] or unnoticed,[83] observational scales may still be ineffective.[52]

Despite multiple studies, the controversy continues about which behaviors truly indicate pain rather than another unmet need.[69,76] For example, facial expressions, especially grimacing,[84] are believed to be reliable indicators of pain in AD as this expression is thought to remain intact,[13,46,47] and the PAIC15 includes facial expressions thought to be specific to pain.[82] However, one study by leaders in this field revealed that facial responses were weakly correlated with self-report in participants with dementia and controls,[85] making this observation only a component of an overall picture of pain rather than a substitute for self-report. Despite their training, familiarity with pain assessment, and work history with older adults, nurses did not have an advantage over laypeople in recognizing facial expressions related to pain.[85] To be

competent in recognizing facial expressions of pain it appears extensive training and time is required.[47] Nurses may not have the appropriate support, resources, or time needed to train or to conduct adequate pain assessments.[86]

There are also issues with recognition of general pain behaviors in people with AD. Pain behaviors in AD are underrecognized and behavioral changes are misunderstood and misinterpreted by nursing staff.[69,70] The beliefs and expectations held by nurses also affect the interpretation of pain behaviors. If a nurse believes that pain management is sufficient they may not be as attentive to changes in pain behavior as a nurse who believes pain management is insufficient.[87] Also, if a pain behavior is not repeated several times with the same caregiver, it may not be recognized or considered a pain behavior.[83] Additionally, a lack of pain behavior may occur as a consequence of damage from AD to the rostral pain network.[4] Although they offer assistance as a proxy measure, the validity and feasibility of observational scales continue to be insufficient,[13] and not enough is known about how pain is experienced and expressed by people with AD for these scales to be used with confidence as a sole indicator of pain.

A recent qualitative study found that physicians and nurses felt that observational pain scales were limited in their use because of their perception that the tools were difficult to integrate into practice and did not add valuable information.[88] Even if scales like the PAIC15 do prove to be more accurate, if the scale appears to be difficult and impractical to use in clinical settings and if clinicians and staff are not trained in or believe in their use, the scale will ultimately remain ineffective. Pain management is also not necessarily improved in people with dementia from the use of pain tools because their use may not prompt appropriate action from the caregiver or clinician.[89] Specific guidance about pain and pain assessment in dementia is

missing from the major annual reports of dementia- and pain-related organizations,[90] and current best practices are not well-known or adequately implemented into clinical practice.[71,88,91,92]

Herr and colleagues recently published an ASPMN position statement regarding pain assessment in people who cannot self-report pain, including dementia, and recommend:[93 (p. 403)]

1. Use the Hierarchy of Pain Assessment Techniques:
    a. Be aware of potential causes of pain including known painful interventions;
    b. Attempt self-report;
    c. Observe patient behaviors;
    d. Solicit reporting of pain and behavior/activity changes;
    e. Attempt analgesic trial.
2. Utilize behavioral pain assessment tools, as appropriate.
3. Minimize emphasis on vital signs.
4. Assess regularly, reassess postintervention, and document.

While these recommendations represent our current best practices, they also demonstrate the time commitment and complexities of assessing and managing pain in people with AD. Despite challenges, these best practices[94] and the new IASP pain definition[77] need to be implemented widely into clinical practice to improve the chances of detecting and managing pain in people with AD.

CHAPTER 2

SCIENTIFIC PREMISE

The BOLD Response

Functional MRI (fMRI) uses standard MRI scanners[95] to indirectly investigate brain activtivation.[96] While "direct measurements" of tissue perfusion, blood-volume changes, and changes in the concentration of oxygen can be achieved via fMRI,[97 (p. 803)] these physiological changes are indirectly correlated with neuronal activity.[95] In 1990, Seiji Ogawa was the first to publish research about blood oxygenation level-dependent (BOLD) as a natural MRI contrast found in increased blood flow from paramagnetic deoxyhemoglobin.[98] Paramagnetic denotes having the property of magnetic field attraction, and deoxyhemoglobin is hemoglobin without oxygen.[95] Without being aware of Ogawa's work, Ken Kwong was the first to conduct an fMRI experiment in 1991.[99,100]

The BOLD signal can be defined as a measure of the ratio of blood that is oxygenated to blood that is deoxygenated[101] from fMRI images and results from a sequence of indirect effects.[95] An increase in oxygenated blood is required to meet the metabolic demands of information transfer between neurons in the brain.[102] Local neural activity is usually paired with "functional hyperemia," which is a temporary oversupply of blood flow causing an increase in blood and tissue oxygenation.[103] The indirect physiological changes demonstrated by fMRI images are correlated with neuronal activity,[95] demonstrating a "time course" of activity by the neurons.[35] The BOLD signal is thought to predominantly reflect excitatory synaptic input to a brain region[104] and increases in oxygenation and blood flow are correlated with excitatory

neuron activity.[103] It may also be that the primary driver of the BOLD response is in the form of interneuron activity levels.[103] However, the correlation of specific neural activity with the BOLD response is not necessarily the same in all areas of the brain.[103]

The BOLD signal is formed within the vasculature of the brain when vessels dilate and carry more oxygen.[103] Previously, the "Balloon Model"[105,106] was accepted as a biomechanical explanation, positing that blood volume changes occurred mainly in the venous compartment which would expand to form a balloon.[103,106] Instead, "more realistic" explanations based on newer research demonstrate a different picture.[103 (p. 61)] A stimulus induces neural activity and within a few hundred milliseconds dilation of arteries occurs with peak arterial dilation in two to three seconds.[103] When the stimulus stops, the arteries return to baseline after a few seconds. Arteries may dilate up to 20-30%, and volume increases are much larger in arteries than veins.[103] The dilation of veins is much slower and they do not dilate as much as arteries, with only up to 10% dilation. Veins take at least tens of seconds to reach peak dilation.[103] How these arteries and veins dilate in the brain is "well understood," however, the workings of capillaries are more speculative.[103 (p. 62)] This uncertainty is because of limitations in microscopy, conflicting experimental results, and discrepancies in the definition of capillary.[103] Because of field strengths in human fMRI scanning, the signal detected is primarily from veins and capillaries.[103]

The underpinnings of BOLD remain an area of investigation[103,107] and not all neural activity causes a BOLD response.[103] Despite this, BOLD has "provided an unparalleled window on human cognition." [103 (p. 61)] Out of all MRI methods, fMRI has had the most impact on the field of neuroscience, and is considered a "reliable, robust, and extremely useful signal." [107 (p. 2975)]

Prior Research

A comprehensive summary of the prior research through 2012 on pain in AD is presented in the only known framework on the topic: the "Conceptual Framework of Pain in People with AD" by Monroe et al (Figure 1).[4] This framework offers a snapshot of the complexities and ambiguities of research findings on the experience of pain in AD. Mild, moderate, and severe AD can have decreased brain volume and metabolism, while mild and moderate AD demonstrate increased brain activation, and severe AD is suspected to have decreased brain activation. Suspected results are presented for severe AD because little study has been conducted on participants in this stage of AD.[4] Both mild and moderate AD can have normal, increased, or decreased affective and/or sensory pain reports. Mild and moderate AD have shown normal or increased behavioral pain reports, and severe AD is suspected to have normal or decreased reports.[4]

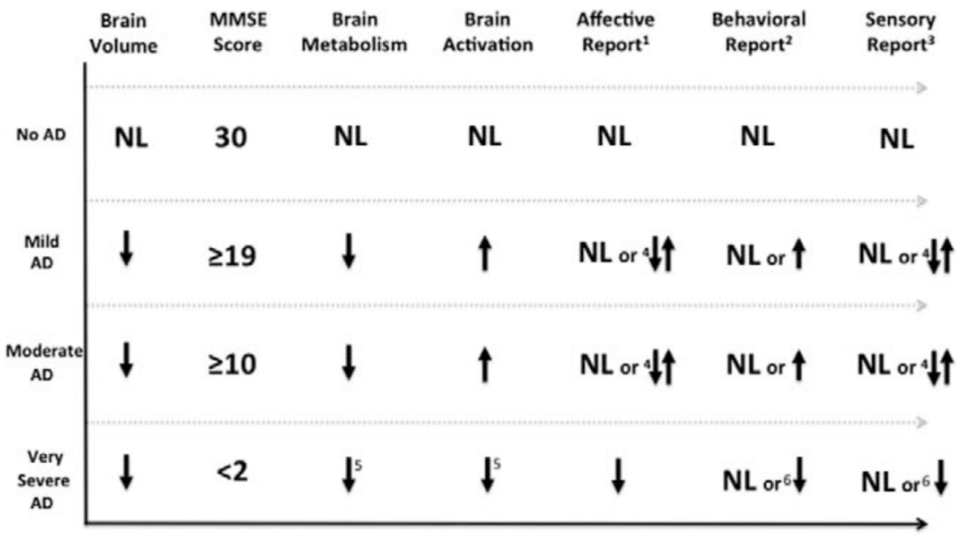


Figure 1. **Conceptual framework of pain in people with AD by Monroe et al**[4] (p. 242)

Overlapping results in each AD stage elucidate that it is difficult to precisely stage AD without an autopsy, and that there can be shared symptoms across stages. Additionally, variation in findings may be because of differences in pain stimuli, study design, self-report vs observational pain report, levels of cognitive impairment, and type of pain. An important finding of prior research is the demonstration that people with AD continue to respond to pain and report pain, regardless of study differences. Weaknesses of prior research include the variation in results and the limited amount of study into the neural basis of pain in AD. The ambiguous findings presented in the framework reflect the need for further study of the experience of pain in AD and deeper investigation of the neural basis of alterations found in AD.

Preliminary neuroimaging studies and controlled experimental studies using psychophysics from a repeated pain stimulus and self-report of pain in AD yielded the most relevant data to inform this study.[1-3,36-38,40-44,48,108] Tables 1[109] and 2 in Appendix A review these important studies of pain in AD, including pertinent studies that were incorporated in the above framework by Monroe et al.[4] Additional supporting psychophysical studies,[39,45-49,110] that include observational pain reports, are also included in Table 1.[109]

The peer-reviewed journal articles in Tables 1 and 2 are scored based on the widely used method presented by Hawker et al[111] to systematically review research, even if from disparate disciplines. Although only one study[43] received the maximum possible points, overall, most individual scores were in the "fair" to "good" range (out of "very poor," "poor," "fair," and good") demonstrating acceptable rigor. Only one study[41] received "very poor" to "poor" scores because it was a poster presentation reporting limited information, however, this was still included because of pertinent information on habituation and because it is cited in the literature. Older studies typically received lower scores. If the older studies were published more recently,

they might have higher scores because of the inclusion of more details resulting from a greater emphasis on rigor, reproducibility, and transparency in the current scientific community, as well as acknowledgment of ethics and bias (e.g. ethics board approval and conflicts of interest). Effect sizes were calculated and p-values were reported for findings whenever adequate information was reported in the article and it included a self-report of pain. Most results had effects sizes indicating meaningful differences between participants with AD and healthy controls (refer to Tables 1 and 2).

Studies were conducted in Italy, Australia, Germany, Denmark, and the US. There were 994 total participants, 520 with AD or another form of dementia, across 19 studies. Each study used a stimulus to induce pain, however, multiple types were used. These included heat, mechanical pressure, electrical, tourniquet-induced ischemia, and cold water and were used on various areas of the hand, arm, or leg (see Table 1). Most of the studies had participants self-report their pain, however, some used observational pain tools, or both, with different pain scales or descriptors. All but two studies[108,110] were case-controlled with healthy older adults that were at least approximately age- and sex-matched, although two other studies did not report sex/gender demographics.[39,41] The two studies without controls were examining MMSE scores and pain[108] or sex-differences and pain[110] in participants with AD. Table 3[109] provides a summary overview of the psychophysical results of all studies that compared participants with AD to controls.[1*]

[1*] Portions of this chapter, including Tables 1 and 3, have been adapted with permission from "A Systematic Review of Experimentally Evoked Pain in Alzheimer's Disease"[109] published in the Journal of the American Association of Nurse Practitioners.

Table 3. Summary of psychophysical outcomes of participants with AD compared to controls*

Conditions	Heat	Pressure	Electrical	Ischemia	Cold water	Overall Totals
Stimulus detection	**AD > HC** Gibson[42] Monroe[43] Monroe[3] **AD = HC** Jensen-Dahm[37] Jensen-Dahm[41] Jensen-Dahm[38] Jensen-Dahm[40]	**AD > HC** Cole[1] Cole[2] **AD = HC** Lints-Martindale[39]	**AD = HC** Benedetti[36] Lints-Martindale[39]	-	-	AD > HC x 5 AD < HC x 0 AD = HC x 7
Pain threshold	**AD > HC** Monroe[43] **AD = HC** Gibson[42] Jensen-Dahm[37] Jensen-Dahm[41] Jensen-Dahm[38] Jensen-Dahm[40]	**AD > HC** Cole[1] **AD = HC** Jensen-Dahm[37]	-	**AD = HC** Benedetti[36]	**AD = HC** Jensen-Dahm[37]	AD > HC x 2 AD < HC x 0 AD = HC x 8
Pain tolerance	-	**AD < HC** Jensen-Dahm[37]	**AD > HC** Benedetti[36]	**AD > HC** Benedetti[36]	**AD < HC** Jensen-Dahm[38] **AD = HC** Jensen-Dahm[37]	AD > HC x 2 AD < HC x 2 AD = HC x 1
Pain unpleasantness	**AD > HC** Jensen-Dahm[41] Jensen-Dahm[38] **AD = HC** Jensen-Dahm[40] Monroe[43] Monroe[3]	**AD > HC** Cole[1] Kunz[49] Kunz[45] Beach[46] Beach[47] Beach[48] **AD = HC** Cole[1] Cole[2] Lints-Martindale[39] Kunz[45]	**AD < HC** Rainero[44] **AD = HC** Benedetti[36] Rainero[44] Lints-Martindale[39]	-	**AD = HC** Jensen-Dahm[37]	AD > HC x 8 AD < HC x 1 AD = HC x 11
Pain habituation	**AD = HC** Jensen-Dahm[41] Jensen-Dahm[38]	-	-	-	-	AD > HC x 0 AD < HC x 0 AD = HC x 2

Numerical superscripts refer to the study's citation; AD = participants with AD; HC = healthy controls; >, <, and = refer to the participant group result compared to the other group (e.g. AD > HC means that participants with AD had greater responses for that condition than HC; and AD = HC means that there were no differences between participants with AD and HC). *Portions of this table have been adapted with permission from "A Systematic Review of Experimentally Evoked Pain in Alzheimer's Disease"[109] published in the Journal of the American Association of Nurse Practitioners.

Stimulus detection between participants with AD and controls was primarily the same or higher in AD. Out of 12 stimulus detection outcomes, seven demonstrated no differences between AD and controls[36-41] and five demonstrated that participants with AD needed higher intensities when detecting and reporting the stimulus at a variety of stimulus intensities.[1-3,42,43] MMSE scores were associated with higher temperatures to detect warmth[43] but were not correlated with electrical stimulus detection.[108] Females with AD detected mild and moderate pain stimuli at lower temperatures than did males with AD demonstrating sex-differences in stimulus detection.[110]

Pain threshold between participants with AD and controls was primarily the same. Of the 10 pain threshold outcomes, eight demonstrated no differences[36-38,41,42] and two demonstrated higher stimulus intensities were needed to reach threshold in AD.[1,43] MMSE scores were not correlated with electrical pain thresholds.[108]

Pain tolerance between participants with AD and controls was effectively about the same but mixed. Of the five pain tolerance outcomes across three studies, tolerance was higher in participants with AD for electrical and tourniquet-induced ischemia in the same study[36] but also lower in AD in two other outcomes[37,38] and the same as controls in another.[37] Of these outcome, of note is that for pain tolerance using cold water, participants with AD only had about half the tolerance of controls.[38] MMSE scores were correlated with greater pain tolerance in electrical and ischemic pain in the same study.[36]

Pain unpleasantness between participants with AD and controls was primarily the same or greater in AD. Of the 20 pain unpleasantness outcomes, unpleasantness was the same for AD and controls in 11 outcomes[1-3,36,39,40,43-45] and more unpleasant for participants with AD in eight outcomes.[1,38,41,45-49] Unpleasantness was less in participants with AD compared to controls in

only one outcome.[44] MMSE scores were not associated with pain unpleasantness.[43] Males with AD reported greater pain unpleasantness compared to females with AD at mild and moderate pain percepts, demonstrating sex-differences.[110]

Pain habituation between participants with AD and controls was the same.[38,41] One of the studies also performed repeated psychophysics to evaluate test-retest results.[37] Because the results were reproducible and demonstrated performance comparable to controls, the test-retest findings indicated that participants with mild to moderate AD are able to understand and cooperate with heat pain and pressure pain testing.[37] The best test-retest results were from heat pain,[37] indicating that it may be the superior choice for experimental studies of pain in AD.

Ancillary findings in three studies demonstrated that participants with AD and related dementias were not able to reliably report their pain.[45,46,49] The ability of these participants to give a self-report of pain decreased as their level of cognitive impairment increased in two of the studies ($p < 0.001$ for both).[45,49] In the third study, no differences were found between mild/moderate AD and severe AD in pain scores given to them by an observer, but participants with severe AD could not reliably self-report their pain.[46]

Overall, the psychophysical results demonstrate that people with AD may be more likely to report pain at similar stimuli to cognitively intact older adults, but may need a greater stimulus to report pain and may have difficulty reporting their pain. Pain threshold, pain tolerance, and pain habituation also appear to be about the same in people with AD compared to cognitively intact older adults. Pain unpleasantness appears more likely to be greater in people with AD compared to compared to cognitively intact older adults, but may also be similar. These results indicate that people with AD continue to experience pain as much as or more unpleasantly than healthy, cognitively intact older adults. Confirmation of pain unpleasantness in people with AD

reveals a significant problem because pain is underrecognized in AD and pain assessment and management in AD remain inadequate. These findings also highlight the need for continued research into the pain experience in people with AD.

Preliminary neuroimaging findings (Table 2) indicate that major brain regions involved in pain processing continue to demonstrate activity in response to painful stimuli in AD,[1-3] however, this activity is altered from that of healthy older adults.[1-3,48] The first study to investigate brain activity during pain in AD found that both participants with AD and healthy controls displayed a common network of pain-induced increased BOLD activity in the medial and lateral regions,[1] both of which are regions known to be involved in pain.[32] The medial pain network (see Table 4 for brain regions) represents the affective/motivational and cognitive/ evaluative components of pain. These components include emotion, arousal, attention, memory, and unpleasant aspects of pain.[4] The affective/motivational component is a crucial part of pain sensation and includes what is thought of as "suffering from pain" which leads a person to communicate their dislike of the pain.[112] The lateral pain network (Table 4) represents the sensory/discriminative components of pain which involve discrimination of the location, intensity, and quality of pain.[4,112] Additionally, more recent work has outlined the rostral pain network (Table 4), which overlaps with the medial and lateral networks, and represents the behavioral component of pain.[4,113] Findings based on pathology and autopsies demonstrate that the rostral[114] and medial[115,116] regions are damaged earlier in AD but the lateral[115,116] regions are damaged late into the disease process.[4]

Table 4. Brain regions included in pain networks

Brain Region	Medial Pain Network	Lateral Pain Network	Rostral Pain Network
Prefrontal cortex	✓		
Insula	✓	✓	✓
Thalamus	✓	✓	✓
Hypothalamus	✓		✓
Periaqueductal gray	✓		✓
Anterior cingulate cortex	✓	✓	✓
Hippocampus	✓		
Somatosensory cortex		✓	
Amygdala			✓
Orbitofrontal cortex			✓
Ventral Striatum			✓

In addition to the common network of activity in the medial and lateral regions, no differences in BOLD signals were found between AD and controls for innocuous pressure.[1] For moderate pain, however, a significant difference was found as the BOLD response was slower to return to baseline in participants with AD compared to controls (see Table 2).[1] This increased brain activity, combined with AD participants reporting pain as more unpleasant, may indicate continued attention to the pain by participants with AD that may represent an amplified pain experience.[1]

A follow-up study investigated the task-evoked functional connectivity of the pain network in AD compared to controls (see Table 2).[2] Per Heuttel, functional connectivity refers to possible "direct or indirect" links between regions in the brain that are "inferred from common changes in activation over time." [95 (p. 522)] Task-evoked whole-brain functional connectivity revealed significant correlations for both AD and controls with the right dorsolateral prefrontal cortex (DLPFC-R) and common brain structures involved in pain including the left DLPFC, and bilateral regions of the anterior cingulate cortex (ACC), secondary somatosensory cortex (S2),

and insula. However, compared to controls, participants with AD had significantly greater functional connectivity than controls between the DLPFC-R and several other pain structures, including the hypothalamus, PAG, and thalamus. Compared to controls, inter-regional connectivity (region-to-region connection) of the regions of interest (ROIs) revealed that participants with AD had stronger connectivity between the hypothalamus, PAG, and thalamus as well as greater connectivity within the remaining network (see Table 2).[2]

As suggested in the first study,[1] results of this second study are thought to indicate that participants with AD had increased attention to pain.[2] Also, both studies are thought to reflect the possibility that participants with AD may experience pain as more threatening because of a diminished ability to recognize and contextualize the pain appropriately due to deleterious effects of AD on memory and judgement. Interpreting pain as a greater threat and giving pain more attention and expectation, also likely means that the individual experiences greater pain as both conditions are known to increase pain.[117]

An additional study investigated resting-state functional connectivity (RSFC) in AD.[3] Compared to controls, participants with AD had increased RSFC between the DLPFC-R and ACC, as was found in the previous task-evoked connectivity study,[2] but decreased RSFC between the right insula and bilateral ACC and between the right S2 and the right amygdala (see Table 2). Only one correlation was found between RSFC and pain reports. For controls, unpleasantness ratings of moderate pain were associated with greater RSFC between the cognitive evaluative/affective structures of the DLPFC-R and the left ACC, but this was not found in participants with AD. As with the previous two studies,[1,2] this further reveals differences in brain function that may alter the experience of pain in AD.

The last neuroimaging study including pain in AD examined behavioral and autonomic pain responses and separate fMRI scanning without a pain paradigm.[48] This study found that participants with AD who had increased behavioral responsiveness to pressure pain in a previous session also had alterations in functional connectivity during a separate fMRI session. Alterations were found between several prefrontal brain structures and within and between the default and salience networks (see Table 2).[48] This may mean that increased behavioral responsiveness to pain implicates dysfunctional prefrontal and temporal limbic affective-behavioral regulation, decreased contextual appraisal for memory, and increased mental activity to pain. Because this study did not include self-report of pain, an active pain paradigm, or the same ROIs, it is not as easy to compare to the other neuroimaging studies, however, increased mental activity in response to pain by participants with AD is suspected in all four studies. As mentioned above, mental attention to pain can increase feelings of pain.[117]

The Periaqueductal Gray

The PAG is critically involved in the neural basis of pain.[19,20,50] It is a cell-rich, poorly differentiated gray matter structure that is subdivided[118] and is approximately 14 mm long and 5 mm wide.[18] As shown in Figure 2, the PAG is part of the midbrain and almost entirely surrounds the cerebral aqueduct.

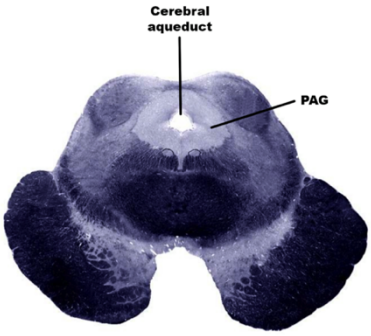


Figure 2. Transverse slice of the midbrain including the PAG[119]

The PAG is anatomically located where ascending sensory information and descending modulatory information from higher brain centers intersect,[18] positioning it to act as a modulatory information collection and transmission center.[22-24] Figure 3 visually demonstrates the relative position within the brain of the PAG via neuroimaging activation estimates.[18]

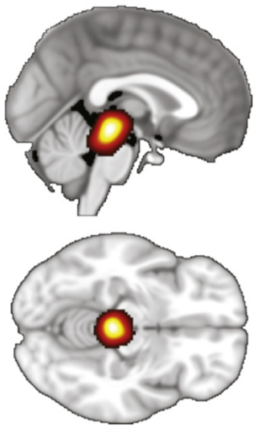


Figure 3. PAG activation from neuroimaging data adapted from Linnman et al[18] (p. 503)

Out of the neurobiological functions of the PAG, pain modulation is the most clearly defined.[18,22] In addition to primary endogenous opioid release[22,26] and mediation of exogenous opioid analgesics,[26-29] the PAG also modulates pain through habituation, expectation, attention, anticipation, distraction, and placebo.[18] A variety of painful conditions including low back pain, neuropathic pain, complex regional pain syndrome, myofascial pain, osteoarthritis, and fibromyalgia are related to pain activation in the PAG.[18] This demonstrates that the PAG is not specific to one type of pain but is involved in general pain processing. Figure 4 is a representation of pain activation in the PAG from neuroimaging data compiled from multiple studies of experimental, evoked pain.[18] Of note is that out of 54 studies reporting PAG activation

during pain, 25 used heat pain.[18] Decreased or altered activation is also found in the PAG in some chronic pain states, such as chronic low back pain[120] and fibromyalgia,[121] suggesting that the inhibitory functions of the PAG are dysfunctional which allows pain to continue.

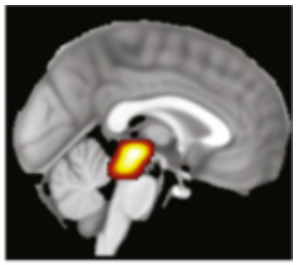


Figure 4. Pain activation in the PAG by Linnman et al[18] (p. 516)

Regional Brain Connections with the PAG

The prefrontal cortex, including the ACC,[122,123] insula, and amygdala provide major inputs to the PAG.[18] The PAG is reciprocally connected to the central nucleus of the amygdala.[18] The PAG projects to the thalamus, hypothalamus, brainstem (e.g. rostral ventromedial medulla (RVM)[122]), and deep layers of the spinal cord via the RVM.[18,122] The regions of efferent and afferent projections of the PAG share overlap with the pain networks and the heat-induced neurologic pain signature (NPS; see Table 5).

Table 5. Brain regions in the pain networks, NPS, and PAG projections

Brain Region	Medial Pain Network	Lateral Pain Network	Rostral Pain Network	Neurologic Pain Signature	Afferent PAG Projection	Efferent PAG Projection
Prefrontal cortex	✓				✓	
Insula	✓	✓	✓	✓	✓	
Thalamus	✓	✓	✓	✓		✓
Hypothalamus	✓		✓			✓
Periaqueductal gray	✓		✓	✓		
Anterior cingulate cortex	✓	✓	✓	✓	✓	
Hippocampus	✓					
Primary somatosensory cortex		✓				
Secondary somatosensory cortex		✓		✓		
Amygdala			✓		✓	✓
Orbitofrontal cortex			✓			
Ventral striatum			✓			
Rostral ventromedial medulla						✓

The fMRI-derived NPS is based on repeated and reproducible BOLD signal activations during an evoked heat pain stimulus.[32] As listed in Table 5, the primary regions included in the NPS are the insula, thalamus, ACC, S2, and PAG, all of which are known to be involved in pain processing.[4,23,32] While the primary somatosensory cortex (S1) is a site of sensory-discriminatory pain processing, lesion and surgical studies demonstrate that pain intensity processing can occur without S1.[124] Brain regions both contralateral and ipsilateral to a unilateral simulation site also process pain intensity, as demonstrated by studies with split-brain patients and of those with surgical removal of a cerebral hemisphere.[124] Bilateral activation in response to experimental pain stimulation was found in brain regions including the insula, thalamus, ACC, and S2 during PET scans,[124] and in the fMRI-derived NPS.[32]

The NPS is relevant to the study of the experience of pain in AD because it is based on brain activation during an acute, evoked pain stimulus, it corresponds to self-reported pain, it is specific and sensitive to physical pain,[32] its accuracy has been replicated across multiple studies,[125-129] and it overlaps substantially with the pain networks (Table 5). Additionally, heat was the pain stimulus used for the NPS and heat is one of the primary and most reliable[37] pain stimuli used in studies of AD and pain.

The insula is a multimodal cortical region deeply buried[130] subjacent to portions of the frontal, parietal, and temporal cortex.[22] It is one of the most consistently activated brain regions during acute physical pain,[33] and is part of all three pain networks.[4] It is involved in visceral and somatic sensory processing,[130] and via projections with the PAG may induce antinociception.[21] The insula may also be involved in all subjective feelings, such as subjective feelings of pain.[131,132]

The thalamus is largest part of the diencephalon and is a multimodal relay station.[133] It is considered a relay, or gateway,[134] because all incoming information (except olfactory[135]) must pass through the thalamus before reaching regions of the cerebral cortex.[136] Information may be altered based on behavioral demands during its passage through the thalamus.[135] Like the insula, the thalamus is consistently activated during acute pain,[134] and is part of all three pain networks.[4] The PAG projects to the thalamus and the thalamus transmits nociceptive information to the insula and ACC which create part of the affective/motivational components of pain.[22]

The ACC is the most anterior portion of the cingulate gyrus[22] which is a thick section of cortex that encircles the corpus callosum.[137] Aversiveness to pain may arise from the ACC[21,138] which may be because the ACC integrates cognitive control, negative affect, and pain.[137] The ACC projects to the PAG,[122] and like the insula and thalamus, it is part of all three

pain networks.[4] Activation in the ACC is consistently seen during acute noxious thermal stimuli and hyperactivation is seen in chronic pain states.[138] The ACC also plays an active role in the creation of fear memories resulting from pain.[138]

The secondary somatosensory cortex (S2) is a functional subdivision of the cerebral cortex.[22] The somatosensory cortex maps the entire body and S2 processes somatosensory information from the primary somatosensory cortex.[22] S2 is part of the lateral pain network,[4] but it does not share direct projections with the PAG. The entire somatosensory cortex is involved in pain, and S2 is involved in recognizing and giving attention to noxious stimuli, particularly stimuli above pain thresholds.[139] S2 can also be directly activated by both noxious and visceral stimuli.[140]

Pain Circuitry

Nociceptors detect stimuli that have the potential to damage tissue[141] and transmit this information to the dorsal horn of the spinal cord.[22] This nociceptive information is routed contralaterally from the dorsal horn via ascending tracts that terminate in the brainstem/midbrain and thalamus.[141] The spinomesencephalic tract (SMT) carries this nociceptive information to the PAG.[142,143] The PAG may also receive nociceptive information from descending projections from higher brain structures such as the ACC[144,145] in response to nociceptive information that was routed through the thalamus.[141]

The SMT terminates in the midbrain[146] and is primarily involved in nociception.[147] Within the SMT, the spinoannular bundle[146] projects directly to the PAG.[123,147,148] Major projections from the SMT terminate in the ventrolateral subdivision of the PAG.[146] The ventrolateral PAG (vlPAG) is associated with pain modulation while other subdivisions are more involved in homeostatic behaviors than pain.[26] The ACC also has greater projections to the

vlPAG than to other subdivisons.[145] The vlPAG is a site of opioid release,[25,145] is densely packed with mu-opioid receptors,[27] and receives the bulk of nociceptive information from C-fibers rather than Aδ-fibers.[149]

The PAG overall contains high levels of opioid receptors and opioid peptides and is sensitive to opioid analgesics,[18,22] which have been in use for over 4,000 years.[27,150] All three families of opioid neuropeptides, the enkephalins, dynorphins, and endorphins, are found within the PAG.[22,118] Enkephalin opioids are the most prevalent in the PAG,[118] and the PAG neurons manufacture enkephalin[149,151] and dynorphin opioids.[149] Results from positron emission tomography (PET) studies indicate that endogenous opioid release within the PAG correlates with pain sensations and correlates with expectations of pain relief from placebo.[18] Additionally, endogenous opioid release may mediate pain relief in response to deep brain stimulation of the PAG in humans.[152,153] Experimental stimulation of the PAG produces analgesia/inhibition of pain,[19,25,154] which was originally identified in rats[155] and then in humans.[156-158]

Crucial to descending pain modulation is the PAG-RVM pathway,[20,50] which is considered the most functionally significant circuit for pain modulation.[159] The PAG-RVM pain circuit contributes to the aversiveness of the pain sensory experience.[154] The connection between the PAG and RVM mediates pain inhibition,[26,118] and if the PAG-RVM connection is disrupted, the PAG is no longer able to inhibit pain.[26] This indicates that the PAG does not directly connect to the spinal cord dorsal horn during pain and must utilize the RVM[26] as a relay station.[24,160] The RVM is often described as a relay station[24,160] made of clusters of brainstem neurons.[21] Activation of the PAG-RVM pathway can originate within the PAG,[20] triggered by a pain stimulus traveling from the SMT.[142,143] Activation of the PAG-RVM to spinal cord pathway preferentially blocks C-fibers, leading to inhibition of pain but preservation of sensory and

discriminative information carried by Aδ-fibers.[161] The PAG-RVM pain circuit also influences withdrawal reflexes meant to protect the body from injury, and inactivation of the RVM alters the threshold of withdrawal reflexes.[154]

The neurons comprising the PAG contain a variety of neurotransmitters, and the most is known about glutamate and gamma-aminobutyric acid (GABA) within the PAG.[118] In the human PAG, glutamate and GABA are found in a similar distribution.[118] Glutamate and GABA are necessary for pain modulation and are involved in endogenous pain mechanisms, however, the functional significance and specific distribution of each neurotransmitter is not clear-cut.[162] Glutamate may also be a neurotransmitter in the SMT because the enzyme glutaminase was found within a subpopulation of cells within the tract,[147] and glutamate is thought to transmit nociceptive information from the SMT to the PAG.[123] Glutamatergic neurons also project from the prefrontal cortex/ACC to the PAG and lead to antinociception.[144] GABA is the primary neurotransmitter involved in projections between the PAG and RVM,[26] and GABAergic neurons within the PAG[163] and RVM[24] are a prominent site of action for endogenous opioids.[26] The pain-relieving effects of exogenously administered opioids are also primarily mediated by the PAG-RVM.[26-28] Serotonin is the main neurotransmitter involved in local control of the RVM,[24] while serotonergic[160] and GABAergic[26] projections connect the RVM to the spinal cord.

The RVM contains pain-facilitatory "ON" and pain-inhibitory "OFF" neurons that modulate pain.[20,24,160] Although GABA is the predominant neurotransmitter regulating ON and OFF cells,[20,164] ON cells can be regulated by serotonin,[24,160] and glutamate[164] and OFF cells can also be regulated by serotonin.[24,160] Exogenous morphine, an enkephalin, acts on mu-opioid receptors within ON cells to suppress ON cell activity, thereby reducing pain facilitation.[141,164] It is unknown if endogenous opioids work the same way,[164] however, it is also likely an

enkephalinergic process.[141,165] It is also unknown if other exogenous opioids work the same way as morphine because of difficulties in their study and inconsistencies in their mechanisms of action.[141]

OFF cells are the primary neuronal projection from the RVM to the spinal cord.[20] Through GABAergic output to the RVM, the PAG can indirectly activate OFF cells and directly inhibit ON cells.[20] Enkephalin opioids,[164] or in some cases endocannabinoids,[20] inhibit GABAergic interneurons within the PAG that would normally inhibit projection/output neurons to the RVM[166] (Figures 5-7). The projection/output neurons are suspected to be glutamatergic.[25] Because they are not inhibited (i.e. "disinhibited") by GABAergic interneurons, the glutamatergic projection/output neurons in the PAG are able to transmit their antinociceptive message to the RVM.[20,25,166] Then GABAergic OFF neurons from the RVM transmit sensory input to GABAergic/enkephalinergic neurons in the spinal cord dorsal horn (Figures 5-6). These dorsal horn GABAergic/enkephalinergic neurons act as "gatekeepers" to painful information.[164] Additionally, endogenous enkephalin opioids act as a lock to keep the gate closed to painful sensory information, thereby reducing pain.[164]

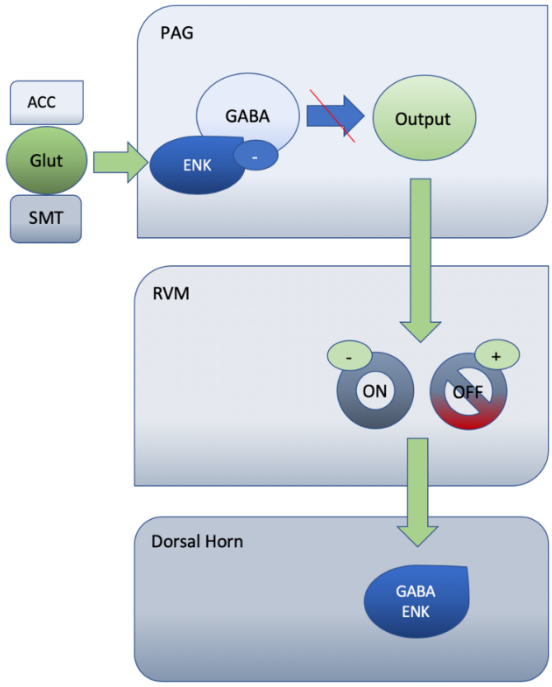


Figure 5. Conceptual schematic of the PAG-RVM analgesia circuit
(ACC = anterior cingulate cortex; Glut/green = glutamate; SMT = spinomesencephalic tract; GABA = gamma-aminobutyric acid; ENK = enkephalin; (-) = inhibit; (+) = activate)

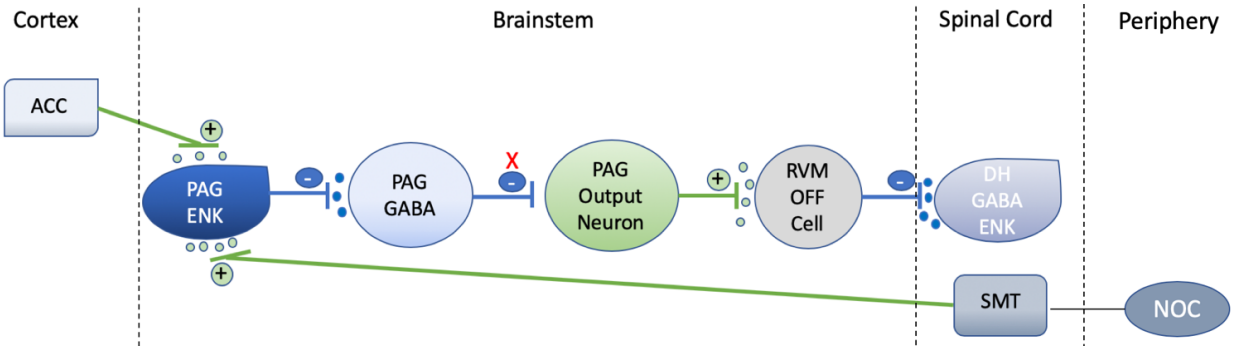


Figure 6. Analgesia circuit of the PAG-RVM
(ACC = anterior cingulate cortex; Glut/green = glutamate; SMT = spinomesencephalic tract; GABA/blue = gamma-aminobutyric acid; ENK = enkephalin; RVM = rostral ventromedial medulla; DH = dorsal horn; NOC = nociceptor; (-) = inhibit; (+) = activate)

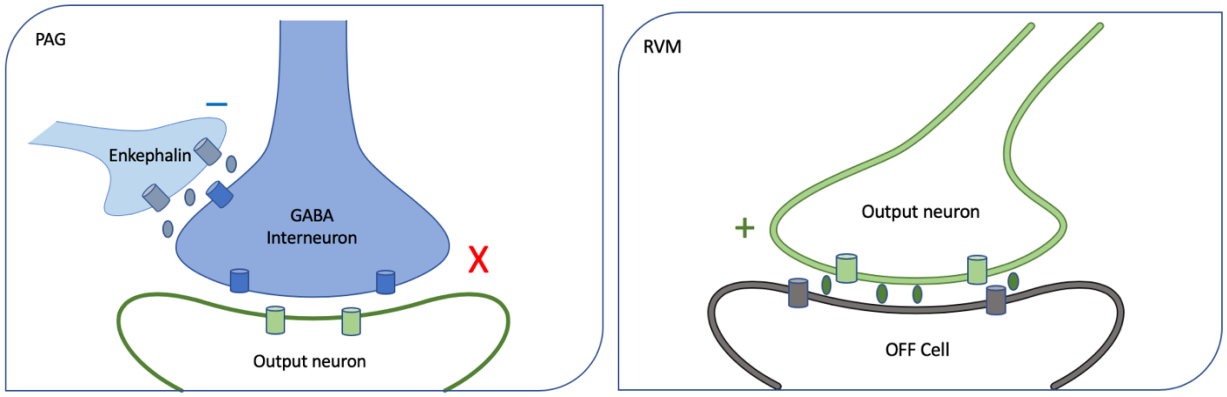


Figure 7. GABA and output neuron activity of the PAG-RVM
(- , X) = inhibit; (+) = activate)

Damage to the PAG in AD

The disease process of AD creates pathology that causes damage to the PAG, however, the extent and consequences of the damage in regard to pain in AD are not yet known. In AD, there are volume decreases in the midbrain gray matter, where the PAG is located.[167] Furthermore, amyloid-β, abnormally phosphorylated tau, and other pathological changes are found on autopsy in the PAG in AD patients.[14-17] Histology from 32 brains from patients with AD found that the PAG had substantial pathological changes in 81% of the samples.[14] The changes were symmetrical and included amyloid-β, abnormally phosphorylated tau, and neurofibrillary tangles (NFTs) (Figure 8). None of the controls without AD demonstrated any of these changes.[14] Duration of AD correlated with the number of plaques and tangles found.[14]

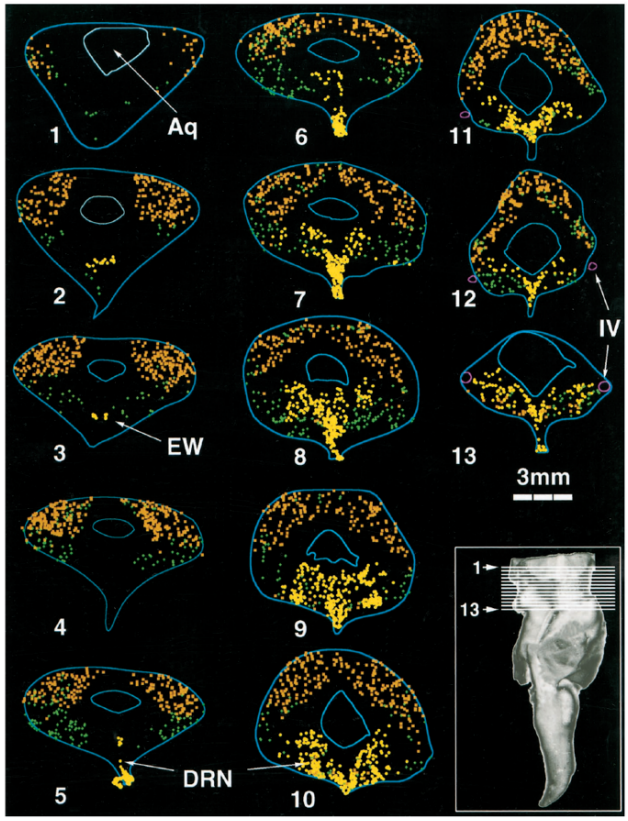


Figure 8. Representation of pathological changes from AD in the PAG by Parvizi et al[14] (p. 349)

(yellow = NFTs, green = "dense core plaques," orange = "diffuse" plaques; note: these plaques together are now commonly referred to as amyloid-β plaques)

An earlier histological study also found amyloid-β and NFTs in the PAG of individuals with AD,[15] and an additional study produced similar findings.[17] More recent work demonstrated that neuropil threads, as well as tau, NFTs, and amyloid-β (Figure 9) are prominently found in the PAG and this pathology increases with disease progression.[16] If the PAG is reduced in volume and damaged by pathology from AD, its ability to modulate and process pain may be compromised which, in turn, may alter the experience of pain in the individual with AD.

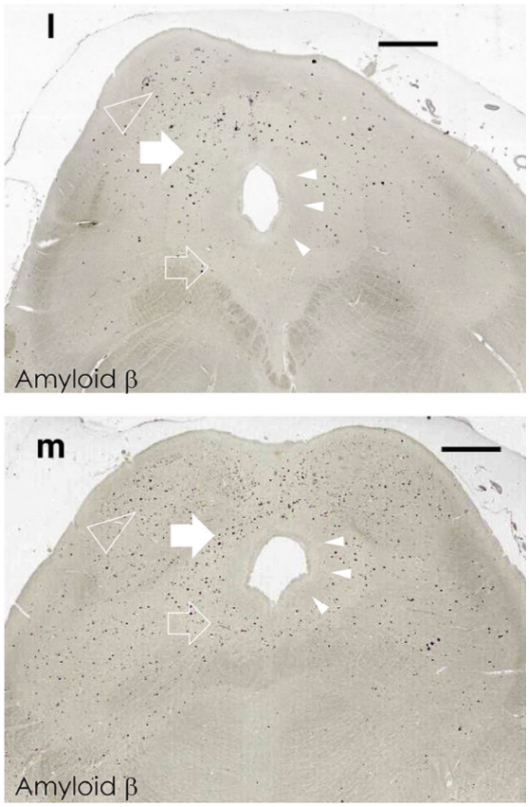


Figure 9. Stained midbrain slice of the PAG with amyloid-β by Uematsu et al[16] (p. 13)

Opioid Dysfunction in AD

There is limited research focused on opioid receptor alterations in AD, and most existing research is aimed at finding treatment targets for disease progression in AD.[168-170] In the only known review of the opioid system in AD, Cai & Ratka[170] conclude that the opioid system is dysfunctional in people with AD and this likely plays a role in the pathogenesis of neurodegeneration. Opioid system dysfunction affects multiple aspects of brain function[168-170] and is related to amyloid-β generation, hyperphosphorylation of tau, neuroinflammation, and cognitive impairment.[168,170,171]

Although the PAG was not studied, of the brain regions that were studied post-mortem, opioid receptor binding and distribution was almost always increased or decreased in AD

compared to healthy controls depending on the receptor and brain region studied.[170] While there were variations, there was an overall/general increase in kappa (κ) receptor binding and decrease in mu (μ) and delta (δ) receptor binding.[170] A recent experimental study concluded that there is an overall "low density" of opioid receptors in AD and this is related to epigenetic methylation processes during AD.[172] Also, opioid peptide precursors appear to be reduced in individuals with AD.[170] Associated with opioid dysfunction is the loss of GABA terminals and receptors as well as disruption of glutamatergic neurotransmission thought to be because of a decrease in opioid receptors in AD.[170] It is unclear how this dysfunction impacts pain processing, but it further establishes that we can expect pain processing to be altered in AD. More recent work elucidates that each kind of opioid receptor plays different and altered roles in AD,[168] but this work does not consider these altered roles in pain modulation.

In experimental mouse models comparing healthy mice to mice with AD, the opioid system seems to behave differently from that of regular mice and these differences appear to cause alterations in pain sensitivity and response to morphine in the mice with AD.[173,174] The mouse model demonstrated that mice with AD had increased inhibition and decreased excitation in the spinal cord which was related to less sensitivity to thermal pain.[174] The mice with AD were also less sensitive to morphine and had no relief from gabapentin.[173] Also of interest is that paracetamol (acetaminophen) was not effective for mice with AD or healthy mice.[173] It is plausible that normally prescribed amounts and/or types (i.e. receptor affinity) of opioid analgesics may be insufficient for pain management in people with AD.

CHAPTER 3

METHODOLOGY

Innovation

This is the first known study to focus on PAG neurophysiology via BOLD signal activation during experimentally induced heat pain in people with AD, thus this study creates new knowledge. Most studies examining pain in people with AD focus on self-report or behavioral pain expressions and/or psychophysical pain responses, and do not investigate how AD pathology affects pain processing.[3,4] However, neuropathological changes that occur in AD likely alter the experience of pain.[1,2,4] Although the PAG is included in overall neuroimaging results in some studies,[2] the only published studies that focus on the PAG in AD are post-mortem histological investigations that demonstrate damage to the PAG during the disease process of AD.[14-17] Advancing our knowledge of the understudied function of the PAG during pain in AD is both innovative and important for understanding the pain experience in this devastating condition.

Overview

This between-groups secondary data analysis includes fMRI brain images captured during a heat-induced experimental pain paradigm. Brain activation in the PAG and responses during experimental pain were compared between participants with AD and healthy age- and sex-matched controls. The PAG contains mechanisms to independently modulate pain,[175] however, additional data were collected from the insula, thalamus, ACC, and S2, all regions from the neurologic pain signature (NPS).[32] This study was feasible because the data were

available, no participants needed to be recruited, analysis could take place within a reasonable timeframe, and expertise and needed resources (e.g. software) were available. Additionally, little to no funding was required and an unpublished pilot analysis verified the feasibility of examining the PAG during pain in AD via fMRI.[176] Major weaknesses of prior research includes the lack of understanding, as well as the lack of study, of the neural basis of the pain experience in AD. This study intended to address this weakness by bringing new insight about possible neural alterations in AD, and therefore advance our understanding of the pain experience in AD.

Sample and Collection Methods

The number of participants from the Monroe and Cowan labs (MCL) that met the inclusion/exclusion criteria for this study and were sufficiently sex- and age-matched was 36 (18 AD, 18 controls), all over the age of 65.[177] Brain images came from data that had not been published and/or analyzed. Inclusion criteria was: (1) full data available, (2) age- and sex-matched controls available, (3) MMSE of 30 for controls and under 23 for AD, and (4) images captured during fMRI scanning of a heat-induced pain paradigm from the MCL. Exclusion criteria was: (1) MMSE scores of 24-29 (to avoid mild cognitive impairment[178]), and (2) not meeting inclusion criteria.

The MCL participants from the original brain image capture were recruited from geriatric faculty practices at Vanderbilt University Medical Center. Inclusion and exclusion criteria from the original data capture follows:

> Participants with AD: (1) diagnosis of probable AD confirmed by medical chart review of standard diagnostic criteria and medical tests, as well as screening for other non-AD causes of cognitive impairment to avoid their inclusion (i.e. hypothyroidism, vitamin B12 deficiency, niacin deficiency, hypercalcemia, neurosyphilis, and HIV infection); AD may

also be evaluated by the Primary Investigator (PI) using a DSM-IV[179] based algorithm, (2) have a designated guardian, and (3) MMSE score of 10-26. Controls: (1) no diagnosis of AD and no apparent cognitive impairment (i.e., MMSE > 26), (2) able to travel to the Vanderbilt University Institute of Imaging Science. Both AD and controls: (1) English speaking, (2) able to provide a pain rating, (3) not taking an analgesic medication within 24 hours of testing, (4) able to see and hear (with or without corrective lenses or hearing aid). Exclusion criteria for AD: (1) any non-AD cause of cognitive impairment. Exclusion criteria for both AD and controls: (1) peripheral neuropathy, uncontrolled diabetes, or stroke, (2) unstable cardiovascular disorder, (3) current alcohol or substance abuse problems, (4) psychotic, bipolar disorder, or posttraumatic stress disorder, (5) claustrophobia, (6) movement disorder, (7) pacemaker or any metal implanted objects that are not 3T MRI compatible, (8) severe spinal curvature, spinal disorder, or severe arthritis, (9) inability to lie flat for at least 10 minutes, (10) acute or chronic pain condition requiring scheduled analgesics, and (11) other reasons as determined by the research team.

Psychophysical heat-induced pain responses were obtained via the Medoc Pathway Pain and Sensory Evaluation System ("Medoc") thermal stimulator. Those responses were collected from all participants before fMRI scanning to determine each participant's personal pain paradigm. Then the pain paradigms were delivered during the fMRI scans. The Medoc is a thermal stimulator that is Food and Drug Administration (FDA) approved to deliver precise heat stimuli.[180] The Medoc has a temperature range of 30°C (86°F) to 55°C (131°F). It can deliver heat at rates of up to 70°C (158°F) per second with cooling rates of up to 40°C (104°F) per second which allows the delivery of a stimulus in less than 300 milliseconds.[181] A thermode is

placed on the thenar eminence of the hand to deliver the stimulus (Figure 10). The thermode is unable to create enough heat to cause tissue damage and is well-tolerated by healthy participants and individuals with AD.[180,182] The Medoc allows participants to rate heat-induced sensations and acute pain levels, such as warmth, mild pain, and moderate pain.

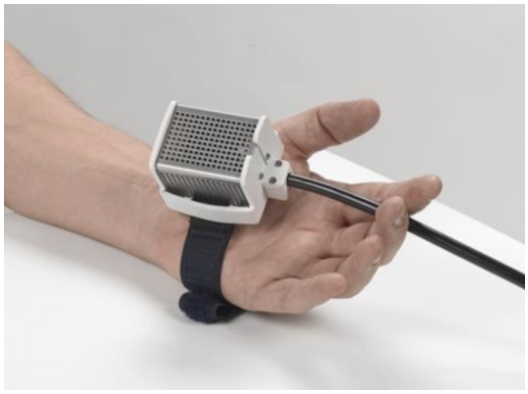


Figure 10. Medoc Thermode[183]

The MCL lab modeled their stimulus intensity pattern for psychophysics after the protocol used by Cole et al.[1,43] Additionally, the Institutional Review Board at Vanderbilt University required the psychophysical pain paradigm to have a maximum pain level of the participant's subjective report of moderate pain. Therefore, pain stimuli were required to be perceptually matched rather than be fixed temperature stimuli.

Instructions were given to participants before psychophysics collection began and during collection. Participants were told that they would be asked "how strong the pain feels" (intensity of pain; warmth, mild, or moderate) and "how unpleasant or disturbing the pain is for you" (unpleasantness of pain; unpleasantness scale).[43] Participants were instructed to notify the research assistant when to stop the heat stimulus when the participant perceived sensations of warmth, mild pain, and moderate pain. Participants were then told, "After you stop the heat, I

will ask you to tell me how unpleasant the previous temperature was." Then participants were shown a 0 - 20 unpleasantness scale (0 = neutral, 20 = very intolerable).[43] Instructions were repeated before each stimulus delivery and ratings of unpleasantness. A practice trial was completed, and then three trials of each of the three stimulus conditions were conducted for psychophysics.[43]

During fMRI scanning, the Medoc delivered two of each pain percept in the form of six pseudorandomized thermal stimulus blocks, to prevent order effects. This occurred during each of four scans (BOLD runs) with an 8℃/second-ramp rate, 16-second duration, and 24-second rest. The 16-second length of the block approximates the hemodynamic response duration that the BOLD signal detects, and the 24-second rest allows the response to return to baseline.[95] This block duration is long enough to allow for the hemodynamic response but short enough to avoid noise from issues with scanner hardware (e.g. scanner drift) that longer duration blocks are more likely to pick up.[95] Therefore, this block length allows for a stronger signal detection with less noise.[95] The MCL brain images were acquired with parameters determined by an MRI physicist based on the goals of the study.[184] Details of the acquisition parameters include:

> 264 second functional run with 28 field echo planar images (EPI), 132 dynamics, 4.50 mm brain slice thickness with 0.40 mm gap between brain slices, 2 second TR (time repetition; time to collect a brain slice), 35 millisecond TE (echo time; time in between each dynamic), 79° flip angle, field of view (FOV) = 240, matrix = 128 × 128, and a standard whole-brain 3-D anatomical T1-weighted/TFE (a time constant) with SENSE coil for alignment.[182]

Data Analysis

Only cases with complete data were used in the analysis of the study aims. Descriptive statistics were used to summarize the characteristics of the group of participants with AD and the matched control participants. Due to skewness of the distributions of several of the continuous variables, median and inter-quartile ranges were used to summarize those values; frequency distributions were used for nominal and ordinal categories. Mann-Whitney (continuous) and Chi-square tests of independence (categorical) were used to compare the characteristics of the two groups. A p-value/type I error rate of 0.05 was used for determining statistical significance.

Aim 1: Median and inter-quartile range summarized the temperature at which each level of pain was reported (temperature) and the perceived unpleasantness of the sensation (affect) at the respective level. Mixed-effects general linear models were used to test the main and interactive effects of AD status and pain level on each of the measures. Data were square-root transformed to normal to meet the assumptions of those models. Mann-Whitney tests compared the temperature values and affect responses between the groups at each pain level. Cohen's d effect statistics were generated for each comparison.

Aim 2: Statistical Parametric Mapping (SPM),[185,186] version 12, was used for the analysis of the fMRI data. SPM was the first widely used analysis software for fMRI.[35] Statistical parametric mapping is a voxel-based technique that is used to identify regional neuroimaging responses to experimental factors that are usually mapped in anatomical space.[186] According to Friston, a final statistical parametric map can be thought of as "an 'X-ray' of the significance of regional effects."[186] (p. 11, plate 1)

A brain image is a large matrix of data composed of voxels.[35] A voxel, an analog to a pixel,[35] is a "three-dimensional volume element" that is a basic sampling unit of MRI.[95] The

smaller the voxel, the greater the ability to identify fine structures in images, but if they are too small the signal may be insufficient.[95] In fMRI images, a voxel is typically 3 to 5 mm,[95] and in this study they were 2 x 2 x 2 which is standard in SPM12. Analysis via SPM12 included preprocessing, first-level analysis, and second-level analysis. Preprocessing prepares the data for analysis. First-level analysis is at the single-subject level, and second-level is at the group level where the groups of participants with AD are compared to healthy controls.

Preprocessing

Preprocessing is the first analysis step that is used to clean[187] and transform the data before task-related analysis occurs.[101] Steps needed in preprocessing are often the same regardless of the fMRI experiment or task.[101] Preprocessing steps include: realigning, slice-timing, coregistration, segmentation, normalization, and smoothing.[187,188] These steps attempt to remove and decrease the variability and noise in the data that is not related to the task/experiment.[95,101]

Realignment corrects for misalignments in the images and puts them all in appropriate orientation.[187] This includes orienting the brain image correctly to the x, y, and z axes and rotation around the axes (pitch (x-axis), roll (y-axis), yaw (z-axis)).[95] This is necessary because of head movement that occurs during scanning.[95]

Brain images are acquired in slices during fMRI scans,[35] with each slice being composed of thousands of voxels.[95] The MCL uses an interleaved, rather than sequential, slice acquisition over a two-second TR (time repetition).[182,188] Interleaving collects every odd-numbered slice then collects every even-numbered slice, which reduces the excitation influence on adjacent slices.[95] Slices are collected over a times-series to be able to detect when in time a voxel may show increased or decreased activity after a stimulus.[95] *Slice-timing* corrects for the mismatch in

the time-series between when the slice was collected (in real-time during scanning) and when the statistical model "thinks" each slice was collected (all at the exact same point in time).[35]

Coregistration aligns the functional and structural MRI images.[101] Structural runs take about 10 minutes to perform, whereas the functional runs scans every two seconds which results in a far smaller resolution of voxels than structural data.[101] The better resolution in structural runs means the visual picture is much clearer for structural images and it is easy to identify brain structures and landmarks, unlike the "blurry blob" picture of functional data.[95] Coregistration links the functional data to the structural data to improve the spatial localization of the functional data, allowing you to know where the functional activity occurred anatomically.[95,101]

Segmentation is a step that helps prepare the images to be normalized.[187] This step assigns the different brain tissues (gray matter, white matter, cerebrospinal fluid, soft tissue, bone, and also air) to tissue probability map templates.[187] Tissue Probability Map (TPM) for 6 tissue classes is the default in SPM12. Assigning the voxels to the correct tissue type helps with the next pre-processing step[187] where spatial normalization to stereotaxic space occurs (e.g. Montreal Neurological Institute (MNI) space).[95]

Normalization corrects for spatial differences among images[101] and moves them to a standardized space.[187] Healthy human brains are consistent across individuals as far as the presence of the same brain structures and organization.[35] However, there are large differences between individuals in size and shape[101] of up to 30% difference in brain size.[95] Normalization aligns brain images to match location, orientation, and size of the participant brain with a brain atlas.[101] Montreal Neurological Institute (MNI) space is the stereotaxic space SPM12 uses as the brain atlas for normalization.[95,101]

Smoothing, or spatial smoothing,[101] is conducted to cancel/reduce noise and increase signal.[187] In each voxel, the BOLD value is replaced by a weighted average BOLD response of its neighboring voxel via a kernel.[101] Weights are greatest at the smoothed voxel and the voxels further away have sequentially decreased weights.[101] Smoothing "blurs" the data by cutting off peaks and filling up valleys at each TR.[101] This blurring across adjacent voxels reduces spatial resolution, but improves the validity of the statistical testing.[95] Smoothing reduces the noise in the data and allows the data to fit assumptions of the statistical models used in future analysis steps.[101] In this study, images were smoothed with a full-width half maximum (FWHM) 8 mm Gaussian kernel. Many methods for dealing with the multiple comparisons problem, such as those of random field theory, assume that data is smoothed with a Gaussian kernel.[101]

First-level Analysis: Single-Subject Analysis

After pre-processing is complete, the data can be fit to a model.[187] Model testing is a nearly universal goal of fMRI data analysis,[95] and a model must be fit to the time-series, or signal measured, of each individual voxel.[187] General Linear Modeling (GLM) is the statistical test used to achieve this goal.[95] GLM evaluates the experimental parameters of the observed fMRI data (dependent variable; voxels by time points from the BOLD signal) that best models the original data with predictor values (independent variables; pain stimuli) while reducing unexplained error and uncorrelated noise.[95]

The results of the GLM represent an estimate of the average amplitude of the BOLD signal in response to the condition in the model.[187] Robust weighted least squares estimation (rWLS) is an SPM12 toolbox that replaces the default first-level analysis. The GLM is conducted by the rWLS process, and rWLS further corrects for motion artifact (e.g. head movement).[188] This process is weighted so that images with higher variance will have less impact on the

results.[189] This technique can be especially useful for older adult populations where sporadic events that create motion artifacts happen more often.[189]

Data from fMRI yields information about changes in brain activation over time and cannot yield information about absolute levels of brain activation.[95] Therefore, the difference in activation between conditions is often compared,[95] in this case the difference between baseline and warmth, mild pain, and moderate pain. SPM12 will use components from the GLM in combination with selected contrast weights using the SPM12 "contrast manager" program. For this study, the contrasts are: warmth > baseline, mild pain > baseline, and moderate pain > baseline. SPM12 then conducts t-tests on each voxel to evaluate the effect of these conditions.[95] The end result of first-level analysis is a statistical parametric map for each subject during the selected conditions[101] of warmth, mild pain, and moderate pain.

Second-level Analysis: Group-Level Analysis

After obtaining a statistical parametric map for each subject during first-level analysis, group differences can be compared during second-level analysis. SPM12 will calculate the mean, and standard error for a contrast, then the statistical significance for a contrast as well as a t-test on the contrast images made during first-level analysis.[187] The SPM12 "contrast manager" program is used again but this time the contrasts are Control < AD and then Control > AD during warmth, then during mild pain, and then during moderate pain. For these contrasts, an alpha level of $p = 0.05$ and voxel-wise threshold of $p = 0.05$ was used. This results in whole brain group statistical parametric maps, and an example of uncorrected "glass brain" outputs from SPM12 from this study are shown in Figure 11. However, the objective of this study was to compare activation in certain ROIs known to be involved in pain processing and selected *a priori*.

Therefore, ROIs were created for the PAG, insula, thalamus, ACC, and S2, and compared between AD and controls to obtain group comparisons.

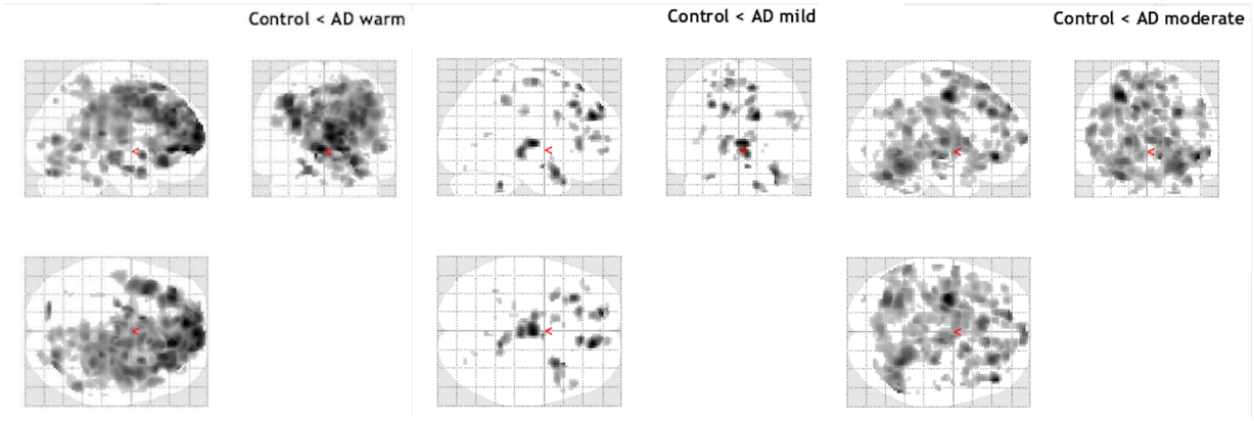


Figure 11. Second-level analysis uncorrected glass brain outputs for control < AD during warmth, mild pain, and moderate pain

The PAG ROI was created from a consensus centroid derived from findings in Neurosynth[190] with MNI coordinates 2, -26, -10, and a 6 mm sphere was created around this centroid using the MarsBaR toolbox for SPM12.[191] Neurosynth is a National Institutes of Health (NIH)-supported database that allows for large-scale, automated synthesis of fMRI data.[190] The insula, thalamus, ACC, and S2 ROIs were created with the Wake Forest University (WFU) PickAtlas.[192] Bilateral ROI mask sections were chosen separately (e.g. "right insula" and "left insula") resulting in eight ROIs, nine ROIs total including the PAG (see Table 6). WFU PickAtlas generates ROI masks based on the Talairach Daemon database and includes Brodmann area, Lobar, Hemisphere, Anatomic Label (Automated Anatomical Labeling; AAL), and Tissue Type.[192] The Talairach Daemon database[193,194] is widely used and is part of the International

Consortium for Brain Mapping (ICBM) and the NIH Human Brain Project in developing a probabilistic atlas of human neuroanatomy.[195]

Table 6. Regions of interest from the WFU-PickAtlas and MarsBaR toolboxes in SPM12

Bilateral ROI	Atlas/Coordinates
PAG	6 mm sphere MNI 2, -26, -10
Insula	Automated Anatomical Labeling
ACC	Talairach Daemon Brodmann Areas 24, 32
Thalamus	Automated Anatomical Labeling
S2	Talairach Daemon Brodmann Areas 40, 44

Cluster correction was used to correct for the problem of multiple comparisons[101] in this study. Cluster correction is used because t-statistics are generated for each voxel, but the data is not spatially independent and occurs in clusters of voxels with spatial correlation.[186] Not only would a Bonferroni correction be far too conservative for thousands of voxels,[186] but the clustering of activation with neighboring statistical correlations makes it inappropriate.[186] Therefore, finding significant clusters is the objective rather than significant single voxels on their own.[187]

There are several ways to achieve cluster correction and common methods are functions within SPM or Analysis of Functional NeuroImages (AFNI).[196] One common option is the small volume correction (SVC) function within SPM12, which is a method that searches for activation in a defined area of the brain (i.e. ROI) thereby reducing the volume of the brain that is searched.[35] SPM12 SVC is well-known to be an extremely conservative Family-wise error (FWE) multiple comparisons correction method. FWE is a common measurement of Type I errors used by SPM for multiple comparisons and represents the chance of one or more false

positives being found within a brain image.[35] While SVC and other methods work for most regions of the brain, they may not function correctly for very small ROIs,[35] such as the PAG. Another method is to use existing literature on the methods used by others for SVC. A cluster of activated voxels can be considered significant if their number surpasses the set threshold, or the "voxel threshold."[101] Therefore, a voxel threshold was chosen for cluster correction based on Kong, Linnman, and colleagues' work.[122,197] A voxel threshold level of 5 was used for a 3 mm PAG sphere[197] and a voxel threshold level of 10 was used for a 6 mm PAG sphere.[122] In this current study, a voxel threshold of 10 contiguous voxels with a voxel-wise $p = 0.05$ was used for the 6 mm PAG sphere.

AFNI has transitioned to a new cluster correction process intending to correct for a statistical concern in their popular program 3dClustSim.[196] However, the neuroimaging community has come to no consensus on this issue and many still use the program. Because of this known concern with AFNI, the MCL is currently using the more conservative option of SPM12 SVC. A voxel-wise $p = 0.05$ corrected FWE with a minimum cluster extent of 0 contiguous voxels was set for the threshold for the insula, thalamus, ACC, and S2 during SVC in SPM12. The FWEc output number was then used to determine if any significant clusters remained above that threshold.

Privacy/Confidentiality

All data records were identified only by a study-specific participant number and accessed from secure Vanderbilt University and Ohio State University servers and platforms. For the original MCL data collections, strict university confidentiality procedures were followed. Any forms with identifiable participant information are digitally maintained on a secure drive on Ohio State University servers, and hard copies were shredded and disposed of securely.

CHAPTER 4

RESULTS

Sample Characteristics

The sample of 36 participants (18 AD, 18 control) was comprised of 50% female participants equally for each group. In addition to exact sex-matching, participants were closely age-matched with zero- to two-year age differences for all but one pair with a three-year age difference. As shown in Table 7, the majority of the sample was Caucasian and right-handed. Given that the MMSE score was used as a criterion for inclusion in the study and assignment to the groups, as expected those scores differed between the groups ($p < 0.001$). Depressive symptoms are known to co-vary with MMSE,[3,198] thus not surprisingly the participants in the AD group had higher Geriatric Depression Scale (GDS) scores than did those in the control group ($p < 0.001$) (see Table 7).

Table 7. Demographic and clinical characteristics by study group

	Total (N=36)	Control (N=18)	AD (N=18)	
	N (%)	N (%)	N (%)	*p-value*
Education				0.395
≤ High school	7 (19.4)	2 (11.1)	5 (27.8)	
Partial college	8 (22.2)	4 (22.2)	4 (22.2)	
College graduate	11 (30.6)	5 (27.8)	6 (33.3)	
Graduate degree	10 (27.8)	7 (38.9)	3 (16.7)	
Handedness				0.070
Right	33 (91.7)	15 (83.3)	18 (100)	
Race/ethnicity				0.546
African-American	3 (8.3)	1 (5.6)	2 (11.1)	
Caucasian	33 (91.7)	17 (94.4)	16 (88.9)	
	Median [IQR] Min, Max	**Median [IQR] Min, Max**	**Median [IQR] Min, Max**	*p-value*
Age	71.0 [68.0, 78.8] 65, 86	71.0 [67.7, 79.3] 65, 86	72.5 [68.0, 77.5] 65, 86	0.799
MMSE score[1]	26.5 [15.5, 30.0] 10, 30	30.0 [29.0, 30.0] 29, 30	16.0 [11.7, 22.0] 10, 24	< 0.001
Average pain[2]	0.5 [0.0, 3.0] 0, 5	1.0 [0.0, 2.3] 0, 4	0.0 [0.0, 3.0] 0, 5	0.709
Pain right now[2]	0.0 [0.0, 0.0] 0, 4	0.0 [0.0, 1.0] 0, 3	0.0 [0.0, 0.0] 0, 4	0.528
GDS-SF score[3]	1.0 [0.0, 3.8] 0, 5	0.0 [0.0, 1.0] 0, 5	3.0 [1.7, 4.0] 0, 5	< 0.001
STAI state score[4]	48.5 [45.0, 50.8] 32, 75	50.0 [46.5, 51.5] 32, 75	48.0 [44.7, 50.0] 42, 68	0.293
STAI trait score[4]	47.0 [44.0, 50.0] 32, 56	47.0 [44.0, 50.0] 32, 56	46.0 [42.7, 49.3] 41, 56	0.339

[1]Folstein Mini Mental State Examination (range = 0-30; 0 = completely cognitively impaired, 30 = completely cognitively intact)
[2]BPI-SF-Brief Pain Inventory Short Form (range= 0-10; 0 = no pain, 10 = most pain)
[3]GDS-SF-Geriatric Depression Scale Short Form (range; 0=no indication of depression, 15 = high possibility of depression)
[4]STAI-Spielberger State or Trait Anxiety Inventory (range; 20 = indicates increased anxiety, 80 = indicates least amount of anxiety)

Analysis of Hypotheses

Aim 1: To determine between-groups differences of heat-induced pain responses during PAG activation between participants with AD and healthy age- and sex-matched controls. **Hypothesis 1a:** Participants with AD will report detecting mild and moderate pain at higher temperatures than controls. **Hypothesis 1b:** Participants with AD will rate mild and moderate pain as just as unpleasant or more unpleasant than controls.

Summaries of the temperature at which each of the pain levels was reported by the respondents and their perceptions of the unpleasantness of those temperatures (pain levels) are shown in Table 8. Statistically significant main effects of increasing threshold levels were observed on both the temperature and affective perceptions confirming the pain-inducing paradigm used in this study ($p < 0.001$). There were also statistically significant main effects of AD status on the temperature necessary to produce the perception of pain ($p < 0.001$) and on the experience of unpleasantness of the pain ($p = 0.039$). The participants with AD did not detect pain until a higher temperature was reached compared to controls and when pain was detected, they reported the pain to be more unpleasant. Contrary to the hypothesized interaction effects, the interaction effect of AD status and pain threshold level on temperature and affect (unpleasantness) were not statistically significant (temperature: $p = 0.622$; affect: $p = 0.168$). The effects of AD on the temperature at which a given pain level was reported were strongest at the warmth and mild levels of pain (Cohen's $d = 0.91$ and 0.90 respectively). While the main effect of AD status on the unpleasantness of pain (affect) was statistically significant as reported above, none of the differences at the specific pain levels were statistically significant ($p > 0.05$). The strongest effect of AD status on those perceptions (affect) was at the level of pain perceived as "mild" (Cohen's $d = 0.60$, see Table 8).

Table 8. Psychophysics of temperature thresholds necessary to produce warmth, mild pain, or moderate pain and unpleasantness ratings (affect) at each condition by study group

	Total (N=36)	Control (N=18)	AD (N=18)	*p-value**	Cohen's *d*
	Median [IQR] Min, Max	Median [IQR] Min, Max	Median [IQR] Min, Max		
Temperature °C					
Warmth	33.0 [32.0, 34.0] 31, 38	32.0 [32.0, 33.0] 31, 35	34.0 [33.0, 35.0] 32, 38	0.002	0.91
Mild pain	36.0 [35.0, 39.8] 33, 46	35.0 [34.0, 37.0] 33, 46	39.0 [35.8, 40.3] 34, 43	0.004	0.90
Moderate pain	41.5 [38.0, 43.8] 34, 48	39.5 [38.0, 42.0] 34, 48	43.0 [39.0, 44.3] 36, 48	0.025	0.71
Affect[a]					
Warmth	0.0 [0.0, 0.0] 0, 4	0.0 [0.0, 0.3] 0, 3	0.0 [0.0, 0.0] 0, 4	0.495	0.01
Mild pain	3.5 [0.2, 5.0] 0, 11	1.5 [0.0, 4.0] 0, 5	4.0 [0.7, 5.0] 0, 11	0.064	0.60
Moderate pain	6.0 [4.2, 8.0] 0, 14	5.0 [3.7, 6.3] 0, 11	7.0 [4.2, 10.3] 1, 14	0.147	0.35

[a]0 - 20 unpleasantness scale (0 = neutral, 20 = very intolerable)[43]
Note: Mixed-effects analyses revealed statistically significant main effects of AD status for both temperature ($p < 0.001$) and unpleasantness ($p = 0.039$). Confirming the sensory inducement paradigm, statistically significant main effects of increasing threshold levels were observed on both temperature and unpleasantness ($p < 0.001$). Neither of the interaction effects of AD status and pain threshold level were statistically significant (temperature: $p = 0.622$; affect/unpleasantness: $p = .168$).
*p-values are for Mann-Whitney tests at each pain threshold level. Values were square-root transformed to meet normal distribution assumptions of Cohen's *d*.

Aim 2: To determine between-group differences in PAG activation in response to heat-induced pain in participants with AD healthy age- and sex-matched controls. **Hypothesis 2:** There will be increased brain activation during heat-induced pain in the PAG in participants with AD compared to controls.

PAG activation during a heat-induced pain paradigm was greater in participants with AD compared to controls during warm temperatures and mild pain. See Figures 12 and 13 for visual results of the fMRI BOLD activation between-groups output from SPM12 displayed with the

avg152T1 template, and Table 9 for comparison data. All visual SPM12 output, with each slice of activation, can be seen in Appendix B in Figures 14 and 15.

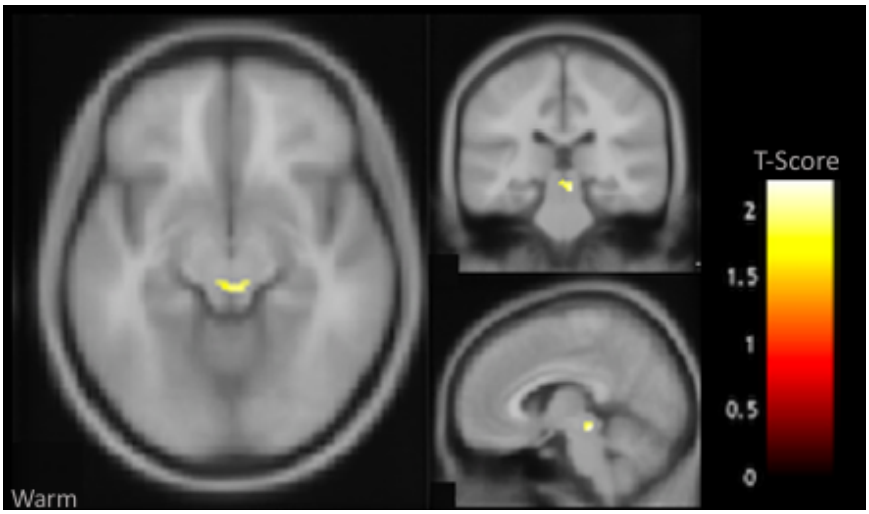


Figure 12. Results indicating greater activation in AD participants relative to controls in the PAG for the contrast comparison of warmth greater than baseline

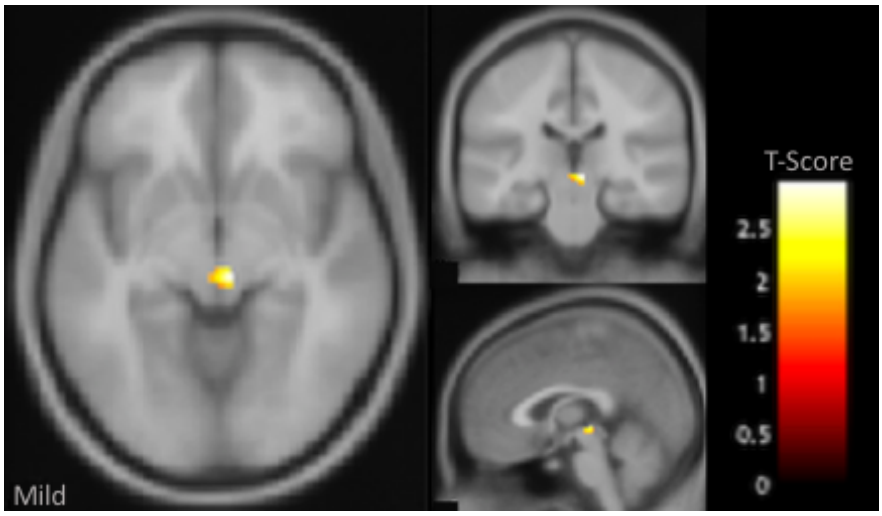


Figure 13. Results indicating greater activation in AD participants relative to controls in the PAG for the contrast comparison of mild pain greater than baseline

Table 9. All PAG activation differences between participants with AD and controls

Comparison of all activations results above the threshold in the PAG	Extent/voxel threshold for cluster correction	Number of voxels in cluster	Peak T (T-score)	Peak coordinates (x, y, z)	p-value
Control < AD during warmth (Figure 12)	K = 10	$K_E = 28$	2.22	6, -30, -12	0.016
Control < AD during mild pain (Figure 13)	K = 10	$K_E = 33$	2.96	6, -24, -6	0.003

Additional Data

Additional fMRI analyses were conducted on the insula, thalamus, ACC, and S2, which are all part of the neurologic pain signature (NPS). These analyses resulted in no statistically significant differences in activation ($p > 0.05$) between participants with AD and controls because no voxels surpassed the FWEc threshold of SPM12 small volume correction (SVC).

CHAPTER 5

DISCUSSION

Meaning of Findings

Psychophysical Findings

The paradigm used in this study was confirmed to induce pain by the statistically significant main effects of increasing threshold levels on both the temperature and affective perceptions ($p < 0$ .001, Table 8). Although the interaction effect of AD status and pain level on temperature and affect were not statistically significant (temperature: $p = 0.622$; affect: $p = 0.168$), participants with AD reported warmth ($p = 0.002$), mild pain ($p = 0.004$), and moderate pain ($p = 0.025$) at higher temperatures than controls as shown in Table 8. All three temperature percept reports were statistically significantly different from controls and had effect sizes that may indicate a meaningful difference ($d = 0.91$, 0.90, 0.71, respectively) as shown in Table 8. Based on previous research, an effect size equal to or greater than $d = 0.30$ may represent a meaningful finding.[43,199,200] There were also statistically significant main effects of AD status on the temperature necessary to produce the perception of pain ($p < 0.001$) and on the experience of unpleasantness of the pain ($p = 0.039$). These results support hypothesis 1a.

While not statistically significant, participants with AD rated mild and moderate pain affect as more unpleasant than controls (mild 1.5 [0, 4] in controls vs 4 [0.7, 5] in AD and moderate 5 [3.7, 6.3] in controls vs 7 [4.2, 10.3] in AD; Table 8). These affect/unpleasantness reports had effects sizes that may indicate a meaningful difference in mild ($d = 0.60$) and moderate ($d = 0.35$) pain, which partially supports hypothesis 1b.

These psychophysical findings demonstrate that participants with AD may need greater experimental stimuli to report perceptually-matched pain, however, their experience of pain may be more unpleasant compared to controls, particularly during mild pain conditions. This indicates that people with AD continue to experience acute pain as much, or more unpleasantly, than age- and sex-matched cognitively intact older adults.

As discussed in Chapter 2, *Pain Circuitry*, the PAG-RVM pathway is crucial for pain modulation,[20,50,159] and influences withdrawal reflexes meant to protect the body from injury.[154] Inactivation of this area can alter withdrawal thresholds,[154] and damage to this area of the midbrain found in AD[14-17] may mean that withdrawal reflexes to pain are altered. This may be a contributing factor as to why participants with AD needed greater stimuli to report pain. Greater pain unpleasantness is discussed in relation to the PAG-RVM below.

fMRI Findings

As discussed in Chapter 2, *The BOLD signal*, the indirect physiological changes demonstrated by fMRI images are correlated with neuronal activity,[95] demonstrating a "time course" of activity by the neurons.[35] Local neural activity is usually paired with "functional hyperemia" which is a temporary oversupply of blood flow causing an increase in blood and tissue oxygenation.[103] The BOLD signal is thought to predominantly reflect excitatory synaptic input to a brain region[104] and increases in oxygenation and blood flow are correlated with excitatory neuron activity.[103] It may also be that the primary driver of the BOLD response is in the form of interneuron activity levels.[103]

The primary afferent projections to the PAG involved in pain,[18,122,123] and the neurologic pain signature (NPS),[32] include the insula[21] and ACC.[144,145] The spinomesencephalic tract (SMT) directly projects pain information to the PAG from the periphery.[142,143,146,147] The

thalamus and S2 do not project directly to the PAG. Therefore, this indicates that the pain stimulus the participants experienced produced excitatory output, likely glutamatergic,[123,147] from the SMT and/or the insula or ACC to the PAG. These excitatory post-synaptic potentials arrived in the PAG, and local excitatory processing within the PAG may have occurred. Excitatory neurons (e.g. glutamatergic[25]) and/or inhibitory neurons (e.g. GABAergic[163]) in the PAG may have fired action potentials.

This activity may represent activation of the PAG-RVM pathway.[20] As discussed in Chapter 2, *Pain Circuitry*, this pathway preferentially blocks C-fibers, leading to inhibition of pain but preservation of sensory and discriminative information carried by Aδ-fibers.[161] The PAG contains high levels of opioid receptors and opioid peptides,[18,22] and PAG neurons manufacture opioids.[149,151] Results from PET studies indicate that endogenous opioid release within the PAG correlates with pain sensations.[18] Direct, experimental stimulation of the PAG results in analgesia/inhibition of pain,[19,25,154] and neuroimaging studies demonstrate that increased pain has been found to scale with increased PAG BOLD activation.[50,51] It may be that increased pain is reflected by an increased PAG BOLD signal and then processes within the PAG cause pain inhibition to occur.

Neuroimaging findings from this study indicate that PAG activation during an experimental heat-induced pain paradigm was greater in participants with AD compared to controls during warmth and mild pain (Figures 12 and 13 and Table 9). This indicates that input to the PAG is at least partially retained in this sample of people with AD but it may be altered from that of healthy older adults. Central pain processing appears to continue in some manner since there was not an absence of activation in the PAG in people with AD. Because there was greater activation in the PAG of participants with AD compared to controls and because

increased pain has been found to scale with increased PAG activation,[50,51] people with AD may be particularly vulnerable to suffering from pain. This may be, at least in part, because the opioid system is dysfunctional in AD and it is unclear if PAG activation means that the endogenous opioids are performing properly during activation. If endogenous opioids are not inhibiting pain, this could mean that people with AD have a greater risk of amplified pain unpleasantness. However, if this dysfunction includes over-active endogenous opioids, the reverse could be true, but this over-active scenario does not fit with the psychophysical research. Taken together, the psychophysical and neuroimaging results suggest that people with AD are at greater risk of amplified pain unpleasantness compared to cognitively intact older adults.

No differences were found in brain activation between controls and participants with AD in the insula, thalamus, ACC, and S2 during the perceptions of warmth, mild pain, and moderate pain. This indicates that these regions may respond in a similar way to pain in AD as they do in healthy older adults. Alternatively, this finding may demonstrate the conservativeness of the small volume correction (SVC) Family-wise error (FWE) multiple comparison correction in SPM12. The conservativeness of this method is well-known and activation that is present may not surpass the SVC statistical threshold, whereas activation might surpass the threshold with less conservative methods. The finding of no differences may also be because of the sample size, where a larger size might reveal differences between groups.

Significance with Prior Research and Implications

Psychophysical experimental pain research

Between-groups differences of heat-induced pain responses between participants with AD and healthy age- and sex-matched controls in this study are similar to previous research discussed in Chapter 2 (Tables 1 and 3) in regard to stimuli detection. The majority of the

previous studies found that participants with AD needed higher intensities to detect and report the stimulus at a variety of stimulus intensities.[1-3,42-44]

In the previous studies evaluating the unpleasantness of pain, unpleasantness experienced by people with AD was the same[1-3,36,39,40,43-45] or worse than controls.[1,38,41,45-49] This study found that, while not statistically significant, participants with AD did rate mild and moderate pain as more unpleasant than controls which is in line with the previous findings.

Neuroimaging Research

Preliminary neuroimaging findings (Table 2) indicated that major brain regions involved in pain processing continue to demonstrate activity in response to painful stimuli in AD,[1-3] and that this activity is altered from that of healthy older adults.[1-3,48] These findings are also supported by the current study. Greater PAG activation was found in AD compared to controls, which also indicates pain processing is altered in people in AD. Additionally, because there were no differences compared to controls in findings from the insula, thalamus, ACC, and S2, this demonstrates continued activity in response to pain similar to that of controls, which supports previous findings confirming that pain processing continues in AD.

An important finding of prior research is the demonstration that participants with AD continue to respond to pain and report pain even among methodological differences in studies.[109] The current study supports this finding in that participants responded to pain verbally with pain reports and also displayed brain activation in response to pain.

These findings represent a significant public health issue because pain is underrecognized and undertreated in AD,[7,8] and pain assessment methods and implementation remain inadequate for people with AD.[71,88,201] Clinicians need to keep in mind that people with AD feel pain but may have difficulty communicating their pain.[202] To provide compassionate care, people with

AD may need to be proactively treated for signs of pain with the clinician erring on overtreatment rather than undertreatment.[90] Adding non-pharmacologic, complementary options[203] may offer safer ways to maximize treatment.[68] Using observational pain scales, such as the PAIC15,[82] a detailed pain and medical history, patient/proxy pain reports, and recommendations from the ASPMN position statement on people who cannot self-report pain[93] will increase the likelihood of recognizing and managing pain appropriately in people with AD.[202]

Limitations

This study is an early step in research with a mechanistic focus to give us insight into basic PAG function in AD. Results from this study can be thought of as feasibility or pilot data and cannot answer questions that are further down the road, such as the full pain experience of a person with AD or how to specifically alter pain assessment tools. The importance of these initial study findings should be interpreted with some caution as there are several factors that could impact the results. The study design included a perceptually matched pain paradigm and participants with AD required higher temperatures before they reported pain. This could be a reason why there is greater activation in the PAG as the higher temperature could potentially cause greater activation.

Another factor is that depression is a potential confounder which was not able to be controlled for in this fMRI analysis. As expected, the participants' GDS score were higher in people with AD and these scores co-vary with MMSE.[3,198] This may be in part because there is overlap in positive questions on the GDS with dementia symptoms.[204,205] Although, the GDS scores were in the non-depressed range (4 and below) at a median of 3.0 and IQR of [1.7, 4.0] for participants with AD, which indicates depression was unlikely to impact the study results.

Another limitation of this study is that only 36 of the 40 available participants met inclusion criteria (age-matching). The sample used has zero- to two-year age differences with only one participant pair having a maximum three-year difference, therefore, to keep age-matching close, four participants could not be used because of nine- to 11-year age differences. The available sample pool also meant that participants with MMSE scores of 29 (instead of only 30) had to be used for five controls and a score of 24 (instead of 23 and below) had to be used for two participants with AD. The median sample size for fMRI studies has grown to N = 28.5 for single-group studies and N = 19 for multiple-group studies, but there is a call for increased sample sizes in fMRI[206] and the sample size of 36 may be too small. A larger sample would be expected to reveal more accurate results that might withstand the conservative SPM12 analysis as described earlier.

Recommendations for Future Research

Future analyses can be conducted on this same dataset to determine if there are any significant correlations between psychophysics and the percent signal change in the PAG, insula, thalamus, ACC, and S2 during pain between the participants with AD and controls. Percent signal change data measures the effect a task (e.g. heat pain) has on the BOLD signal,[101] and is the percentage of BOLD signal change across an ROI[35] rather than peak voxel activations within an ROI.[207] Percent signal change data can be extracted through a different SPM analysis than that conducted for this study. The brain analysis results from this study cannot be correlated with psychophysics, and correlation between percent signal change and psychophysics was not part of the aims of this study. This future analysis would yield additional information about how brain activation and pain unpleasantness might be linked in participants with AD. Additionally,

Apolipoprotein E (APOE) status may affect pain[208] and this genetic status could be analyzed to see if there were any associations with pain responses.

No differences in activation were found in the insula, thalamus, ACC, and S2 between participants with AD and controls, however, the highly conservative SPM12 method may have eliminated differences that would be revealed with other methods. Future steps could include analysis using AFNI to determine if more nuanced between-group differences for these ROIs exist.

Future research should include larger sample sizes and, if possible, participants in more severe stages of AD. Also, rather than a perceptually matched heat-induced pain paradigm, a fixed heat-induced pain paradigm would further expand knowledge about the pain experience in AD. Fixed heat pain would mean each participant receives the same stimulus at the same time during fMRI scanning, rather than an individualized, subjective pain stimulus. This would yield data about brain activation during these specific heat temperatures in people with AD compared to healthy older adults. Fixed heat pain would also avoid potential cognitive burden on participants with AD from deciding at what point to report pain and what pain rating to assign to this variable pain. Fixed heat pain may also better allow for the inclusion of participants with more severe AD, which is greatly needed.

There is evidence of opioid system dysfunction in AD[170] but how this impacts pain processing and the experience of pain has not been studied. This system should be a focus of future research. Also important for future research is how exogenous opioid analgesics perform and are processed in AD during this opioid system dysfunction. This knowledge is needed to inform clinicians on proper prescribing of opioid analgesics in patients with AD.

Sex and gender differences are an NIH/National Institute on Aging (NIA) priority and

special interest in the AD population.[209] Biological sex is a genetic factor known to influence the experience of pain[117,209-215] and the processing and antinociceptive effects of exogenous opioid analgesics.[27,210,215-217] Preliminary neuroimaging and psychophysical work in older adults and adults with AD demonstrate that sex differences continue in older age.[110,199,218] Just as pain processing in AD appears to be altered from that of healthy older adults, sex differences in pain in AD also appear to be altered from what is seen in healthy older adults.[110,199] Further research into sex differences in pain in AD may help improve and individualize pain management.[219] Continued research of pain in AD can yield important information that may lead to improvements in pain assessment and management. In turn, this can improve the quality of life of people living with AD and their caregivers. This may also reduce costs and lessen burdens on the healthcare system.

Appendix A

Table 1. Psychophysical data from experimentally evoked pain in AD*

Investigator, Year, Country, Title	Design, Sample, Psychophysical Aim	Psychophysical Methods	Psychophysical Findings	Scoring[111]
Benedetti et al.[36] **1999** **Italy** **Pain threshold and tolerance in Alzheimer's disease**	Between-group cross-sectional N = 48 AD = 24 (14 females) Healthy controls = 24 (13 females) AD MMSE range: 10-19 To test pain thresholds and tolerance in AD compared to age-matched controls and to evaluate these with degree of cognitive impairment	Stimuli: electrical (non-dominant wrist) and tourniquet-induced ischemia (non-dominant upper arm) 12 AD subjects were tested with electrical stimuli and the other 12 with ischemic pain; all 24 controls were tested with both. Subjects reported detection of tactile sensation if the sensation was painful and if the sensation was unbearable	Electrical: No difference between AD and controls was found for stimulus detection ($p = 0.522$, $d = 0.23$) or stimulus experienced as painful ($p = 0.92$, $d = 0.04$); however, pain tolerance was significantly higher in AD ($p < 0.001$, $d = 1.61$). MMSE correlation to electrical pain tolerance: Significant correlation ($p < 0.02$, $d = 1.76$) of MMSE scores with greater electrical pain tolerance Ischemic: No difference between AD and controls for pain threshold ($p= 0.929$, $d = 0.03$), but pain tolerance was significantly higher in AD ($p < 0.001$, $d = 1.19$) compared to controls. MMSE correlation to pain ischemic tolerance: Significant correlation ($p < 0.03$, $d = 1.62$) of MMSE scores with greater ischemic pain tolerance	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims - 3 3. Method and data - 3 4. Sampling - 3 5. Data analysis - 3 6. Ethics and bias - 1 7. Findings/results - 4 8. Transferability/ generalizability - 3 9. Implications and usefulness - 3 Overall score: 27 out of 36

Rainero et al.[44] **2000** **Italy** **Autonomic responses and pain perception in Alzheimer's disease**	Between-groups cross-sectional N = 40 AD = 20 (12 females) Healthy controls = 20 (10 females) AD MMSE range: 8-18 To analyze pain perception and autonomic responses in AD compared to age-matched and mostly sex-matched healthy controls	Stimulus: electrical (wrist on non-dominant arm) Stimuli were randomly delivered, then a series of three stimuli with increasing intensity was given to determine pain threshold, with subjects rating pain with the NRS (0-10). For autonomic responses, stimuli were given as just above the pain threshold or at twice the threshold.	No differences were found between subjects with AD and controls for pain perception just above the threshold (which could be considered weak pain; d = 0.4), but those with AD had diminished autonomic responses (d = 2.09) at this level of pain. Pain perception at twice the threshold (which could be considered moderate pain) resulted in diminished pain perception (with lower ratings of pain) in AD (d = 2.89) but no difference in autonomic response compared to controls (d = 0.65).	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 3 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 1 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 26 out of 36
Gibson et al.[42] **2001** **Australia** **An examination of pain perception and cerebral event-related potentials following carbon dioxide**	Between-group cross-sectional N = 30 AD = 15 (12 female) Healthy controls = 15 (10 female) AD MMSE mean (range): 12.7 (2-19)	Stimulus: radiant heat from CO2 laser (dorsal surface left hand) Random staircase delivery of stimuli, with subjects rating sensations based on descriptors of unpleasantness and warmth.	Subjects with AD had higher stimulus detection thresholds for just noticeable sensations compared to controls (p = 0.003, d = 1.22). No difference between AD and controls was found for pain threshold intensity (pain sensitivity) (p = 0.364, d = 0.35).	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 1 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 27 out of 36

laser stimulation in patients with Alzheimer's disease and age-matched control volunteers	To examine pain thresholds and reports in AD compared to age-matched controls			
Benedetti et al.[108] **2004** **Italy** **Pain reactivity in Alzheimer patients with different degrees of cognitive impairment and brain electrical activity deterioration**	Cross-sectional N = 30 AD = 30 (15 females) (No control) AD MMSE range: 8-21 To examine correlations among several variables related to pain in AD; MMSE and pain responses	Stimulus: electrical (non-dominant wrist) Series of random stimuli given, and the subject rated the pain on a numerical scale (0-10)	Impaired cognition from AD did not appear to impair pain perception, as no correlation was found between MMSE scores and pain threshold ($p = 0.627$, $d = 0.2$) or stimulus detection ($p = 0.40$, $d = 0.33$)	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 3 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 1 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 28 out of 36
Cole et al.[1] **2006** **Australia** **Pain sensitivity and fMRI pain-related**	Between-group cross-sectional N = 29 AD = 14 (7 females) Healthy controls = 15 (6 females)	Stimulus: mechanical pressure (right thumbnail) Five seconds of pressure applied in a triple random staircase procedure at 20 seconds apart to determine thresholds for just noticeable pain (JNP), weak pain (WP) and moderate pain (MP);	Compared to controls, subjects with AD needed higher pressure intensities to report JNP, WP, and MD ($p < 0.05$, $d = 0.93$) but rated JNP ($p < 0.05$, $d \sim 3.6$) and WP ($p < 0.05$, $d \sim 0.88$) as more unpleasant than controls.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 3 7. Findings/results – 4 8. Transferability/

brain activity in Alzheimer's disease	AD MMSE mean (SD): 19.4 ± 5.7 Psychophysical aim was to determine thresholds for just noticeable pain (JNP), weak pain (WP), and moderate pain (MP) and unpleasantness in response to mechanical pressure in subjects with AD compared to age- and mostly sex-matched healthy controls	subjects rated pain on a 0-20 numerical scale with descriptors.		generalizability – 3 9. Implications and usefulness – 4 Overall score: 31 out of 36
Kunz et al.[49] **2007** **Germany** **The facial expression of pain in patients with dementia**	Between-group cross-sectional N = 96 Dementia/AD = 42 (22 females), 16 AD, 17 Vascular Dementia, 9 Mixed Dementia Healthy Controls = 54 (43 females)	Stimulus: pressure (left and right forearms, Fischer algometer) Pressure stimuli (1-5 kg) applied 4 times each to the left and right forearm in a pseudorandom order. Ramp of 1 kg per second and held at target intensity for 5 seconds. Inter-stimulus interval between 20s and 30s. Self-report used a 6-point verbal scale for peak	Participants with dementia showed an increased frequency and intensity of facial responses in response to pain, and these facial responses were greatest for pain-relevant rather than pain irrelevant responses. Additionally, there was a greater increase in facial responses with increasing stimulus intensity in patients with dementia relative to controls. Participants with dementia were less reliable in giving self-report	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 4 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 34 out of 36

	Dementia MMSE Mean (SD): 16.3 ± 5.5 To explore if patients with dementia show differences in facial responses to pain relative to controls and if any change is specific to facial pain responses.	sensation after each stimulus application. Sessions recorded for scoring using the Facial Action Coding System.	ratings (mean: 91% of stimuli). Ability to self-report decreased with increasing cognitive impairment ($p < 0.001$). No differences were found between the value of self-reported ratings between patients with dementia and healthy controls ($p = 0.929$).	
Lints-Martindale et al.[39] **Australia** **2007** **A psychophysical investigation of the facial action coding system as an index of pain variability among older adults with and without Alzheimer's disease**	Between-group cross-sectional Total N = 49 Total AD = 27 Total Healthy Controls = 22 Electrical: N = 35 AD = 13 Healthy Controls = 22 AD MMSE Mean (SD): 21.2 ± 5.4 Mechanical: N = 28 AD = 14	Stimulation: electrical: electrodes 3 cm apart on retromalleolar ankle (Gorman ProMed), pressure: hydraulically driven pressure on thumbnail Random staircase procedure used to find participant's thresholds for warmth, mild pain, and moderate pain based on intensity scores from the Numeric Rating Scale. Affective pain measured through unpleasantness on the Numeric Rating Scale and the method of constant stimulation. Sessions (except for first administration) video-taped in order to score facial responses using the	Unpleasantness ratings increased with stimulus intensity for both types of stimulation (electrical: $p < 0.001$, mechanical: $p < 0.001$) with no differences between groups. For electrical and mechanical stimulation, FACS frequency and intensity scores increased with stimulus intensity ($p < 0.001$) with no differences between groups. No differences were found between participants with AD and healthy controls for the level of stimulus needed to evoke each percept. FACS frequency higher for electrical stimulation than pressure stimulation in both groups across all pain levels ($p < 0.01$). FACS intensity higher for electrical	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 3 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 3 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 28 out of 36

	Healthy Controls = 14 AD MMSE Mean (SD): 22.8 ± 3.91 The authors explored the differences in psychophysical perception between participants with AD and healthy controls as well as exploring unpleasantness and facial responses to psychophysical stimuli.	Facial Action Coding System (FACS).	stimulation than pressure stimulation in both groups for JNP ($p < 0.001$) and weak pain ($p < 0.05$). No significant correlations were found between FACS and pain unpleasantness scores for either group or stimulus type. While the following results do not give information regarding differences between AD and healthy controls, it does offer information about different stimulus types: For FACS frequency with electrical stimulation, post-hoc comparisons found significant differences ($p < 0.05$) between all stimulus levels (baseline – moderate pain) except JNP and weak pain. For pressure stimulation, post-hoc comparisons found significant differences ($p < 0.01$) of baseline with JNP, weak pain, and moderate pain. For FACS intensity, post-hoc comparisons found significant differences of baseline with JNP, weak pain, and moderate pain ($p < 0.001$) as well as between JNP and moderate pain ($p < 0.05$). For pressure, post-hoc comparisons	

			found significant differences ($p < 0.001$) of baseline with JNP, weak pain, and moderate pain as well as JNP – moderate pain ($p < 0.05$) and weak pain – moderate pain ($p < 0.05$).	
Kunz et al.[45] **2009** **Germany** **Influence of dementia on multiple components of pain**	Between-group cross-sectional N = 81 Dementia/AD = 35 (17 females), 13 AD, 14 Vascular Dementia, 8 Mixed Dementia Healthy Controls = 46 (36 females) Dementia/AD MMSE Mean (SD): 16.4 ± 5.3 This study explored the following 4 responses to pain between participants with dementia and controls: 1) self-report, 2) nociceptive flexion reflex (NFR), 3) facial	Stimulus: electrical stimulation (left calf over the pathway of the sural nerve) Stimuli applied to left calf over sural nerve, recording taken from bicep for the NFR. NFR threshold determined using impulse train with a 20-30 second inter-stimulus interval and an up-down staircase method. 10 stimuli 5 mA above threshold used for recording. Participants rated each stimulus using a 6-point verbal scale for peak stimulus sensation. Facial responses video recorded and scored using the FACS. Autonomic response measured via heart rate and sympathetic skin response.	Participants with dementia gave self-reports of pain that were similar to that of controls; however, those with dementia were less reliable in giving self-report ratings (mean: 79% of stimuli). Ability to self-report decreased with increasing cognitive impairment ($p < 0.001$). No differences were found between the value of self-reported ratings between dementia and healthy controls ($p = 0.146$). Facial responses of pain were significantly increased in participants with dementia compared to controls. Participants with dementia showed decreased NFR thresholds relative to healthy controls ($p \leq 0.001$) but no differences in suprathreshold NFR responses ($p = 0.158$). Participants with dementia showed decreased sympathetic skin response amplitude ($p = 0.006$), with no significant differences in heart rate response ($p = 0.190$). However, the authors did find a	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 4 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 33 out of 36

	responses, and 4) autonomic response		moderate effect size for heart rate response in the 6-9s window post-stimulus, but this did not reach significance (p = 0.067).	
Cole et al.[2] **2011** **Australia** **The impact of Alzheimer's disease on the functional connectivity between brain regions underlying pain perception**	Between-group cross-sectional N = 29 AD = 14 (7 females) Healthy controls = 15 (8 females) AD MMSE mean (SD): 19.4 ± 5.7 Psychophysical aim was to determine thresholds for just noticeable pain (JNP), weak pain (WP), and moderate pain (MP) in response to mechanical pressure in subjects with AD compared to age- and mostly sex-matched healthy controls	Stimulus: mechanical pressure (right thumbnail) Five seconds of pressure applied in a triple random staircase procedure at 20 seconds apart to determine thresholds for just noticeable pain (JNP), weak pain (WP) and moderate pain (MP); subjects rated pain on a 0-20 numerical scale with descriptors	No differences were found in reports of MP between subjects with AD and controls (p=n. s., d = 0.37). However, subjects with AD differed in their pain thresholds compared to controls (p < 0.05, d = 0.93), with AD subjects needing significantly greater stimulus intensity levels to report JNP (p < .01, d = 1.07) and WP (p < .05, d = 0.86) compared to controls.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 3 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 3 7. Findings/results – 4 8. Transferability/ generalizability – 4 9. Implications and usefulness – 4 Overall score: 33 out of 36
Jensen-Dahm et al.[37]	Between-group cross-sectional	Stimuli: heat (Medoc, volar side of the lower left arm), pressure (middle phalanx of	No difference was found in pain thresholds between subjects with	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4

2014a **Denmark** **Quantitative sensory testing and pain tolerance in patients with mild to moderate Alzheimer disease compared to healthy control subjects**	with repeated measures N = 58 AD = 29 (15 females) Healthy controls = 29 (15 females) AD MMSE mean (SD): 22.4 ± 1.18 To estimate test-retest reliability and agreement of three pain stimuli, and then to investigate the effects of mild to moderate AD on pain processing	the left index finger), cold (right hand in cold water) Sequence of pain stimuli: thermal stimulation (5 random stimuli for warmth detection and pain threshold, rated with colored analog scale (CAS)), pressure algometry (3 measurements), cold test (1 submergence) with one hour between each test on day one and repeated on day two.	AD and controls for all 3 tests (heat, pressure, cold). No difference was found in warmth detection between the subjects with AD and controls. Although no difference was found in pain threshold for pressure, pain tolerance was lower for pressure in AD compared to controls. No difference was found in cold pain or cold tolerance between the two groups. Test-retest intraclass correlations (ICC): ICC interpretations - slight/poor (<0.2), fair (>0.2 to 0.4), moderate (>0.4 to 0.6), substantial (>0.6 to 0.8), and almost perfect (>0.8) Warmth detection thresholds (the point at which warmth is detected) AD: 0.72 (same day), 0.45 (separate days), indicating moderate to substantial reproducibility Control: 0.56 (same day), 0.62 (separate days), indicating a moderate reproducibility Heat pain thresholds (the point warmth turns to pain)	2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 3 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 32 out of 36

			AD: 0.70 (same day), 0.50 (separate days), indicating moderate to substantial reproducibility Control: 0.86 (same day), 0.84 (separate days), indicating almost perfect reproducibility Pressure pain thresholds (the point sensation turns to pain) AD: 0.72 (same day), 0.34 (separate days), indicating fair to substantial reproducibility Controls: 0.84 (same day), 0.50 (separate days), indicating moderate to almost perfect reproducibility Pressure pain tolerance (the point pain become intolerable) AD: 0.79 (same day), 0.61 (separate days), indicating substantial reproducibility Controls: 0.79 (same day), 0.46 (separate days), indicating moderate to substantial reproducibility Cold pain threshold AD: 0.73 (same day), 0.32 (separate days), indicating fair to substantial reproducibility Controls: 0.31 (same day), 0.52 (separate days), indicating fair to moderate reproducibility	

			Cold pain tolerance AD: 0.12 (same day), 0.68 (separate days), indicating poor to substantial reproducibility Controls: 0.18 (same day), 0.70 (separate days), indicating poor to substantial reproducibility Results indicate mild to moderate AD subjects are able to understand and cooperate with heat pain and pressure pain testing, as the results were reproducible and demonstrated performance comparable to control subjects. Heat pain had the best test-retest results.	
Jensen-Dahm et al.[41] **2014b** **Denmark** **Stimulus-response function to heat pain in patients with mild-moderate Alzheimer's disease**	Between-group cross-sectional N = 65 AD = 33 Healthy controls = 32 (sex-matched but no report of numbers) AD MMSE range: 16-26 To examine habituation, sensitization, and supra-	Stimulus: heat (unknown) Series of stimuli given for warmth and pain threshold with the participants rating pain on a colored analog scale (CAS)	There were no differences in warmth detection, pain thresholds, or habituation to heat in AD compared to controls. At supra-threshold heat stimulation participants with AD rated pain higher which could indicate increased pain sensitivity.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 1 3. Method and data – 1 4. Sampling – 1 5. Data analysis – 1 6. Ethics and bias – 1 7. Findings/results – 2 8. Transferability/ generalizability – 2 9. Implications and usefulness – 2 Overall score: 15* out of 36 *Poster Presentation report published in the journal Alzheimer's & Dementia

	threshold responses to heat pain stimuli compared to healthy age- and sex-matched controls			
Beach et al.[46] **2015** **USA** **Autonomic, behavioral, and subjective pain responses in Alzheimer's disease**	Between-group cross-sectional N = 71 AD = 38 (28 female) Healthy Controls = 33 (21 female) AD MMSE (Mini Mental State Exam) Mean (SD): 11 ± 9.1 Mild/moderate AD defined as MMSE 18-23 Severe AD defined as ≤ 10 This study explored autonomic, pain behavior, and self-report in AD vs healthy controls as well	Stimulus: pressure (volar surface of distal forearm, Wagner Instruments Force Dial FDK 20 Force Gauge) Stimuli between 1-5 kg applied 4 times each to the left and right forearms using a pseudorandom order (same for all participants). Stimuli held for 5 seconds with an inter-stimulus interval of 50 seconds. Pain behavior was measured through the modified Pain Assessment in Advanced Dementia (mPAINAD) scale. Self-report measures of pain collected using the Faces Pain Scale-Revised (FPR-R, scale consisting of six faces). Autonomic response measured through heart rate.	For the 2-5 kg stimuli, the AD group showed higher mPAINAD scores relative to healthy controls ($p < 0.001$) with no differences between severe AD and mild/moderate AD. At these levels of stimulus, AD patients showed significant differences in all mPAINAD domains (vocalization $p < 0.003$, facial expression $p < 0.005$, bodily response $p < 0.003$). Severe AD patients were unable to reliably give FPR-R ratings. Differences were found between mild/moderate AD and healthy controls in that the mild/moderate AD group had higher FPR-R ratings for 1 kg ($p = 0.003$) and 2 kg ($p = 0.005$) relative to healthy controls. For autonomic responses, there were no differences between AD and healthy controls for heart rate response ($p = 0.64$). Blunted heart rate response in severe AD relative to mild/moderate AD & healthy controls ($p = 0.009$), with no post-	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 4 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 34 out of 36

	as in mild/moderate AD vs severe AD.		hoc comparisons surviving correction at $p < 0.01$. There was a trend towards lower heart rate responses in severe AD relative to mild/moderate AD ($p = 0.016$) and healthy controls ($p = 0.016$).	
Jensen-Dahm et al.[38] **2015** **Denmark** **Discrepancy between stimulus response and tolerance of pain in Alzheimer disease**	Between-group cross-sectional N = 65 AD = 33 (17 females) Healthy controls = 32 (18 females) AD MMSE range: 16-26 To examine stimulus-response function to suprathreshold heat stimuli, habituation and sensitization to heat stimuli, and cold pain tolerance in subjects with AD compared to age- and sex-matched healthy controls	Stimuli: heat (volar side of the lower left arm with Medoc), cold (left hand in cold water) Series of random heat stimuli given for warmth detection, pain threshold, and suprathreshold with the subject rating pain with the colored analog scale (CAS); cold test for pain tolerance with pain rating with the CAS	There was no significant difference in AD compared to controls in warmth detection threshold, heat pain threshold, or habituation to heat pain ($p < 0.05$, means very similar, no SD reported), but subjects with AD rated pain as more intense than controls at suprathreshold levels ($d = 2.78$). Differences were found on the cold test, with only 9 AD subjects compared to 20 controls being able to keep their hand submerged in the cold water (27.3% vs 62.5%, $p = 0.006$).	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 3 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 2 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 27 out of 36
Beach et al.[47]	Between-group cross-sectional	Stimulus: pressure (volar surface of distal forearm,	Participants with AD showed more pain-related facial responses than	Good (4) Fair (3) Poor (2) Very Poor (1)

USA **2016** **Effects of Alzheimer's disease on the facial expression of pain**	N=68 AD = 35 (25 female) Healthy Controls = 33 (21 Female) AD MMSE Mean (SD): 11 ± 9.1 This study aimed to explore the facial response to pressure pain in patients with AD relative to healthy controls in addition to exploring the correlation between facial responses to pain and clinical pain scales.	Wagner Instruments Force Dial FDK 20 Force Gauge) Stimuli between 1-5 kg applied 4 times each to the left and right forearms using a pseudorandom order (same for all participants). Stimuli held for 5 seconds with an inter-stimulus interval of 50 seconds. Sessions were video recorded and facial responses to pain scored using the Facial Action Coding System (FACS). Pain behavior was measured through the modified Pain Assessment in Advanced Dementia (mPAINAD) scale. Self-report measures of pain collected using the Faces Pain Scale-Revised (FPR-R, scale consisting of six faces).	healthy controls as pressure increased ($p < 0.001$). Subsequent testing showed this was significant for all for all levels of pressure between 1-5 kg ($p < 0.01$). Participants with AD also showed a greater increase in pain-related facial responses relative to pain-irrelevant facial responses with increasing levels of pressure. Between 2 and 5 kg of pressure participants with AD showed increased pain behaviors measured by mPAINAD ($p < 0.001$). Participants with AD rated 1 and 2 kg of pressure as more painful relative to healthy controls ($p < 0.01$). FACS scores had a significant direct correlation with mPAINAD scores for both participants with AD and healthy controls ($p < 0.01$), with no significant correlation between FACS and FPR-S scores being noted. FPR-S slopes had a significant direct correlation with mPAINAD scores for both participants with AD and healthy controls ($p < 0.05$).	1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 4 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 33 out of 36
Jensen-Dahm et al.[40] **2016**	Between-group cross-sectional N = 37	Stimuli: heat (dorsum of the left-hand Medoc) Thermal stimulation with 5 random stimuli for warmth	No significant differences were found between AD and controls in their ratings of pain intensity and quality ($p = 0.82$, AD: mean 4.1, confidence interval [2.6–	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims - 4 3. Method and data - 3

Denmark **Contact Heat Evoked Potentials (CHEPs) in Patients with Mild-Moderate Alzheimer's Disease and Matched Control—A Pilot Study**	AD = 20 (8 females) Healthy controls = 17 (7 females) AD MMSE median (IQR): 21.5 (17.5–24.0) Psychophysical aim was to examine painful stimuli using contact heat in AD compared to age- and sex-matched healthy older adult controls	detection and pain threshold, rated with numeric rating scale (NRS), on volar side of left lower forearm was completed the same way as in their previous study "Quantitative sensory testing and pain tolerance in patients with mild to moderate Alzheimer disease compared to healthy control subjects." Multiple stimuli were given with a rest period of 32 to 40 seconds in between each stimulus. Pain intensity and pain quality ratings were obtained for each heat stimulus and rated on the NRS (0-10), or yes or no to pain if they could not understand the NRS (5 out of 20 answered with yes or no).	5.6]; controls: mean 4.3, confidence interval [3.2–5.5]) or for warmth detection or pain threshold.	4. Sampling - 3 5. Data analysis - 4 6. Ethics and bias - 4 7. Findings/results - 3 8. Transferability/ generalizability - 3 9. Implications and usefulness - 3 Overall score: 31 out of 36
Monroe et al.[43] **2016** **USA** **Contact heat sensitivity and reports of unpleasantness in communicative**	Between-groups cross-sectional N = 80 AD = 40 (20 females) Healthy controls = 40 (20 females)	Stimulus: heat (thenar of right hand, Medoc) Series of stimuli given that were perceptually matched to the subject based on their feedback (rather than a fixed temperature paradigm), and subjects reported pain intensity ("how strong the pain feels") and unpleasantness ("how	Compared to controls, subjects with AD needed higher temperatures (greater stimulus) to report detecting warmth ($p = 0.002$, $d = 0.64$), mild pain ($p = 0.016$, $d = 0.51$), and moderate pain ($p = 0.043$, $d = 0.45$), but at any stimulus intensity, there were no statistically significant differences between AD and controls in ratings of unpleasantness ($p = 0.823$, $d = 0.04$-0.18).	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 4 5. Data analysis – 4 6. Ethics and bias – 4 7. Findings/results – 4 8. Transferability/ generalizability – 4

people with mild to moderate cognitive impairment in Alzheimer's disease: A cross-sectional study	AD MMSE median (IQR): 19.5 (14–24) To compare psychophysical responses to heat pain in AD to age- and sex-matched healthy controls	unpleasant or disturbing the pain is for you") on a 0-20 numerical scale for warmth, mild pain, and moderate pain.	Within the AD subjects, lower MMSE scores were not associated with lower ratings of unpleasantness to mild pain ($p = 0.966$, $d = 0.02$) or moderate pain ($p = 0.942$, $d = 0.02$) but were associated with higher temperatures to detect warmth ($p = 0.02$, $d = 0.80$). Also, temperatures for detection and pain unpleasantness were not associated with depressive symptoms ($r = 0.06–0.22$, $p > 0.20$).	9. Implications and usefulness – 4 Overall score: 36 out of 36
Beach et al.[48] **2017** **USA** **Altered behavioral and autonomic pain responses in Alzheimer's disease are associated with dysfunctional affective, self-reflective and salience network connectivity**	Between-group cross-sectional N = 44 AD = 20 (14 females) Healthy controls = 24 (16 females) AD MMSE mean (SD): 15.3 (7.6) To further elucidate neural underpinnings of alterations in pain responses in participants with AD compared to healthy, mostly	Stimulus: mechanical pressure (bilateral volar surface of the distal forearm) No self-report of pain collected. Video recordings of the pressure stimuli testing were obtained to use for an observational measure of pain using the Pain Assessment in Advanced Dementia Scale (PAINAD)	Subjects with AD received greater PAINAD scores than controls, and group differences were found between mean PAINAD scores ($p = 0.004$) and slope of change in PAINAD scores ($p = 0.002$), suggesting subjects with AD had greater pain than controls.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 3 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 32 out of 36

	age- and sex-matched controls			
Cowan et al.[110] **2017** **USA** **Sex differences in the psychophysical response to contact heat in moderate cognitive impairment Alzheimer's disease: A cross-sectional brief report**	Between-group cross-sectional N = 28 AD only (14 females) AD MMSE median (IQR): 16 (12-20) This study aimed to explore sex differences in response to experimental pain in participants with AD.	Stimulus: heat (thenar eminence of right hand, Medoc) Perceptually matched paradigm for the percepts of warmth, mild pain, and moderate pain applied using the Medoc "Method of Limits" with a ramp rate of 1 degree Celsius/second. Participants self-reported unpleasantness following each stimulus application using a 0-20 scale.	Females with AD reported the percepts of mild pain (p = .051, Cohen's d = .72) and moderate pain (p = .036, Cohen's d = 0.80) at lower temperatures than males with AD. Males reported higher unpleasantness rating for the percepts of mild pain (p = 0.072, Cohen's d = 0.82) and moderate pain (p = 0.006, Cohen's d = 1.32) relative to females with AD.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 4 7. Findings/results – 4 8. Transferability/ generalizability – 3 9. Implications and usefulness – 4 Overall score: 34 out of 36
Monroe et al.[3] **2017** **USA** **The Impact of Alzheimer's Disease on the Resting State Functional Connectivity of Brain Regions Modulating Pain: A Cross**	Between-groups cross-sectional N = 46 AD = 23 (13 females) Healthy controls = 23 (13 females) AD MMSE median (IQR): 21 (14–24) To compare	Stimulus: heat (thenar of right hand, Medoc) Series of stimuli given that were perceptually matched to the subject based on their feedback (rather than a fixed temperature paradigm) and subjects reported pain intensity ("how strong the pain feels") and unpleasantness ("how unpleasant or disturbing the pain is for you") on a 0-20 numerical scale for just	Compared to controls, detection levels were less sensitive in subjects with AD for warmth (p = 0.030) and mild pain (p = 0.039) (decreased thermal sensitivity) compared to controls; however, no differences were found in ratings of unpleasantness (p > 0.05). Effect sizes could not be calculated; however, these participants were drawn from the above 2016 study,[43] and it was reported that the findings were similar to that study's findings.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 4 3. Method and data – 4 4. Sampling – 4 5. Data analysis – 3 6. Ethics and bias – 3 7. Findings/results – 3 8. Transferability/ generalizability – 4 9. Implications and usefulness – 4 Overall score: 33 out of 36

Sectional Study	psychophysical responses to heat pain in AD to age- and sex-matched healthy controls	noticeable warmth, mild pain, and moderate pain		

*Portions of this table have been adapted with permission from "A Systematic Review of Experimentally Evoked Pain in Alzheimer's Disease"[109] published in the Journal of the American Association of Nurse Practitioners.

Table 2. Neuroimaging data from experimentally evoked pain in AD

Investigator, Year, Country, Title	Design, Sample, Aim	Neuroimaging Methods	Findings	Scoring[111] for neuroimaging portion
Beach et al.[48] **2017** **USA** **Altered behavioral and autonomic pain responses in Alzheimer's disease are associated with dysfunctional affective, self-reflective and salience network resting-state connectivity**	Between-group cross-sectional N = 44 AD = 20 (14 females) Healthy controls = 24 (16 females) AD MMSE mean (SD): 15.3 (7.6) To further elucidate neural underpinnings of alterations in pain responses in participants with AD compared to healthy mostly age- and sex-matched controls by investigating the relationship between resting state network (RSN) connectivity and differences in pain	Stimulus: mechanical pressure (bilateral volar surface of the distal forearm) No self-report of pain collected. No pain paradigm during scanning. Observational pain scores from a separate day were compared to resting state fMRI scans. Video recordings of the pressure stimuli testing were obtained to use for an observational measure of pain using the Pain Assessment in Advanced Dementia Scale (PAINAD) and heart rate during pressure pain was measured with an infrared monitor. Then in a separate session up to a week later, resting state fMRI scans were obtained that did not include a pain paradigm. During a single 30 minute session, anatomical and resting state scans were obtained then two 7 minute resting state scans were obtained. 14 pain relevant RSNs and 9 autonomic-related RSNs were compared to PAINAD scores.	Participants with AD received greater PAINAD scores than controls and group differences were found between mean PAINAD scores (large effect d = 0.95, p = 0.004) and slope of change in PAINAD scores (large effect d = 1.05, p = 0.002) suggesting participants with AD had greater pain than controls. Out of just the AD participants, those with more severe MMSE scores had reduced heart rate responses during pain (large effect d = 1.18, p = 0.004). RSN functional connectivity group differences associated with greater behavioral reactivity to pressure pain were found within the temporal limbic network (TLN), between the TLN and ventromedial prefrontal cortex, between the default mode network (DMN) subcomponents, and between the DMN and the ventral salience network. This may mean that increased behavioral responsiveness to	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title – 4 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 4 6. Ethics and bias – 3 7. Findings/results – 3 8. Transferability/ generalizability – 3 9. Implications and usefulness – 3 Overall score: 30 out of 36

	and autonomic behaviors		pain implicates dysfunctional prefrontal and temporal limbic affective-behavioral regulation, decreased contextual appraisal for memory, and increased mental activity to pain.	
Cole et al.[1] **2006** **Australia** **Pain sensitivity and fMRI pain-related brain activity in Alzheimer's disease**	Between-groups cross-sectional N = 29 AD = 14 (7 females) Healthy controls = 15 (6 females) AD MMSE mean (SD): 19.4 ± 5.7 To compare mechanical pressure pain-induced BOLD activity in regions of interest (ROI) located in medial and lateral pain pathways in participants with AD compared to age- and mostly sex-matched healthy controls	Stimulus: mechanical pressure (right thumbnail) Psychophysical data obtained during an initial visit, then at the second visit participants underwent fMRI scanning. During the scan participants were exposed to innocuous pressure, weak pain (WP), and moderate pain (MP) based on the previously collected psychophysical data. Stimuli were randomly given in 30 second blocks with 24 second stimulus free periods over three fMRI scans (one session) ROIs included anterior midcingulate cortex (aMCC), insula cortex (IC), somatosensory cortices I (S1) & II (S2), the primary motor cortex (M1), and dorsolateral prefrontal cortex (DLPFC)	Compared to controls, participants with AD needed higher pressure intensities to report JNP, WP, and MD (large effect, $d = 0.93$, $p < 0.05$) but rated JNP (large effect ~3.6, $p < 0.05$) and WP (large effect ~0.88, $p < 0.05$) as more unpleasant than controls. Both participants with AD and controls displayed a common network of pain-induced increased BOLD activity in the medial and lateral regions (aMCC, IC, medial thalamus, S1 and S2). No differences in BOLD signals were found between AD and controls for innocuous pressure. Compared to controls, participants with AD also had greater peak response amplitude in their BOLD signal bilaterally in the DLPFC (no exact stats reported), which may indicate greater attention to pain from participants with AD.	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 2 7. Findings/results – 3 8. Transferability/ generalizability – 4 9. Implications and usefulness – 4 Overall score: 30 out of 36

			For moderate pain (MP) a significant difference was found as the BOLD response was preserved/slower to return to baseline after MP in participants with AD compared to controls in the: aMCC (intermediate effect d = 0.72, p < 0.01), aIC (int. effect d = 0.52, p < 0.05) mid IC (int. effect d = 0.56, p < 0.05), S1 (large effect 0.80, p < 0.005), S2 (int. effect d = 0.78, p < 0.005), left DLPFC (int. effect d = 0.61, p < 0.05), right DLPFC (int. effect d = 0.57, p = n. s.), and M1 (int. effect d = 0.58, p < 0.05).	
Cole et al.[2] **2011** **Australia** **The impact of Alzheimer's disease on the functional connectivity between brain regions underlying pain perception**	Between-groups cross-sectional N = 29 AD = 14 (7 females) Healthy controls = 15 (8 females) AD MMSE mean (SD): 19.4 ± 5.7 To investigate functional connectivity within the brain in response to mechanical	Stimulus: mechanical pressure (right thumbnail) Psychophysical data obtained during an initial visit, then at the second visit participants underwent fMRI scanning. During the scan participants were exposed to innocuous pressure, weak pain (WP), and moderate pain (MP) based on the previously collected psychophysical data. Stimuli were randomly given in 30 second blocks with 24 second stimulus free periods over three fMRI scans (one session)	No differences were found in reports of MP between participants with AD and controls (small effect d = 0.37, p = n. s.). However, participants with AD differed in their pain thresholds compared to controls (large effect d = 0.93, p < 0.05) with AD participants needing significantly greater stimulus intensity levels to report JNP (large effect d = 1.07, p < .01) and WP (large effect d = 0.86, p < .05) compared to controls. Whole-brain functional connectivity with the DLPFC-R revealed that both participants	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 3 3. Method and data – 4 4. Sampling – 3 5. Data analysis – 3 6. Ethics and bias – 3 7. Findings/results – 3 8.Transferability/ generalizability – 4 9. Implications and usefulness – 4 Overall score: 31 out of 36

	pressure pain in participants with AD compared to age- and mostly sex-matched healthy controls	Seed-based functional connectivity analysis was performed at the whole-brain level to identify functionally connected networks of brain regions without a priori selection. Additionally, as a second approach, a priori ROIs were chosen to investigate BOLD signals within known pain regions, and specific connectivity with the DLPFC-R was included because of their 2006 findings[1] ROIs included 17 structures: the anterior mid cingulate cortex (BA24), supplementary motor area (SMA), periaqueductal grey (PAG), medial dorsal thalamus (dmTh), hypothalamus (HyTh), dorsolateral prefrontal cortex (DLPFC-L and DLPFC-R), primary motor cortices (M1-L and M1-R), primary somatosensory cortices (S1-L and S1-R), secondary somatosensory cortices (S2-L and S2-R), anterior insula (aINS-L and aINS-R) and posterior insula (pINS-L and pINS-R)	with AD and controls had significant correlations with activity in the DLPFC-L, ACC, S2-L and S2-R, and aINS-L and aINS-R (no exact stats reported). However, compared to controls, participants with AD had significantly greater functional connectivity between the DLPFC-R and the aMCC (large effect d = 1.43), SMA (large effect d = 1.36), M1 (large effect d = 1.25), PAG (large effect d = 1.45), HyTh (large effect d = 1.60), and thalamus (large effect d = 1.52) (no p-values reported). Compared to controls, inter-regional connectivity of the ROIs revealed that participants with AD had stronger connectivity between the HyTh, PAG and thalamus as well as greater connectivity within the rest of the network (no exact stats reported as used graphs/visuals).	
Monroe et al.[3] **2017** **USA**	Between-groups cross-sectional N = 46 AD = 23 (13 females) Healthy controls = 23 (13 females)	Stimulus: heat (thenar of right hand, Medoc) Psychophysics were collected, then the MRI scanning was obtained. A survey and structural scan was obtained first, then participants underwent a 16 minute functional	Compared to controls, detection levels were less sensitive in participants with AD for warmth ($p = 0.030$) and mild pain ($p = 0.039$) (decreased thermal sensitivity) compared to controls, however, no differences were found in ratings of	Good (4) Fair (3) Poor (2) Very Poor (1) 1. Abstract and title - 4 2. Introduction and aims – 4 3. Method and data – 3 4. Sampling – 4 5. Data analysis – 3 6. Ethics and bias – 3

The impact of Alzheimer's disease on the resting state functional connectivity of brain regions modulating pain: A cross-sectional study	AD MMSE median (IQR): 21 (14–24) To determine the impact of AD, compared to age- and sex-matched healthy controls, on responses to heat pain and resting-state functional connectivity (RSFC) among sensory, affective, descending modulatory, and default mode structures	task paradigm based on the collected psychophysics (that was not analyzed for this publication), then after resting for 1 minute a 5 minute resting state sequence was obtained (which was analyzed for this publication). Four pain networks were chosen consisting of three ROIs each for a ROI-to-ROI approach: Lateral/sensory: S1, S2, and posterior insula (pINS) Medial/affective: ACC, dlPFC, and anterior INS (aINS) Descending pain modulation: PAG, hypothalamus (HYPO), and amygdala (AMY) Default Mode Network (DMN): posterior cingulate cortex (PCC), hippocampus, and precuneus	unpleasantness ($p > 0.05$). Effect sizes could not be calculated, however, these participants were drawn from their 2016 study,[43] and it was reported that the findings were similar to that study's findings. Compared to controls, participants with AD had increased RSFC between the right DLPFC and ACC in the medial pain network ($d=-0.97$, $p=0.0020$), and decreased RSFC between the right pINS and bilateral ACC (L $d = 0.95$, $p = 0.0025$, R $d = 0.79$, $p = 0.0096$) and between the right S2 and the right AMY ($d = 0.81$, $p = 0.0085$). Only one correlation was found between RSFC and psychophysical results. For controls, unpleasantness ratings of moderate pain were associated with greater RSFC between the right dlPFC and the left ACC. This was not found in participants with AD.	7. Findings/results – 3 8. Transferability/ generalizability – 4 9. Implications and usefulness – 4 Overall score: 32 out of 36

Appendix B

Figure 14. All visual SPM12 output for control less than AD PAG activation during warmth

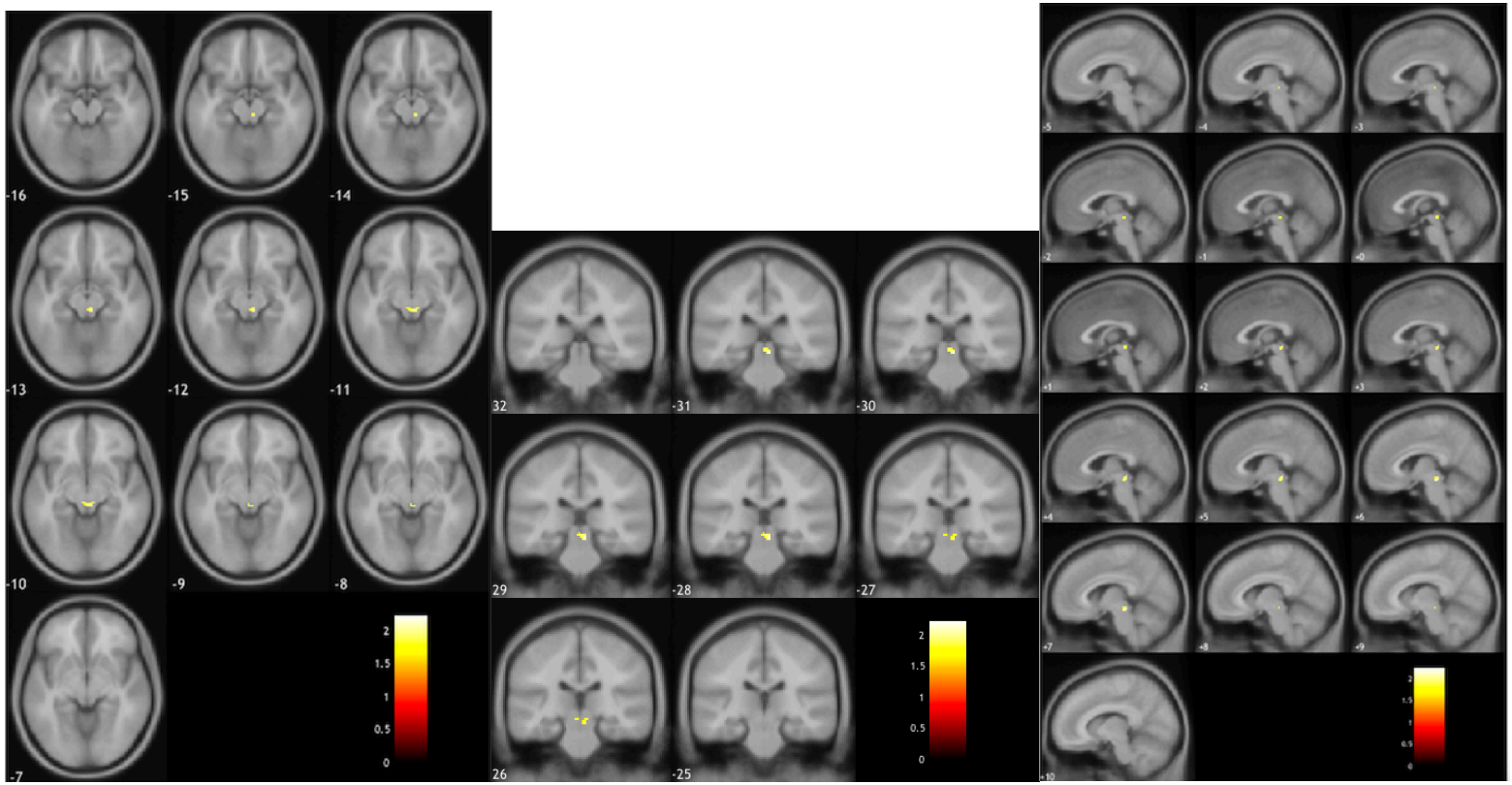
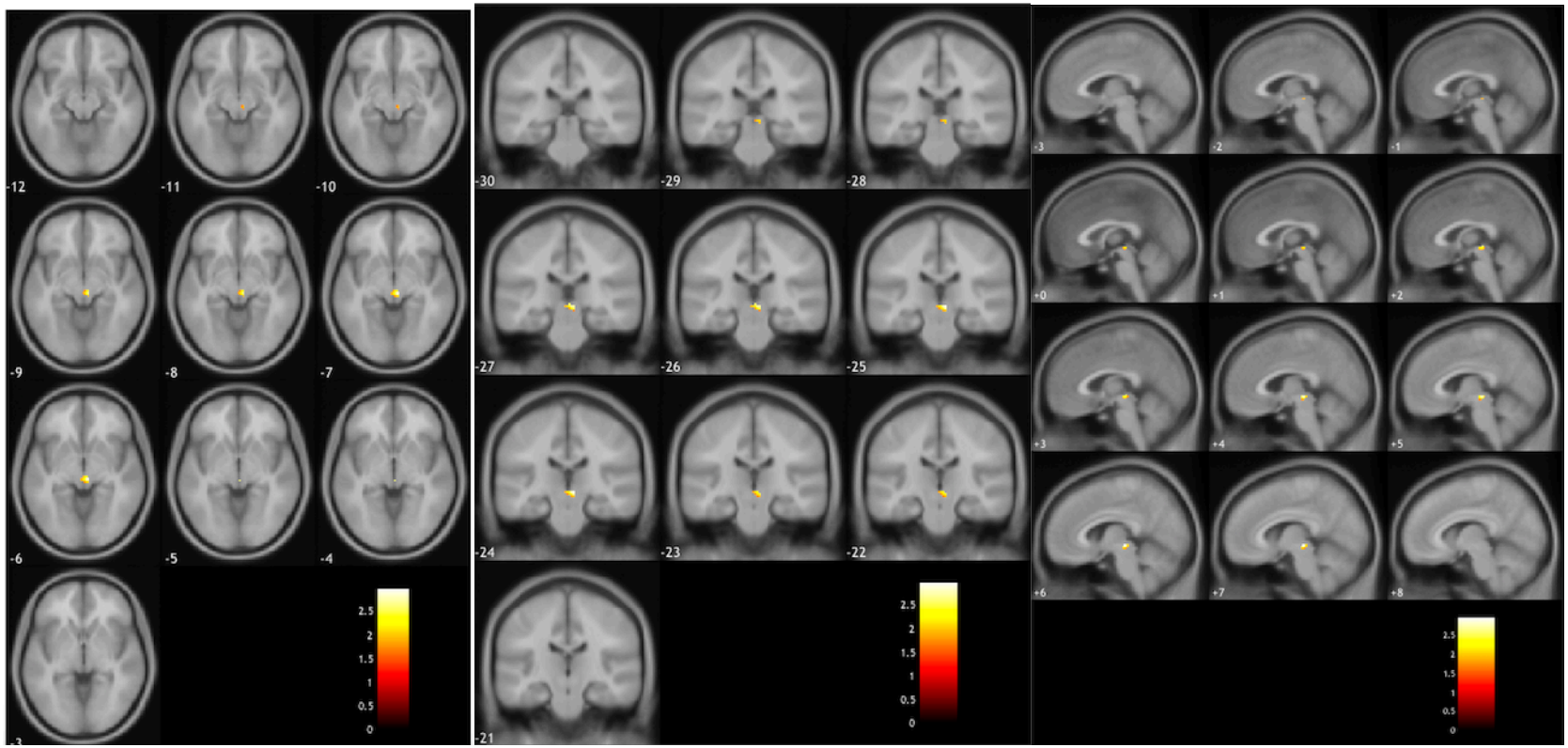


Figure 15. All visual SPM12 output for control less than AD PAG activation during mild pain



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