PAIN IN ALZHEIMER'S DISEASE: A STUDY OF BEHAVIOR AND NEURAL CORRELATES

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ABSTRACT

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Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by insidious and progressive impairment of cognition, emotion, and memory. Though pain in patients with AD is a major medical concern it is under diagnosed and under treated in patients, compared to cognitively healthy elderly. Further complicating matters, subjective self-report of pain by becomes increasingly compromised with disease progression; this often leaves clinicians and caregivers no choice but to rely on discerning pain from behavior alone. Patients also report pain at a lower frequency and intensity than healthy seniors (HS). These findings, coupled with recognition that AD pathology affects many pain processing brain regions, have prompted examination of whether AD alters pain perception. While there is evidence that AD actually predisposes heightened perception of pain, several issues remain: experimental work is limited to a handful of studies, whose results have been inconsistent; few examinations of pain in AD have included patients with advanced disease; the neural mechanism underlying altered pain in AD is not clear. I addressed these gaps in the literature by examining subjective, behavioral, and autonomic pain responses in 33 HS and 38 patients with varying severities of AD. A subset of these subjects (24 HS and 20 AD) were scanned, using fMRI. I then determined how the functional connectivity of various resting-state networks (RSNs) were associated with measured pain responses. I found that AD patients rated low-level stimuli as more painful than HS. Also, patients, regardless of severity, showed greater degrees of pain behaviors than HS – both with respect to global behaviors as measured by a clinical pain scale and facial responses as measured

by an experimental tool. In contrast, autonomic responses were blunted with advancing AD. Altered pain responses in AD were associated with altered function of RSNs involved in attention and internal mentation, affect, somatosensation, and interoception ($p<0.05$, FWE corrected). These findings provide further evidence and an improved understanding of the neural basis for heightened pain sensitivity in patients with AD. They also emphasize the necessity to improve pain assessment and treatment strategies for a vulnerable patient population set to expand greatly in the coming decades.

For mom and my Papa, who set me on this path years ago

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KEY TO ABBREVIATIONS

Alzheimer's disease	AD
Spintothalamic Tract	STT
Medial dorsal thalamic nucleus	MD
Ventromedial posterior thalamic nucleus	VMpo
Ventroposterior lateral thalamic nucleus	VPL
Anterior Cingulate Cortex	ACC
Secondary Somatosensory Cortex	S2
Primary Somatosensory Cortex	S1
Parabrachial Nuclei	PBN
Periaqueductal Gray	PAG
Rostral Ventrolateral Medulla	RVLM
Posterior Insula	pINS
Parietal Operculum	PO
Anterior Insula	aINS
Prefrontal Cortex	PFC
Dorsolateral Prefrontal Cortex	DLPFC
Severe Alzheimer's disease	sAD
Healthy Senior(s)	HS
Mild/Moderate Alzheimer's disease	mAD
Mini-Mental State Examination	MMSE
Clinical Dementia Rating	CDR

Cornell Scale for Depression in Dementia	CSDD
Neuropsychiatric Inventory Questionnaire	NPI-Q
Heart Rate	HR
Pain Assessment in Advanced Dementia	PAINAD
Modified Pain Assessment in Advanced Dementia	mPAINAD
Faces Pain Scale-Revised	FPS-R
Generalized Linear Mixed Modeling	GLMM
Intraclass Correlation Coefficient	ICC
Standardized Test Statistic	STS
Facial Action Coding System	FACS
Action Unit	AU
Functional Magnetic Resonance Imaging	fMRI
Resting-State Functional Magnetic Resonance Imaging	rs-fMRI
Family-Wise Error	FWE
Generalized Linear Modeling	GeLM
fMRI Expert Analysis Tool	FEAT
FMRIB's Software Library	FSL
Multivariate Exploratory Linear Decomposition into Independent Components	MELODIC
Gray Matter	GM
General Linear Model	GLM
Independent Components Analysis	ICA
Group-Level Independent Components Analysis	GICA
Resting-State Networks	RSNs

Threshold Free Cluster Enhancement	TFCE
Pregenual Anterior Cingulate Cortex	pACC
Anterior Default Mode Network	aDMN
Ventral Salience Network	vSN
Posterior Cingulate Cortex	PCC
Ventral Default Mode Network	vDMN
Somatomotor Network	SMN
Retrosplenial Cortex	Rsp
Ventromedial Prefrontal Cortex	vmPFC
Electroencephalogram	EEG
Central Autonomic Network	CAN
Region of Interest	ROI
Seed Correlation Analysis	SCA
Center for Statistical Training and Consulting	CSTAT
Heart Rate Variability	HRV
Middle Cingulate Cortex	MCC
Amygdala	Amyg
Self-Referential Processing	SRP
Minimally Conscious State	MCS
Vegetative State	VS

INTRODUCTION

Acute or chronic pain is one of the most common medical complaints confronting clinicians, afflicting more Americans than diabetes, heart disease, and cancer combined[1]. This is especially true in aging populations, where the prevalence of pain conditions ranges from 25% to 80%, depending on whether seniors are in community dwelling or assisted living situations, respectively[2]. The most common pain syndromes in the elderly are musculoskeletal, neuropathic, and rheumatologic conditions[3]. Unrelieved pain in elderly persons can cause: reduced mobility and decreased lower body strength that can lead to falls; reduced appetite; sleep disturbances; psychiatric disturbances like anxiety, depression, and agitation/disruptive behaviors[2,4]. This increased morbidity makes proper pain assessment and treatment in this population imperative, particularly for the most vulnerable elderly, those with cognitive impairments like Alzheimer's disease (AD).

Patients with AD suffer gradual, but progressive declines in cognition, reasoning skills, and memory. Patients eventually lose functional autonomy and become fully reliant on professional caregivers or heroic family members for care. As the disease progresses, memory, executive function, language, and comprehension abilities deteriorate in kind. Patients may eventually become bedbound, greatly increasing risks of painful conditions, such as pressure ulcers[5].

There are over five million Americans with AD, currently; and this number is set to expand greatly in the coming decades[6]. These patients consume a disproportionate fraction of healthcare resources due to their reliance on caregivers and the duration in which patients live prior to succumbing to AD or other medical conditions (often greater than 10 years)[5,7]. Health maintenance, particularly with respect to pain assessment and treatment, thus becomes essential

for maintaining quality of life. Indeed, there is increasing evidence that proper management of pain in AD or other dementia patients reduces behavioral and psychological symptoms of dementia[8–10]. Unfortunately, there is evidence suggesting that pain is under assessed and treated in patients with AD or other dementias[11–15]. This makes an understanding of pain and its assessment in these patients critical.

Pain and its multiple dimensions

Currently, pain is understood to be both a discriminative sensory experience and a behavioral motivator, making it a unique amalgamation of sensation and emotion. Indeed, the International Association for the Study of Pain defines it as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[16]. This definition illustrates the uniqueness of pain for the human condition, in that the experience does not require an actual noxious stimulus or actual tissue damage. This quality is found in those with phantom-limb or post-stroke pain. Further, use of the term '*experience'* within the definition demonstrates that pain is a subjective and conscious percept.

The experience of pain has been conceptualized to have distinct functional components, or dimensions, described by Melzack and Casey as sensory, affective-motivational, and cognitive[17]. The sensory dimension entails the location, duration, and intensity of a painful stimulus; it is also associated with descriptors of the *type* of pain one is experiencing (burning, stinging, sharp, etc.). The affective-motivational dimension encapsulates the aversive, or unpleasant aspect of pain and the desire to escape it, or its inciting event. This dimension also encompasses emotional feelings directed toward the implications of experiencing pain, i.e. suffering[18,19]. Finally, the cognitive dimension consists of a control system that interacts with the previous two dimensions. Specifically, one's beliefs, attitudes, and past painful experiences

shape the evaluation of and strategies for modulating or escaping from a painful stimulus. Therefore, the cognitive dimension exerts some regulatory authority over the degree of intensity and affect felt during a painful experience. Conceptually, the sensory, affective, and cognitive dimensions converge to create the global pain experience. However, putting this psychological conceptualization into the context of neural processing requires an understanding of nociception, the neuronal encoding of noxious stimuli.

Pain processing

Nociception begins peripherally with small diameter afferents responding to a noxious mechanical, thermal, electrical, or chemical stimulus. These fibers (slow, unmyelinated C-fibers and faster, sparsely myelinated Aδ fibers) transmit information about the physiological status of their innervating tissues to nociceptive specific and wide-dynamic-range neurons in the superficial and deep laminae of the dorsal horn of the spinal cord (laminae I & V-VII) or to the bulbar trigeminal spinal nuclei[19,20]. From here, contralateral ascending projections of the spinothalamic tract (STT) further transmit nociceptive information to higher levels of the CNS. Lamina I contributes heavily to the lateral STT, which, when joined by the trigeminothalamic tract, projects to medial dorsal (MD), ventromedial posterior (VMpo), and ventroposterior lateral (VPL) thalamic nuclei, which in

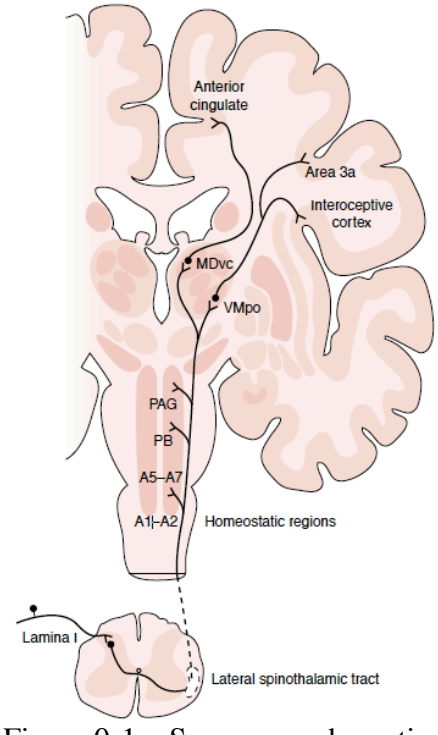

Figure 0-1 – Summary schematic of the primary lamina I-based tracts in the anterolateral spinal cord, brainstem autonomic nuclei, thalamus, and cortex. Adapted from[20].

turn project to anterior cingulate (ACC), interoceptive[1] cortex (dorsal insula and Brodmann area 3a, aka secondary somatosensory cortex (S2)), and primary somatosensory cortex (S1) respectively. Branches of ascending lamina I fibers also project to sympathetic cell columns of the thoracolumbar spinal cord as well as autonomic hubs within the brainstem (catecholamine cell groups, parabrachial nuclei (PBN), periaqueductal gray (PAG), and rostral ventrolateral medulla (RVLM)) as part of the spinomesencephalic tract. These brainstem hubs also receive input from the solitary nucleus (a hub for cranial nerve and autonomic-related input) and help integrate autonomic responses through communication with the hypothalamus, amygdala, and insula. A summary of the major paths and destinations of lamina I can be found in Figure 0-1. Laminae V-VII ascend as part of the anterior spinothalamic tract before sending projections to the cerebellum as well as the reticular nuclei (spinoreticular tract) to provide proprioceptive context and to increase arousal, respectively[19,20].

Relating the three-dimensional functional conceptualization of pain (sensory, affective-motivational, and cognitive) to brain structures has lead researchers to divide much of the supraspinal anatomy described above into lateral and medial systems. The major cortical structures for these two systems are visualized in Figure 0-2. The lateral system consists of lateral STT projections from lateral thalamic nuclei to S1/S2, the posterior insula (pINS), and the parietal operculum (PO)[19,21]. This system is thought to mediate the *sensory* dimension of pain[19]. The medial system consists of ascending spinoreticular, spinomesencephalic, and lateral STTs that are relayed from brainstem nuclei (PBN and PAG) and MD/VMPo thalamic nuclei to PO,

[1] *Interoception* is the process by which one becomes aware of the internal physiological status of one's own body. Thus, *interoceptive cortex* provide a cortical image of this physiological status. *Interoceptive stimuli* thus provoke interoception.

anterior insula (aIns), and ACC[22–25]. The medial system is responsible for the *affective* dimension of pain, but also overlaps with the *cognitive* dimension.

Many components of the medial pain system are also part of the central autonomic network; PAG, hypothalamus, amygdala, and cingulate, insular, and orbitofrontal cortices all contribute to pain-autonomic interactions and responses[26,27]. Considering this high degree of anatomical overlap, it us not surprising that increased sympathetic drive is typically higher correlated with subjective pain[28,29]. Autonomic responses to noxious stimuli are thus thought to be a potential indicator to pain *affect*[27]. The insula, ACC, amygdala, and orbitofrontal cortex orchestrate these autonomic responses as part of the overall affective and cognitive processing of noxious stimuli[26,27,30,31].

The ACC is crucial for attending to and evaluating noxious stimuli, making it a key component of pain *affect* as well as the *cognitive* dimension of pain[18,19,32]. The prefrontal cortex (PFC), also part of the medial system, contributes to the *cognitive* dimension[33,34]. PFC is thought to aid in anticipation of the unpleasantness of the pain experience as well as modulation of pain affect[34,35].

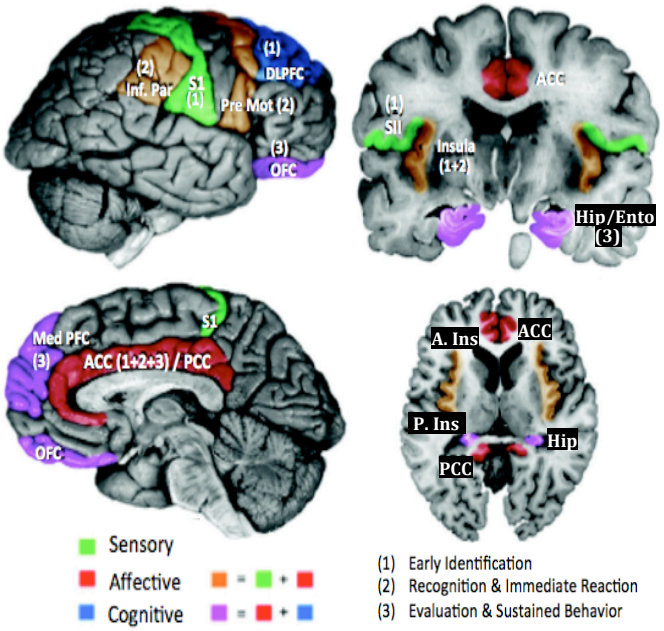

Figure 0-2 – Several brain regions involved with pain processing. Color-coding reflects the hypothesized role of each area in processing different components of pain. Numbers in parentheses indicate the relative involvement of these areas during different stages of the pain experience. Areas highlighted of import for this proposal include anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), primary somatosensory cortex (SI), secondary somatosensory cortex (SII), dorsolateral prefrontal cortex (DLPFC), medial prefrontal cortex (Med. PFC), posterior insula (P. Ins), anterior insula (A. Ins), hippocampus (Hip).: **Adapted from Creative Commons:** Borsook et al. Molecular Pain 2007

Memory related to past painful experiences allows for appraisal of an event by giving it context, making it a key component of the *cognitive* dimension[19]. Pain memories are mediated through a relay consisting of memory systems and the medial pain system. Connections between the PBN and Amyg may be the first step in retaining nociceptive information[24]. From here, projections from medial temporal lobe structures (amgydala and hippocampus) activate S2, PO, and aIns, invoking memories of painful experiences[33]. Posterior parietal cortex collates sensory input for threat evaluation[19], while ACC and PFC are thought to be involved with evaluating possible consequences of pain[18].

The above regions involved in pain processing, particularly affective processing, are highly interactive with structures that modulate pain. Pain modulatory regions include cortical, subcortical, and brainstem structures. The dorsolateral prefrontal cortex (DLPFC - Brodmann areas 9 and 46 - Figure 0-2), is a cortical region involved in pain modulation. Increased DLPFC activity, in one study[35], was associated with reduced medial thalamic-midbrain activity. Further, activity in the DLPFC correlated with a weaker relationship between aINS activity and pain affect/intensity. Given these results, authors posited that the DLPFC is involved in protecting momentary behavioral goals during the pain experience by reducing cortical pain processing (through the aIns) and increasing subcortical pain-inhibitory mechanisms. Primary motor cortex activity may also modulate pain through similar thalamic interactions[36–38]. Subcortical structures are also are involved in pain modulation. Animal studies show that the striatum receives nociceptive and non-nociceptive sensory information from ascending afferents. Further, the striatum is activated during pain testing of human subjects[39,40]. One possible mechanism for striatum-based pain modulation includes D-2 based striatopalladal projections to the brainstem,

particularly to the RVLM, leading to opioid-based inhibitory input to spinal nociceptive activity, and thus reduced pain[41].

There is an extensive relationship between the PAG, a midbrain structure, and pain[42]. Through afferent and efferent spinal projections, the PAG is *highly* connected to cortical, subcortical, and brainstem regions involved in pain processing. The PAG is activated by noxious stimuli, and is somatotopically organized by ascending nociceptive inputs[43]. In receiving input from cortical, subcortical, and brainstem pain regions (ACC, insula, amygdala, prefrontal cortex, RVLM), the PAG exerts both excitatory and inhibitory control over dorsal horn and trigeminal nociceptive activity[43]. In particular, C-fiber input is typically inhibited while Aδ fiber input may be enhanced[44,45]. The PAG also is involved in autonomic responses to noxious stimuli[46]. PAG output, primarily through the RVLM, may lead to either opioid analgesia and sympathodepression or non-opioid analgesia and sympathoexcitation, dependent on whether noxious stimuli are deep-somatic/visceral/repetitive-superficial or short-lasting, respectively[44,47,48]. Aside from being a critical pain modulatory center, the PAG plays a large role in behavioral responses to noxious stimuli, such as vocalizations and fight or flight strategy (reviewed in[42]).

Pain in Alzheimer's disease

Pain is a subjective experience. Clinically, this makes self-report the gold standard for its assessment. However, language use is compromised by AD. Thus, self-report of pain is increasingly unreliable as the disease progresses; it is often not feasible in severe AD (sAD)[49,50]. Loss of self-report requires clinicians to use observational scales to assess pain. However, making inferences about a subjective experience based only on behavior is problematic and may contribute to the aforementioned reduction in analgesic dispensation in AD/dementia patients,

compared to cognitively intact elderly. Reduced analgesia in AD was initially proposed to be a direct result of unreliable self-report. After all, AD patients were found to report pain less frequently and at a lower intensity than non-demented seniors[51]. However, a recent study on the amount of pain medications given to AD patients vs. HS in assisted living conditions found that, whereas both groups received equal amounts for acute pain, AD patients received fewer analgesics for chronic pains[52]. This suggests an interesting possibility: that signs of reduced pain in AD patients could result from AD altering the various aspects of pain, such as its *affective* or *cognitive* dimensions, which in turn would lead to altered subjective and behavioral manifestations of pain in patients[11,21,53].

Imaging[54–56] and pathological studies[57–60] have confirmed that AD preferentially targets medial pain structures. In contrast, lateral pain structures are relatively well preserved. These considerations would seem to portend reduced pain-related affect, but preserved sensory function in AD[11,21]. Experimental work has partially confirmed this hypothesis. Multiple studies have found no difference between AD and HS subjects for pain threshold[61–64]. In contrast, one early study found increased pain tolerance in AD patients, relative to HS. Further, several studies have found that autonomic responses are blunted in patients with more advanced AD[62,65–67]. Because tolerance and autonomic response are associated with pain afect[19,68,69], it was initially suggested medial pain function (and by extension pain affect) is reduced in AD patients[11,62,66]. However, some have questioned initial pain tolerance results as the self-report measures required may have been too complicated for AD patients[64,70]. Studies incorporating more simplistic pain ratings scales or quantitative sensory testing have found similar[70,71] to increased[67,72] pain ratings or reduced pain tolerance[64] in AD patients, compared to controls. Consistent with these findings, AD patients of primarily mild/moderate severity showed increased levels of pain behaviors (e.g.

facial expressions), compared to controls[65,70,71]. CNS activation during noxious stimulation was also preserved in patients[63] compared to controls in one study. In an fMRI study by Cole and colleagues[72], activation in medial pain structures was increased, relative to HS. These findings suggest that, though autonomic responses are increasingly blunted as AD worsens, aspects of pain processing are actually increased in AD patients, compared to HS. What changes in patient brain function may be responsible for this result?

Cole and colleagues, in an extension of their initial activation study, examined temporal synchronicity (i.e. functional connectivity) of canonical pain processing structures during pain-induced activation. They found increased connectivity in AD patients, relative to HS, centered around the hypothalamus, PAG, DLPFC, and sensorimotor cortices. These and original activation results were interpreted as representing increased vigilance toward noxious stimuli in AD patients, compared to HS, owing to impaired appraisal of their threat level. Thus, a neural mechanism for indications of altered pain perception in AD may involve altered dynamics between pain-related affective, memory, and cognitive processes.

This interpretation is supported by in rodent models, which also suggest that impairments of memory-pain context lead to altered pain responses. One study, whereby scopolamine and ketamine were utilized to impair the cholinergic/NMDA system in mice, showed that hotplate reactivity was significantly affected compared to saline injected animals[73]. Specifically, over several days of testing, drug-injected animals did not show decreased latency to react to the hotplate, suggesting that impairment of the memory-pain learning process may be responsible for the appearance of altered pain affect. Rodent studies also provide support for findings in human research. For example, in a one transgenic AD mouse model pain threshold was unchanged[74]. Further, Tau+/+ mice, compared to Tau null mice, show reduced latency to paw licking during

heat pain, as well as increased paw jerks and time of pain-related activity during formalin testing[75].

A majority of findings thus suggest that pain processing is increased in AD patients, and that patients are thus more sensitive pain. However, conclusions about how pain processing is altered in AD are limited for several reasons. First, few sAD patients have been included in behavioral pain studies, and none have been included in neuroimaging studies of pain. This leaves open the question of how AD progression may affect pain. Second, studies examining observable pain behaviors (e.g. facial expressions) have primarily taken place in the context of mixed populations of dementia patients[70,71]. This is problematic because not all dementias target the same brain regions affected by AD[21]. Third, a comprehensive understanding of pain requires integration of behavioral and functional correlates, particularly when examining cognitively and communication-impaired population. Thus examining the relationship between various pain responses (subjective, behavioral, and autonomic) and functional integrity of pain-related networks in the brain would place altered pain responses of patients within the context of AD neuropathology; conclusions about a relevant neural mechanism would thus be better substantiated. However, no neuroimaging studies thus far have related behavioral, subjective, and autonomic pain responses in AD to neuroimaging correlates.

The contents of this dissertation represent an effort to address these gaps in our understanding of how AD alters pain. First, in Chapter 1, autonomic, subjective, and behavioral pain responses in AD patients of various severities were characterized, in comparison with HS. Chapter 2, in an extension of the first, presents an examination of pain-related facial expressions took place; an emphasis was also given to comparing experimental facial analysis to clinical pain scales. Chapter 3 describes results from scanning a subset of subjects with resting-state fMRI to

examine how differences in resting brain function between groups was associated with measured pain responses. Chapter 4 discusses, first various methods utilized throughout the study that failed to provide solid results, second, why they likely failed, third what could changes have been made to improve overall study design. Finally, general study conclusions and future directions are discussed.

CHAPTER 1

AUTONOMIC, BEHAVIORAL, AND SUBJECTIVE PAIN RESPONSES IN ALZHEIMER'S DISEASE

INTRODUCTION

Reliable detection and treatment of pain in elderly persons with Alzheimer's disease (AD) is an important means of improving quality of life and reducing behavioral and psychological symptoms of dementia[76–78]. However, under-treatment of pain in AD patients is common[12,14,15]. AD patients are often impaired in their ability to provide reliable subjective pain ratings[65,70], particularly as the disease progresses, and they report pain less frequently and at a lower intensity than healthy seniors (HS) in clinical settings. These findings, coupled with recognition that AD pathology affects many pain processing brain regions[21] have prompted examination of whether AD alters pain perception.

Results of studies testing pain threshold and tolerance in AD patients, which requires subjective pain ratings, are mixed. A consistent result has been a lack of change in acute pain threshold of AD patients[61,63,6413–15][12–14], indicating AD leaves sensory aspects (intensity/localization) of pain intact. Pain tolerance or unpleasantness findings, thought to reflect pain affect[18], have been inconsistent. For example, Benedetti and colleagues[61] found increased tolerance to electric and ischemic pain; however, Jensen-Dahm et al. and Cole et al.[72] found decreased tolerance and increased unpleasantness, respectively, to pressure pain. It has been proposed that these mixed results are in part due to impairments in pain self-report and comprehension of self-report scales[64].

This variance in the literature highlights the necessity of including non-verbal indicators, such as autonomic responses and pain behaviors, together with self-report when conducting

assessments of pain in AD patients[80], particularly those with severe AD (sAD). Prior studies indicate that, while pain-related autonomic responses tend to decline as AD progresses[62,66], pain behaviors, such as facial expressions and guarding[65,70,71,81], are generally increased in patients relative to HS. The effect of sAD on pain is unclear as few experimental studies have included sAD subjects, though reduced affect, behavior, and sensation have been hypothesized[53]. However, there is evidence of sAD patients exhibiting increased pain behavior and intact pain processing through late disease stages[63,82]. These findings suggest that severely demented patients may be more vulnerable to under-detection and under-treatment of pain[83,84].

AD progressively affects brain structures associated with different aspects of pain (e.g. affective, cognitive, and sensory)[21]. Limbic structures are affected early by AD, likely causing changes in pain affect and memory[21]. Functional brain networks associated with pain affect/motivation and cognition, such as the salience and default mode networks, become increasingly disconnected as AD progresses[85,86]. Finally, sensory cortices, associated with processing pain's intensity and localization aspects[19], are affected at late stages of AD[57]. Loss of brain structural integrity supports the premise that clinical indicators of pain may vary based on disease severity[50]. However, experimental studies further characterizing pain indicators in sAD and the modulating effects of dementia severity are sparse.

To address this gap in the literature, the primary aim of this study was to examine multiple acute pain responses (autonomic, pain behaviors, and potential self-report) in mild/moderate (mAD) and sAD patients during repeated application of multiple forearm pressure intensities. Mechanical pressure algometry was the applied modality as it has been utilized in multiple studies of pain in the elderly with and without dementia[64,70,71,87]. In conjunction with

testing for differences between AD patients and HS, the secondary aim was to determine if any pain responses varied according to AD severity.

METHODS

Subjects

General subject demographics are found in Table 1-1. Thirty-eight patients with chart-confirmed DSM-IV diagnoses of probable AD (28 ♀) and thirty-three HS controls (21 ♀) participated. Ages of HS subjects were 74.4±6.6 (mean ± SD) years while AD patients were 79.5±8.9 years. Clinical Dementia Rating (CDR)[88] and Mini-Mental State Examination (MMSE) scores[89] separated groups, cognitively (HS: MMSE 26-30, CDR 0; AD: MMSE≤23, CDR 0.5–3). HS subjects were required to have no history of subjective memory complaints. Mean HS MMSE was 29±1.1. Mean AD MMSE was 11±9.1.

HS were recruited via ads in newsletters and local AD support groups. AD patients were recruited through local nursing facilities and the Michigan State University Department of Neurology & Ophthalmology's Cognitive & Geriatric Neurology Clinic. Subjects were required to abstain from all analgesic medication for 24 hours prior to testing if deemed unlikely to have baseline pain from analgesic abstinence (via chart review or caregiver discussion). Subjects receiving beta-blocker medications were allowed if their primary physician agreed to temporarily discontinue drug treatment for a period equal to three half-lives prior to study. Further exclusions included history of: Type II diabetes, major depression, history of stroke or transient ischemic attack, central or peripheral neuropathy, diagnosis of neurological or psychiatric disorders other than AD. We excluded those with current arthritic pain, those with arthritis in the stimulus application region (distal forearms), and those requiring daily analgesics for arthritis. Care was

taken to exclude patients with probable mixed dementia through chart review of prior brain imaging and/or clinical evidence of vascular, frontotemporal, or Lewy body dementia.

Table 1-1 Average subject demographics (+/-) standard deviation

	Healthy seniors (n=33)	Alzheimer's disease (n=38)
Age (years)	74.4 (+/-) 6.6	79.5 (+/-) 9.9
Sex (F | M)	21 | 12	28 | 10
Mini Mental State Examination (MMSE)**	29 (+/-) 1.1	12.4 (+/-) 9.0
Clinical Dementia Rating (CDR)**	0.0 (+/-) 0.0	2.1 (+/-) 1.1
Cornell Scale for Depression in Dementia (CSDD)**	1.1 (+/-) 1.2	7.3 (+/-) 4.5
Neuropsychiatric Inventory Questionnaire (NPI-Q)**	0.52 (+/-) 0.09	7.2 (+/-) 0.92
Severity (mild vs. severe)	- - -	17 | 21

MMSE ranges: normal scores 26-30; AD: ≤23. CDR normal score: 0. CSDD normal range: 0-12. Mild/moderate Alzheimer's range: MMSE 11-23, CDR 0.5-2. Severe Alzheimer's range: MMSE ≤10, CDR 3.
**$p<0.001$; $p<0.01$ considered significant after Bonferroni correction for multiple comparisons.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Michigan State University Internal Review Board. Written informed consent was obtained for all HS as well as for AD subjects via named health care proxies identified as a power of attorney for health care or guardian. We obtained assent from all participants (verbal or non-verbal) before beginning testing. Testing was discontinued if any subjects became inconsolably agitated or verbally stated that they wished to end participation. This occurred with one AD subject, who was excluded from analysis.

Materials and procedure

Testing occurred between 1-5:00pm for 1-1.5h. The protocol began with MMSE/CDR testing and completion of the Cornell Scale for Depression in Dementia (CSDD) for AD subjects[90]. Any individuals with CSDD >12, indicative of probable depression[90], were excluded. Behavioral and psychological symptoms were further probed via the Neuropsychiatric Inventory Questionnaire (NPI-Q)[91]. Pressure testing occurred last.

Pressure stimuli were applied using a Force Dial FDK 20 Force Gauge (Wagner Instruments, Greenwich, CT), which allows accurate recording of pressure (kg/cm^2). Here integer pressures shall be referred to in units of 'kg' as per device scaling. The instrument is fitted with a 1cm wide rubber disk to prevent skin abrasion. Pressures were applied to the lateral volar surface of the distal forearm, 2-5cm from the wrist. Subjects were seated, upright, during testing. Because standardization of pain levels is not feasible in sAD subjects, pressure application was adapted from a previous dementia-related pain study[71]. Twenty pseudorandomized stimuli of 1-5kg intensity were applied to left and right forearms (four stimuli per intensity). Stimulus order was determined once for use in all subjects. No intensity occurred more than twice, sequentially, nor was any intensity repeated on the same arm twice in sequence. Subjects were first familiarized to the stimuli via single application of each intensity to the thigh. Pressure application occurred at a rate of ~1kg/s to peak intensity, which was held for 5s. Interstimulus intervals were ~50s. Video recording allowed for scoring of behavioral responses and heart rate (HR) changes. Two trained investigators performed all testing in a standardized manner (PAB, MM).

Behavioral acute pain responses were scored using portions of the Pain Assessment in Advanced Dementia (PAINAD) scale, a validated observational scale for assessing acute pain in

demented patients[80,92,93]. The full version of the PAINAD assesses breathing, negative vocalization, facial expression, body language, and consolability. Each domain is scored 0-2 for a maximum score of 10 points. Several studies have questioned the utility of breathing and consolability due to low internal consistency or low probability of indicating pain[94]. For purposes of this experimental study, breathing and consolability were not incorporated, yielding a maximum modified PAINAD (mPAINAD) score of 6. Scoring was aided by descriptors provided as part of the PAINAD[94]. Two trained PAINAD raters (PAB, MM) initially scored half of the sessions. However, as they were not blinded to stimulus order or group designation a third rater (JH) was added who was. This rater re-scored all original sessions and those remaining. Final mPAINAD scores for double-scored subjects were determined through rater consensus. Doubly scored sessions were used as part of rater reliability testing.

Immediately after stimulus completion (<5s) subjects provided subjective pain ratings with the Faces Pain Scale-Revised (FPS-R)[95]. The FPS-R consists of six faces corresponding to feeling no pain to 'very much pain.' Each face after the initial 'zero' face represents a two-point stepwise increase, creating a 0-10 numerical match-up. The FPS-R has been shown to be reliable in assessing pain in cognitively impaired patients, including those with MMSE scores<11[84,96]. However, AD subjects had to pass a 3-question quiz as part of the FPS-R to be deemed a reliable informant[97].

Autonomic responses were monitored by way of HR. A portable infrared monitor (ePulse2^{TM}–Impact Sports Technologies) displayed HR throughout testing. A response was determined by subtracting the HR at stimulus onset (baseline) from the maximum response within 30s after offset, resulting in an overall positive or negative response. Interstimulus intervals allowed for return to resting HR.

Statistical analysis

Because mPAINAD and FPS-R data distributions were non-normal they were recoded for purposes of statistical modeling. mPAINAD scores were recoded by clustering scores: '0', '1-2', '3-4', and '5-6'. FPS-R scores were recoded by clustering scores: '0', '1-3', '4-6', '7-9', and '10'.

Rater reliability testing of mPAINAD scores occurred three ways. First, average absolute agreement intraclass correlation coefficients (ICC) were calculated for those subjects whose data were scored by two raters (N=36) to determine inter-rater reliability. ICC was calculated for overall mPAINAD as well as individual domains and the overall average ICC was calculated from those scores. Second, for subjects scored by a single rater (N=35), a randomly selected 15% were re-scored by the single rater who was blinded to stimulus order, group designation, and original scores prior to calculation of ICC as above. Third, internal consistency of all subject scores over repeated applications of each intensity was determined by calculating Crohnbach's Alpha.

Generalized linear mixed modeling (GLMM) in SPSS™ (Version 22.0, Armonk, NY: IBM Corp) determined impact of level-two effects (subject group – HS and AD) on level-one effects (mPAINAD/FPS-R scores and HR changes), with subject and stimulus intensity as predictors. GLMM accounts for repeated measures (trials) and nuisance covariates (age, gender, and CSDD, NPI-Q severity score, stimulus applicant). Significant 'group' or 'group*stimulus intensity' interaction effects ($p<0.05$) were followed by post-hoc nonparametric Kruskal-Wallis testing between groups under each stimulus intensity for mPAINAD and FPS-R scores. To control for family-wise error, post-hoc results were considered significant if they met a correct

Bonferroni correction threshold of $p<0.01$. HR data were normally distributed, allowing GLMM to perform post-hoc analysis and Bonferroni correction.

Previous studies of pain in AD indicated increased pain-specific facial expressions, compared to controls[65,70]. We attempted to extend this finding by examining whether groups differentially utilized mPAINAD domains (verbal, facial, and body) to behaviorally express pain. Individual mPAINAD scores were dissected for domain-specific percentages. Domain percentages were recoded into clusters of: '0', '≤25%', '26-50%', '51-75%', and '>75%.' GLMM and subsequent post-hoc testing, described above, were then utilized.

To probe potential AD severity-dependent effects a secondary analysis was performed whereby AD patients were split into subgroups: mAD (CDR 0.5-2; MMSE 11-23; 17 subjects) and sAD (CDR 3; MMSE≤10; 21 subjects). A GLMM, incorporating level-one effects and nuisance covariates described above, was performed to distinguish mAD and sAD subgroups (level two effects) as well as HS. Significant effects were further investigated with appropriate post-hoc testing, described above. Family-wise error was controlled for as described above.

RESULTS

General subject demographics are found in Table 1-1. AD subjects were somewhat older, on average, than HS, though this failed to meet corrected significance threshold ($p=0.02$). AD subjects scored higher on the NPI-Q and CSDD ($p<0.01$). However, no subjects had CSDD scores indicating clinical depression (>12).

ICC for rater reliability testing was calculated at 0.93 (+/- 0.04, SD, $p<0.005$), suggesting strong agreement between raters. Intra-rater ICC calculation between original and a re-scored subjects' mPAINAD scores was 0.86 (SD: 0.08, $p<0.005$), indicative of strong intra-rater reliability. Both inter and intra-rater ICC results are consistent with prior studies of PAINAD

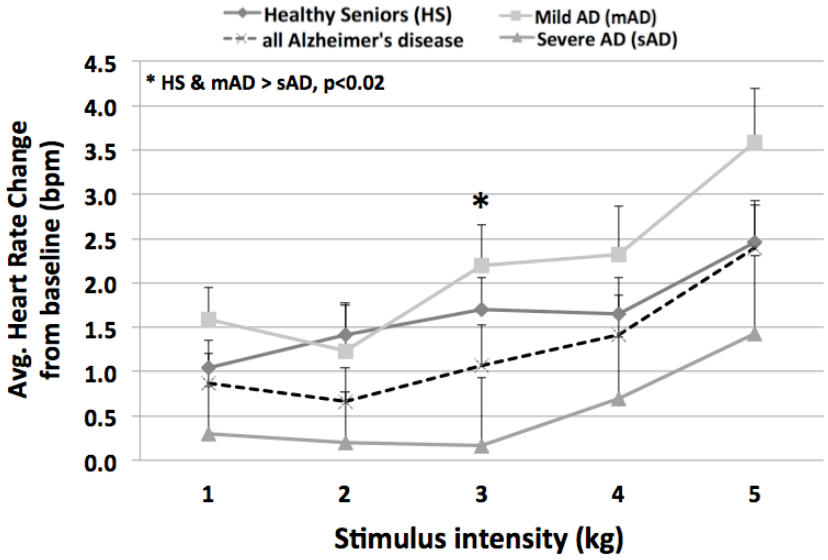

Figure 1-1 - Average heart rate changes from baseline (beats per minute, bpm) across stimulus intensities (kilograms, kg). Error bars represent standard error of the mean (SEM). $p<0.01$ considered significant after Bonferroni correction for multiple comparisons.

consistency[94]. Crohnbach's Alpha testing yielded an overall average score of 0.84 indicating a high level of internal consistency for subject mPAINAD scores over repeated trials.

There were no significant main effects comparing HR responses of HS and AD, (group: $F=0.21$, $p=0.64$ | group*stimulus intensity: $F=1.74$, $p=0.093$). Figure 1-1 shows a plot of average HR responses for HS, AD, and AD subgroups (mAD/sAD) for each stimulus intensity. Secondary GLMM testing confirmed a severity-dependent effect for HR: sAD responses were, in general, diminished compared to HS and mAD ($F=4.7$, $p=0.009$). No post-hoc comparisons met Bonferroni correction threshold of $p<0.01$. However, sAD subjects tended to have reduced responses compared to mAD at 3kg ($t=-2.6$, $p=0.016$;) and HS at 3kg ($t=-2.8$, $p=0.016$ &). No significant differences between mAD and HS were found ($t<1.1$, $p>0.2$).

GLMM testing of mPAINAD data yielded significant effects of group and group*stimulus intensity ($F=34.4$, $p<0.001$; $F=270.6$, $p<0.001$, respectively). Figure 1-2 shows average mPAINAD scores (non-recoded) for HS and AD. Kruskal-Wallis post-hoc testing

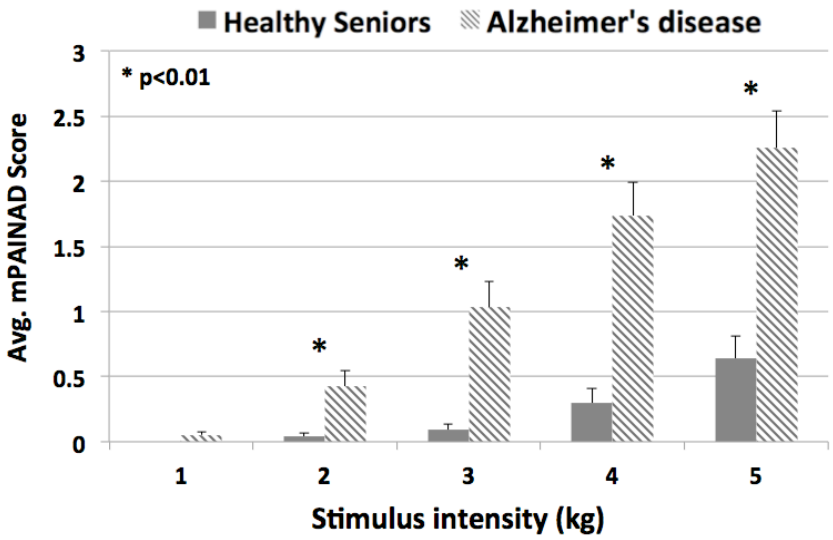

Figure 1-2 - Average modified Pain Assessment in Advanced Dementia (mPAINAD) scores across each stimulus intensity (kg). Error bars represent SEM. p<0.01 considered significant after Bonferroni correction for multiple comparisons.

showed significantly greater mPAINAD scores for AD subjects at stimulus intensities 2-5kg (Chi-Sq all > 12; p<0.001). Secondary GLMM found no significant effects (F=0.10, p=0.75) indicating no AD subgroup differences for mPAINAD scores – thus subgroups are not plotted in Figure 1-2.

Significant effects of group and group*stimulus intensity were found for all mPAINAD domains (vocal: group–F=22.2, p<0.001–interaction–F=19,663.1, p<0.001 | facial: group–21.2, p<0.001–interaction–F=223.5, p<0.001 | body: group–F=83.1, p<0.001–interaction–F=1,959.9, p<0.001). These results suggest that each domain contributed to the overall increase in mPAINAD scores for AD subjects. Average domain responses (non-recoded) for HS and AD are plotted in Figure 1-3a-c. Post-hoc Kruskal-Wallis testing of each domain yielded significant increases for AD subjects in: vocalization for 2-5kg (Chi-Sq=7.7, 17.4, 19.2, 27.9, respectively, p<0.006); facial expression at 2-4kg (Chi-Sq=6.7, 12,1, 7.6, p=0.01, 0.001, 0.006, respectively); and bodily response at 2 (marginally, Chi-Sq=5.6, p=0.018) and 3-5kg (Chi-Sq=11.0, 15.0, 11.9,

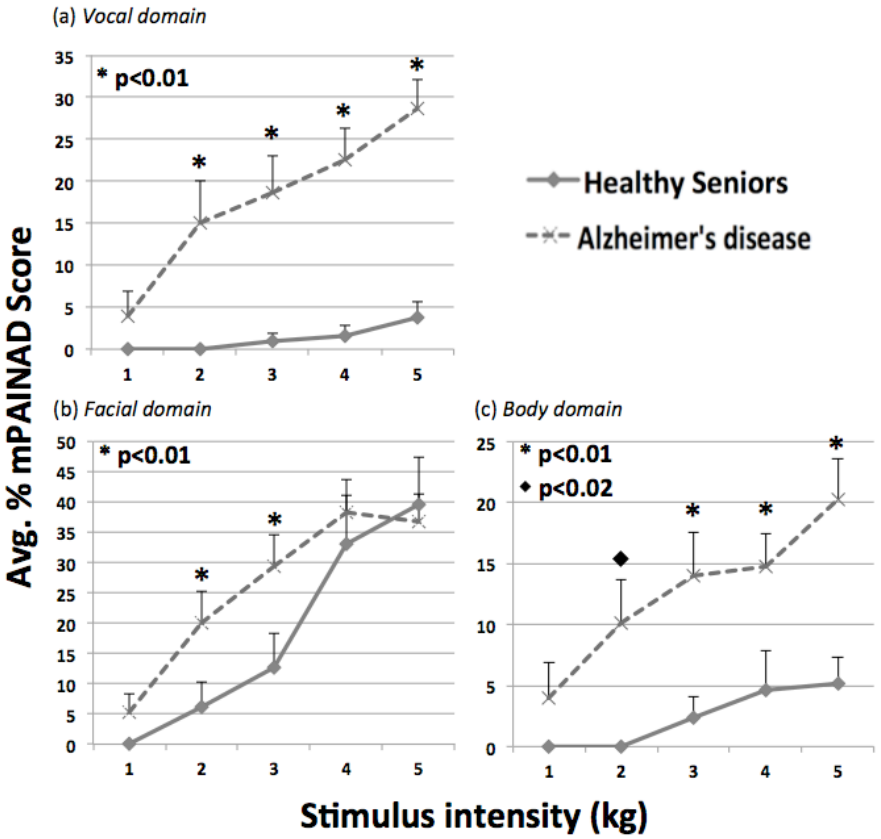

Figure 1-3 - Average mPAINAD domain use (average % of total mPAINAD points per stimulus) across stimulus intensities (kg). (a) mPAINAD vocal domain; (b) mPAINAD facial domain; (c) mPAINAD bodily domain. Error bars represent SEM. p<0.01 considered significant after Bonferroni correction for multiple comparisons.

respectively, p<0.001). Secondary GLMM found no severity-dependent effects for mPAINAD domains (vocal: F=0.31, p=0.58 | facial: F=0.001, p<0.97 | body: F=1.32, p=0.25), thus subgroup responses are not plotted in Figure 1-3.

AD patients with high levels of cognitive impairment (MMSE≤10/CDR=3, sAD subgroup) were unable to pass FPS-R reliability testing. FPS-R results therefore represent differences between the mAD subgroup and HS. Here, GLMM testing found a non-significant group effect (F=1.48, p=0.22) but a significant group*stimulus intensity interaction (F=14.1, p<0.001). Figure 1-4 shows average (non-recoded) FSP-R scores for HS and AD. Kruskal-Wallis

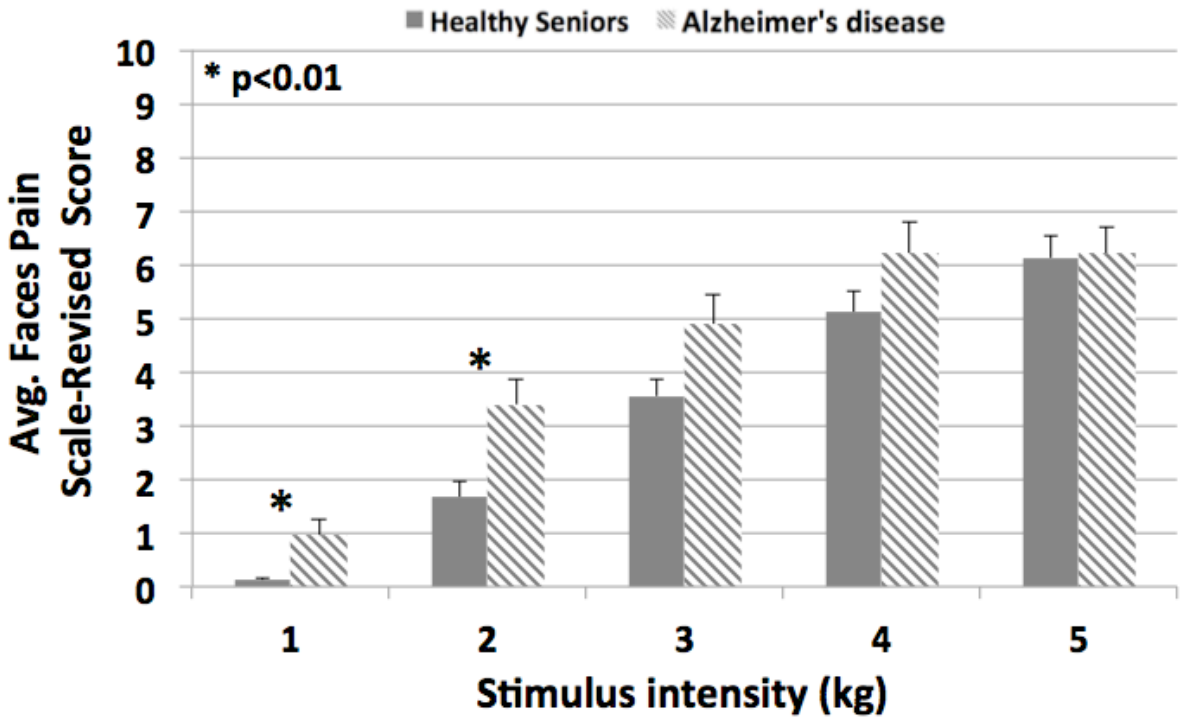

Figure 1-4 - Average subjective pain-report scores for each stimulus intensity (kg). Scores of AD patients represent those who passed reliability testing for the FPS-R. Error bars represent SEM. p<0.01 considered significant after Bonferroni correction for multiple comparisons.

testing showed FPS-R ratings were higher in AD patients for lower level stimuli 1&2kg (Chi-Sq=8.7, p=0.003; Chi-Sq=8.0, p=0.005, respectively). Ratings for 3&4kg intensities were slightly higher in AD subjects, but did not reach Bonferroni correction threshold (Chi-Sq=3.6, p=0.057; Chi-Sq=3.5, p=0.067). Ratings at 5kg were very similar (Chi-Sq=0.32, p=0.57) between groups.

DISCUSSION

Though recent studies have reported behavioral and neuroimaging evidence of increased pain sensitivity in AD, few studies have included severe patients (MMSE≤10/CDR=3). We therefore examined acute pain responses (autonomic, pain behaviors, and potential self-report) in mild/moderate (mAD) and sAD patients, as well as HS, during repeated application of multiple

forearm pressure intensities. A secondary analysis probed for severity-dependent differences for mAD and sAD subgroups.

There was no overall difference in HR response between HS and AD subjects as a whole. However, secondary analyses found that sAD patients had diminished responses compared to both HS and mAD. A tendency for AD patients to show blunted autonomic responses to mild pain is a consistent finding in the literature[62,65,66,70]. In studies including patients with MMSE as low as 8, increasing cognitive impairment was associated with autonomic blunting[62,65,66], with higher levels of noxious stimulation required for 'quasi-normal' autonomic responses. Our findings extend these prior results to patients with MMSE as low as 0. Blunted autonomic responses have been interpreted by some authors as evidence of reduced pain affect in AD [62,66]. However, it is equally likely that central autonomic dysfunction is responsible. Altered autonomic function has been described in AD[98,99], and cortical and subcortical autonomic regulators are affected by AD pathology[21]. The result may be a disconnect between pain-related autonomic and affective-behavioral responses that worsens with AD progression. Overall it would appear that autonomic responses are not a reliable predictor of pain in AD.

AD subjects had higher mPAINAD scores than HS for all but the lowest pressure intensity indicating greater overall behavioral responsiveness. No severity-dependent differences in mPAINAD scores were found. Prior studies also reported increased behavioral expression of pain in AD and other dementia patients. Multiple studies have found increased pain-related facial expressions in AD/dementia patients, relative to HS[65,70,71,81]. Greater degrees of body-based pain responses, namely stiffness, guarding, and nociceptive flexion, were also found in prior studies of cognitively impaired patients[70,81]. Using portions of the PAINAD, which scores behaviors such as facial expressions on a more approximate level, we also found increases in pain-related facial

responsiveness, bodily responses, and negative vocalizations that contributed relatively equally to overall increased pain behaviors in AD patients, regardless of severity.

The level of cognitive impairment played a role in whether subjects could self-report, as no sAD subjects could reliability rate pain with the FPS-R. However, mAD subjects, all reliable reporters, rated low-level stimuli, and to a lesser degree mid-level stimuli, as more painful than HS. Our findings are in accordance with similar recent studies using pressure stimuli, that showed increased unpleasantness to low level pain and reduced pain tolerance in mAD patients[64,72]. These results contradict early findings of increased pain tolerance in AD patients[61,62,66], which included some advanced patients (MMSE 8-10). These early studies utilized electrical and ischemic pain modalities, which may account for some differences in results. It should be noted that increased cognitive deterioration was associated with impaired subjective pain report here and in other studies[70,71] pain in these studies may have been under-reported in early studies, even in mild patients[100]. Further, reduced pain-related semantic memory in AD is associated with reduced self-report of pain[101]. Overall, recent experimental findings and considerations of under-reported pain in patients support the premise that AD increases acute pain sensitivity.

A neural mechanism for increased acute pain sensitivity in AD is currently not known. One fMRI study of healthy subjects found that facially expressive individuals had greater activation in pain processing and motor regions, with less activity in prefrontal & striatal regions compared to "stoic" subjects[102]. While expressive subjects had a higher sensory/affective experience, stoic individuals, investigators concluded, were better able to maintain self-reflective/introspective states and suppress motor responses in accordance to learned display rules. AD affects the function of networks and structures associated with cognitive control,

introspection/self-reflection, and pain processing[21,85,86]. In advanced stages even sensory cortices are affected[57]. AD could therefore increase acute pain sensitivity and pain behavior through its impairment of cognitive control and self-reflective processes. Pain-processing structures may also become sensitized. Evidence for this comes from the only neuroimaging study of pain in AD thus far, which found mild pressure pain induced greater activation of pain-processing regions in mAD vs. HS[72]. Further examination of AD-related brain function in the context of acute pain would be advantageous to further test this hypothesis.

A strength of this study is its inclusion of a relatively large number of sAD subjects. However, this precluded a more detailed determination of pain threshold and tolerance. As late AD pathology does affect somatosensory cortex[21,57] altered pain threshold may have contributed to behavioral findings in sAD patients. We also only tested pain responses using one stimulus modality, pressure, and only in one session. Interestingly, a majority of studies finding greater pain sensitivity in AD patients used pressure stimuli, and it is possible that pressure pain is altered in AD differently than in other modalities. It should be noted though, that increased behavioral pain responses and similar pain ratings in AD compared to HS have also been found using electrical, laser, and needle stick modalities[63,65,70]. It is also possible that pain behavioral responses measured in AD patients could be related to psychosocial distress, which has been proposed to confound PAINAD scoring[103]. Here, a linear rise in mPAINAD scores with increasing stimulus intensity, along with co-occurring increases in pain ratings by patients, suggests that discomfort/pain was in fact measured. Finally, this study is somewhat limited in the number of included patients with more mild/moderate disease. Conclusions regarding differences between AD subgroups for HR responses and between HS and mAD for FPS-R scores must then

be taken with some caution. However, our findings are in agreement with multiple prior studies in AD patients and as such do not appear spurious.

This study examined various biobehavioral pain indicators including autonomic responses, and behavioral and subjective pain ratings in mild, moderate, and severe AD patients. We found that while sAD patients had overall blunted autonomic pain responses, both sAD and mAD patients showed robust behavioral evidence of increased acute pain sensitivity. Mild/moderate AD patients also rated low-level stimuli as more painful than HS. These findings, in conjunction with recent studies, provide accumulating evidence that AD patients experience greater sensitivity to acute pain than HS. This evidence should be taken into account when considering clinical pain assessment and treatment strategies for AD patients of all severities.

CHAPTER 2

EFFECTS OF ALZHEIMER'S DISEASE ON THE FACIAL EXPRESSION OF PAIN

INTRODUCTION

Traditional means of pain assessment are often difficult to perform in patients with Alzheimer's disease (AD). As AD progresses patients lose the capacity to provide subjective pain-report and often have impaired comprehension of clinical pain scales[70,84]. This leaves patients highly vulnerable to under-detection and under-treatment of pain and highlights the necessity to include non-verbal indicators, such as facial expressions, in pain assessments[10,104].

Most existing clinical observational pain scales use an approximate, globally integrated assessment of facial response in determining whether an individual is manifesting a "pained" expression[94]. Because of the potential examiner bias inherent in this approach, the Facial Action Coding System (FACS)[105], a more objective, quantitative measure of facial expression has been adopted by many studies of pain in healthy and demented individuals[65,106–110]. The FACS scores discrete components of facial expression by their intensity and frequency. However, with its rigorous scoring criteria, the FACS requires extensive and prolonged rater training, making it a strong experimental tool with limited clinical utility; though some have suggested it could help improve observational clinical pain scales with facial expression domains[81,109–111]. Thus far, no experimental pain studies have examined the relationship between data analyzed both with the FACS and validated clinical pain scales for AD patients. It is therefore unknown how clinically relevant FACS-based findings may be in this population.

Pain response studies of 'dementia' patients typically find increased facial expressions, compared to healthy seniors (HS), during painful procedures such as venipuncture[65], physical exercise in those with chronic musculoskeletal pain[81], or experimental pain testing using

mechanical pressure or electrical stimuli[70,109,110]. However, many of these studies included mixed dementia groups[70,81,109], and it is unclear whether their general findings apply equally to those diagnosed exclusively with probable AD. In studies that examined only AD patients, results have been inconsistent: an early study that applied venipuncture to patients of multiple severities found increased facial responsiveness compared to healthy seniors (HS)[65]; however, a more recent study that used mechanical pressure and electrical stimuli found no frequency or intensity facial response differences between mild/moderate AD (mAD) and HS participants[110]. Neither of these studies differentiated between pain-relevant and pain-irrelevant facial expressions. Finally, though some postulate that patients with advanced AD show fewer pain behaviors than those with milder AD[53,82,112], very few experimental studies have included severe AD patients. It is thus unclear whether and how pain-relevant and irrelevant facial responsiveness may change with AD progression.

We attempted to fill these gaps in understanding by comparing pain-related facial expressions of AD patients of mild to severe stages to those of HS using the FACS and varied levels of mechanical pressure. In doing so we took care to differentiate between pain-relevant vs. pain-irrelevant facial responses prior to group analyses. Alongside probing for general group differences, we examined whether AD progression (i.e. decreasing Mini-Mental State Examination, MMSE, scores) was associated with changes in the intensity of pain-relevant or irrelevant responses. We also examined whether and to what degree scores from two clinically validated pain scales, the Pain Assessment in Advanced Dementia (PAINAD) scale and Faces Pain Scale-Revised (FPS-R), correlated with the intensity of pain-relevant and irrelevant facial expressions.

In consideration of prior examinations of facial expressions in AD patients, as well as our own prior analyses of pain responses as AD progresses[67], we predicted that: 1) AD would be associated with greater intensity and/or frequency of pain-relevant facial responses; 2) PAINAD and FPS-R indices would strongly correlate with FACS scores for pain-relevant, but not pain-irrelevant responses; 3) AD progression would not be associated with changes in the intensity of pain-relevant facial responses.

METHODS

Subjects

Thirty-five patients with diagnoses of probable AD under DSM-IV criteria (25 ♀, mean age 74.4±6.6SD) and thirty-three HS controls (21 ♀, mean age 78.5±9.7SD) participated. HS controls were recruited through senior newsletters and local AD support groups. They were required to have no current pain or history of subjective memory complaint. AD subjects were recruited through local nursing homes and the outpatient Cognitive & Geriatric Neurology Clinic at Michigan State University. All participants were screened for baseline pain via subject interview, chart review, or caregiver discussion as inclusion required abstinence from analgesics for 24 hours prior to study. As a separate analysis involved examining autonomic responses, individuals taking beta-blocker medications were excluded unless temporary discontinuation for a period equal to three-half lives prior to study was agreed upon by their primary physician. Further study exclusions included history of the following: Type II diabetes, history of stroke or transient ischemic attack, central or peripheral neuropathy, diagnosis of neurological (e.g. seizure disorder) or psychiatric disorders (e.g. major depression, schizophrenia) other than AD. We further excluded individuals with current arthritic pain, those with a history of arthritis in distal forearms (the stimulus application region), and those taking daily arthritic pain medication.

Patient medical histories were also scrutinized to ensure exclusion of those with clinical evidence of probable mixed, vascular, frontotemporal, or Lewy body dementia.

Once subjects passed screening they underwent neuropsychological testing, including completion of Clinical Dementia Rating (CDR)[88], MMSE[89], Cornell Scale for Depression in Dementia (CSDD)[90] and Neuropsychiatric Inventory Questionnaire (NPI-Q)[91]. Mean MMSE and CDR for HS was 29±1.1SD and 0±0.0SD, while mean MMSE and CDR for AD subjects was 11±9.1 and 1.99±1.1. HS scored, on average 1.1±1.2SD on the CSDD while AD subjects averaged 6.8±4.6SD. No subjects had a CSDD score indicative of probable depression (>12)[90]. Mean NPI-Q scores were 0.52±0.51SD and 7.7±5.7SD for HS and AD subjects, respectively.

All testing procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Michigan State University Internal Review Board. Written informed consent was obtained for all HS as well as for AD subjects via named health care proxies identified as a power of attorney for health care or guardian. We obtained assent from all participants (verbal or non-verbal) before beginning testing. Testing was stopped if a subject became inconsolably agitated or verbally declared the wish to discontinue participation. The former occurred with one AD subject, who was subsequently excluded from analysis.

Procedures

Testing procedures took place between 1-5pm and, including neuropsychological testing and experimental pressure pain testing, lasted 1-1.5 hours. Pressure stimulation took place using a Force Dial FDK 20 Force Gauge (Wagner Instruments, Greenwich, CT), which allows accurate recording of pressure (kg/cm^2), though the device is scaled in units of 'kg.' The instrument is fitted with a 1cm wide rubber disk to prevent skin abrasion. Stimulus application occurred on the lateral volar aspect of the distal forearm, 2-5cm from the wrist. Subjects were seated, upright,

during testing. Pressure application was adapted from a previous dementia-related pain study[109]. Pressure stimuli ranged from 1-5kg. Each pressure level was applied four times each, between the right and left forearms, in a pseudorandom fashion with the order determined once for use in all subjects. Stimulus order was limited by the following rules: no intensity could occur more than twice, sequentially; any sequential intensity repetition could not occur on the same arm. Stimuli were applied at a rate of ~1kg/s to peak intensity. Pressures were held at peak intensity for 5s prior to an ~50s interstimulus interval. Two trained investigators (PAB, MM) performed all testing in a standardized manner.

Responses measured

Continuous video recording of experimental pressure testing, with the face the primary object in view, allowed for scoring of facial expressions and other behaviors. Subjects were asked to focus their attention on a red recording light just below the camera lens during stimulus application. The camera was set to be level with the subject's face less than 2m away. Stimulus onset and offset was marked audibly.

Facial expressions were analyzed using the FACS[105], which allows one to determine the frequency and intensity of facial expressions through analysis of 44 anatomically discrete Action Units (AUs). The intensity of each AU (except 45 and 46, which have no intensity score) was scored on a 5-point A-E scale. An 'A' designation represented a trace degree of contraction and E being the maximum degree of contraction. A trained coder who passed qualification testing by the FACS developers (JTH) scored the frequency and intensity of AUs present in each 5s pressure trial for all subjects. The coder was blinded to stimulus order as well as group designation.

As has been done in prior studies utilizing the FACS in the context of pain[106,108,109], we initially performed a data reduction procedure whereby AUs that involve contraction of the same muscular group were combined. Specifically, we combined AUs 1&2, AUs 6&7, AUs 9&10, and AUs 25-27 prior to determining which AUs were pain-relevant and irrelevant. Determination of pain-relevance vs irrelevance also involved steps utilized in prior studies of pain-related facial expressions[81,108,109]. First, we determined which AUs met a critical threshold of occurrence (at least 5%) in pressure stimulations deemed "pain-segments," separately for HS and AD subjects. Pressure intensities 4&5kg, which on average reached "high moderate" pain levels in HS and reliable AD self-reporters, were chosen as "pain segments" for purposes of this analysis in order to increase the likelihood of capturing pain-specific facial expressions. AUs that met this threshold level of occurrence are found in Table 2-1. Second, we computed effect sizes (*Cohen's d*) for each of these AUs, by comparing their average frequency of occurrence in pain-segments vs. the lowest stimulus level (1kg). Those AUs whose effect sizes reached a level of $d > 0.35$ in both groups were considered pain-relevant (Table 2-1, shaded in gray), while those that did not were considered pain-irrelevant. Prior studies utilized a threshold of $d \geq 0.5$. However, as no HS effect sizes reached this level the decision was made to lower the threshold to the mid-point between a typical 'medium' and 'small' effect size. Nevertheless, our discrimination procedures did yield pain-relevant and irrelevant AU designations similar to those in prior studies[106,108,109]. Next, we created weighted scores for each AU frequency to account for the disproportionate occurrence of AU 45 (blink) relative to others. This was accomplished by dividing the frequency of each AU for each stimulus trial by the mean frequency of that AU across all stimulus trials. Finally, composite frequency and intensity scores were calculated by averaging pain-relevant and pain-irrelevant AUs separately for each stimulus trial.

Table 2-1 – Facial Action Coding System Action Units (AUs) that met threshold of occurrence (at least 5%) in considered "pain segments" (4&5kg pressures).

Action Unit (AU)	Description	Healthy Seniors		Alzheimer's disease	
		Percentage[1]	Effect Size (d)	Percentage[1]	Effect Size (d)
AU 1&2	Brow raised	10.7	0.21	36.1	0.32
AU 4	Brow lowered	**11**	**0.38**	**44.6**	**0.80**
AU 6&7	Orbit tightened	**23.9**	**0.44**	**58.3**	**0.65**
AU 9&10	Levator contracted	8.7	0.26	**21.4**	**0.36**
AU 25-27	Mouth open	5	0.16	**13**	**0.41**
AU 45	Blink	8.8	0.10	34	0.31

[1] Indicates the percent occurrence across all pain segments
Bolded lines indicate critical effect size threshold of d > 0.35
Effect size (d) indicates the relative frequency difference of occurrence between pain and non-pain segments (4-5kg vs 1kg)
Gray shaded AUs are those that, for both groups' pain segments, were considered significantly more frequent (d > 0.35) in both groups and thus were considered "pain-relevant" for purposes of further statistical analysis. Non-shaded AUs were considered "pain-irrelevant."

A secondary goal of this study was to determine whether FACS measures correlate with clinical pain scales. Therefore we applied data from a prior analysis[67] of general behavioral responses to the same pressure stimuli using portions of the PAINAD, a validated observational acute pain assessor for dementia patients[80,92,93]. The full version of the PAINAD assesses breathing, consolability, negative vocalizations, facial expression, and bodily responses. Each domain's score ranges from 0-2 with a maximum of 10. Many have questioned the utility of the breathing and consolability items with respect to internal consistency and probability of measuring pain[94]. We therefore elected in the prior analysis to consider only vocal, facial, and bodily domains for purposes of this analysis, yielding a maximum modified PAINAD (mPAINAD) score of 6. mPAINAD scoring was aided by descriptors provided as part of a

review panel of observational pain scales for dementia patients[94]. Two trained raters[113] (PAB, MM) performed initial mPAINAD scoring for half of tested subjects. However, as they were un-blinded to stimulus order and group designation, the FACS rater, also a trained PAINAD rater, re-scored all initial sessions as well as the remainder of subjects. Final mPAINAD scores for double-scored subjects were determined through rater consensus.

We also obtained subjective pain ratings from subjects. After each stimulus application (<5s after) HS and reliable AD pain reporters were given the opportunity to rate their subjective pain levels via the Faces Pain Scale-Revised (FPS-R)[95]. The FPS-R is a subjective pain assessor that has been utilized in cognitively impaired patients from mild to early severe status[84,96]. Each of the five faces after the initial 'no pain' face of the scale represents a stepwise two-point increase in numerical value, for a maximum score a 10. AD subjects able to pass a 3-question quiz that is part of the FPS-R[97] were deemed reliable pain reporters.

Both the FACS and PAINAD have been shown to have high degrees of intra and inter-rater reliability[94,114]. In the current and prior study we performed multiple measures of rater reliability for FACS and mPAINAD scores, respectively. As performed in prior FACS studies of pain in cognitively impaired elderly[81,110], intra-rater reliability for the FACS took place through a re-scoring of a randomly selected 15% of subjects with equal representation of both HS and AD groups. The rater was again blinded to group designation and stimulus order as well as original scores. Absolute intraclass correlation coefficients (ICC) were then calculated for AU selection, frequency, and intensity. The average ICC score for FACS re-scoring was 0.79±0.10SD ($p<0.005$), indicating good to very good agreement. mPAINAD reliability testing involved examining inter-rater reliability for those subjects scored by more than one rater (N=36) with ICC. Intra-rater reliability was also tested through the same re-scoring procedure as described for

the FACS. ICC was calculated for overall mPAINAD score as well as each domain (vocal, facial, and bodily) and then averaged. Mean inter-rater ICC was 0.93±0.04SD ($p<0.005$), while mean intra-rater ICC was 0.86±0.08SD, indicative of strong agreement between and within raters.

Statistics

Generalized linear mixed modeling (GLMM) in SPSS™ (Version 22.0, Armonk, NY: IBM Corp) determined impact of level-two effects (subject group – HS and AD) on level-one effects (FACS intensity and frequency composites, mPAINAD, and FPS-R scores), with subject and stimulus level as predictors. GLMM accounts for repeated measures (trials) and nuisance covariates (age, gender, and CSDD, NPI-Q severity, stimulus applicant). PAINAD and FPS-R data were each recoded into clustered scores to improve modeling of their non-normal distributions. Significant 'group' (HS vs AD) or 'group*stimulus level' interaction effects ($p<0.05$) were followed-up with post-hoc nonparametric independent samples testing between groups under each stimulus level for FACS frequency and intensity composites as well as mPAINAD and FPR-r scores. The exact test for each comparison was determined by SPSS based on their distributions. FACS frequency and intensity post-hoc comparisons involved Mann-Whitney U testing, while mPAINAD and FPS-R comparisons involved Kruskal-Wallis testing. To control for family-wise error, primary GLMM tests were sequential Bonferroni corrected and post-hoc results were considered significant if they met a Bonferroni correction threshold of $p<0.01$. We also tested whether the frequency and intensity of pain-relevant AUs was significantly higher than pain-irrelevant AUs, both across and between groups, using non-parametric related samples (Wilcoxon signed rank) and independent samples (Mann-Whitney U) testing, respectively. These latter tests provided validation of whether measured responses were

pain-specific or not and whether AD responses represent a pain-specific or non-specific increase in facial expressions.

We then performed a series of correlation analyses between pain-relevant and irrelevant AU composite intensity scores to mPAINAD scores, the percentage of each mPAINAD score attributed to the facial domain, and FPS-R scores. We did this in order to determine the degree to which clinical indicators of pain in AD are associated with FACS indices of pain-relevant and irrelevant facial expression. Next, regression and correlation analyses were utilized to examine relationships between FACS indices and clinical measures of AD severity. Within AD subjects and across pressure levels, linear regression was utilized to examine an overall ('main effect') association exists between AD severity (MMSE score) and pain-relevant and irrelevant frequency and intensity facial responses. Because a much larger, though non-significant, association was found between MMSE and pain-irrelevant indices, compared to pain-relevant ones, we also computed Pearson correlation coefficients for individual pressure level. This was done to examine whether a particular stimulus level was driving this marginal association.

RESULTS

FACS findings

As seen in Table 2-2, there were significant effects for both the frequency (significant group effect & group*stimulus level interaction) and intensity (significant group*stimulus level interaction) of pain relevant AU composite scores. These results indicate that AD patients generally showed more frequent pain-relevant responses than HS. Further, with increasing stimulus level AD patients showed greater increases of pain-relevant facial expressions, both in frequency and intensity, relative to HS. There were also significant pain-irrelevant frequency differences between groups (significant group*stimulus level interaction, Table 2-2), such that

increased stimulus level saw more frequent pain-irrelevant responses in AD patients compared to HS. Post-hoc Mann-Whitney U testing indicated significantly increased pain-relevant frequency and intensity composite scores for each individual pressure level (Figure 2-1.a&b; pain-relevant frequency Standardized Test Statistics [STS] for 1-5kg: 3.5, 3,9, 5.9, 6.2, 3.9 – $p<0.001$ for all; pain-relevant intensity STS for 1-5kg: 3.5, 3.9, 6.2, 6.3, 4.4 – $p<0.001$ for all). Meanwhile, as shown in Figure 2-1c & 2.1d, pain-irrelevant facial responses were increased to a lesser extent,

Table 2-2 – Results of primary general linear mixed modeling (GLMM) analyses of composite scores for pain-relevant and irrelevant AU frequency and intensity as well as mPAINAD and FPS-R scores from a prior analysis.

	AD vs HS		(AD vs HS)*Stimulus level	
FACS results	*F*	*p*	*F*	*p*
Pain-Relevant Scores				
Frequency	17.9	**<0.001**	3.05	**0.016**
Intensity	1.7	0.197	5.04	**<0.001**
Pain-Irrelevant Scores				
Frequency	0.82	0.36	2.4	**0.045**
Intensity	0.83	0.36	2.0	0.089
Clinical Scale results				
mPAINAD	71.1	**<0.001**	17,158.7	**<0.001**
FPS-R	15.7	**<0.001**	1,415.2	**<0.001**

FACS – Facial Action Coding System
mPAINAD – modified Pain Assessment in Advanced Dementia scale; FPS-R – Faces Pain Scale-Revised
Significant results (p<0.05) in bold.

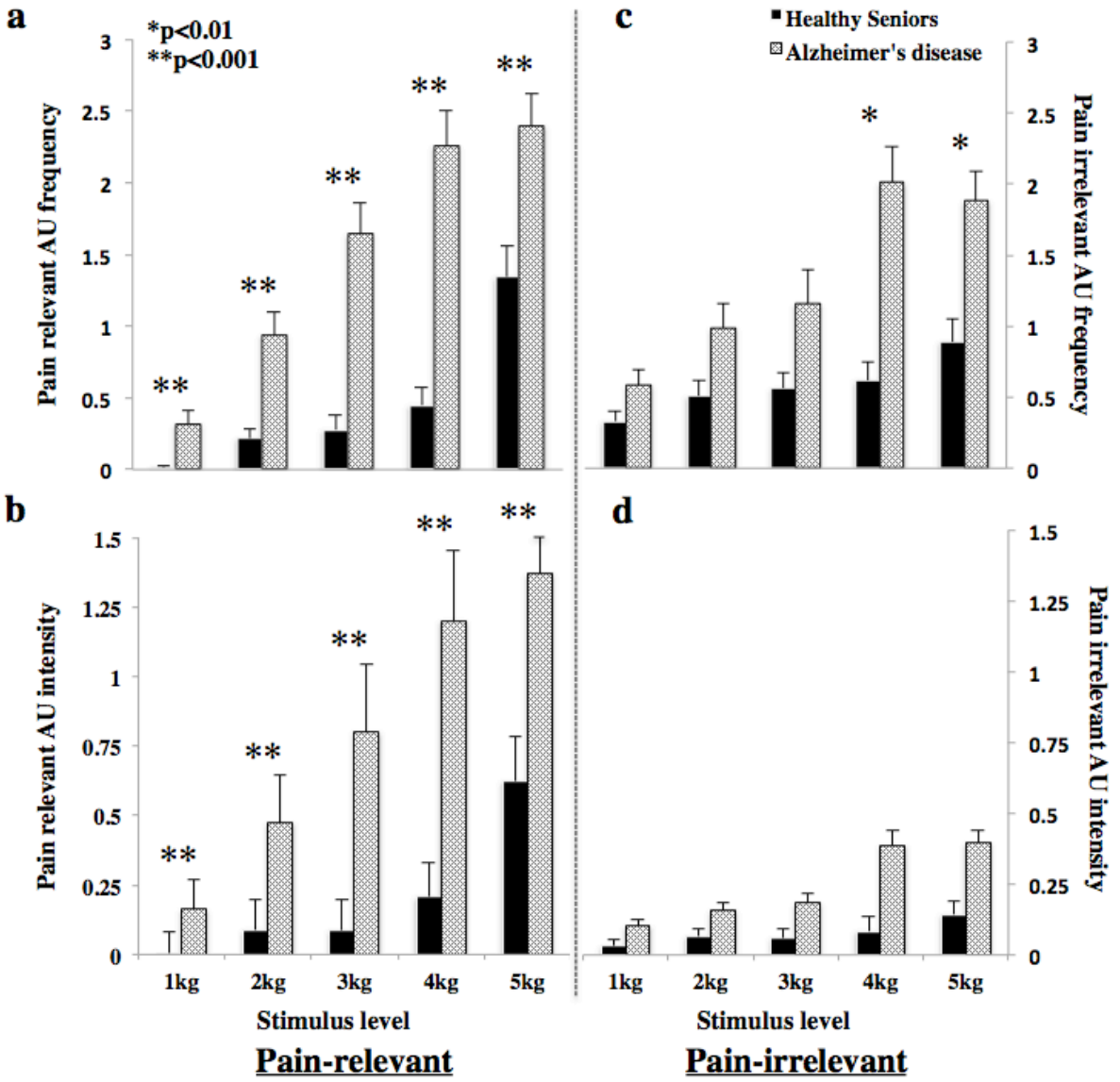

Figure 2-1 - Mean (+ SEM) composite pain-relevant frequency (a) and intensity (b) as well as pain-irrelevant frequency (c) and intensity (d) facial responses (action units – AUs) for Alzheimer's disease (lighter, crossed bars) and healthy senior control subjects (dark bars) for individual stimulus levels (1-5kg). Threshold for appropriate post-hoc testing significance set to p<0.01 after Bonferroni correction for multiple comparisons.

particularly for intensity. Post-hoc Mann-Whitney U testing was only done for pain-irrelevant frequencies as no significant effect was found for intensity. Figure 2-1c shows that the significant interaction effect for pain-irrelevant frequency scores was likely driven by AD responses at pressure levels 4&5kg (STS: 2.9, 3.0, respectively – p=0.003).

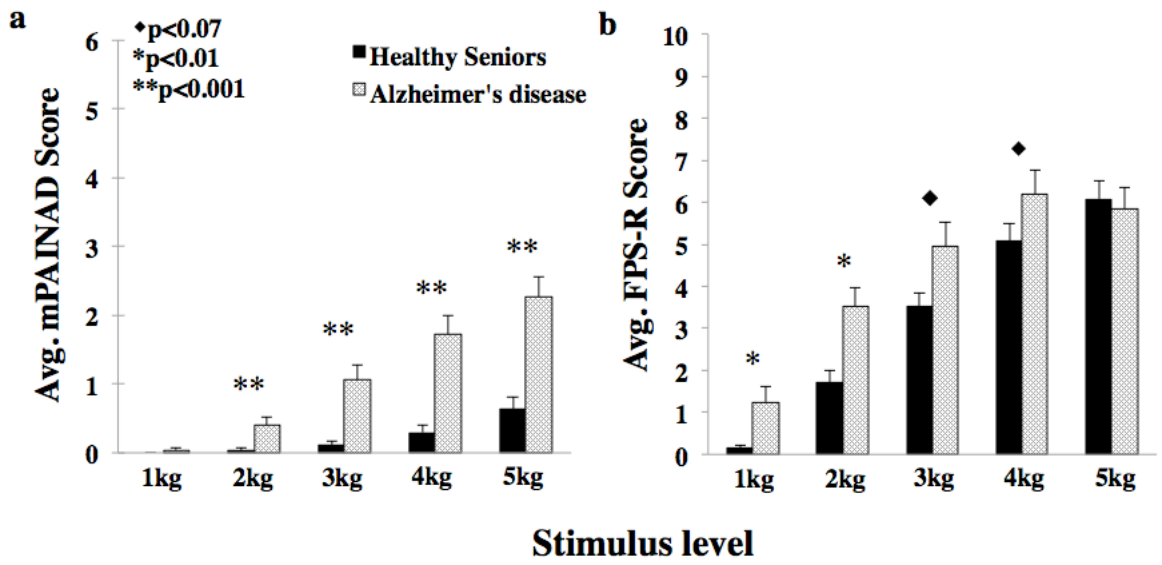

Figure 2-2 - a) Average modified Pain Assessment in Advanced Dementia (mPAINAD) scores across individual stimulus levels (kg); b) Average subjective pain ratings results under individual stimulus levels for HS and AD patients who were able to reliably report using the Faces Pain Scale-Revised (FPS-R). Error bars represent SEM. $p<0.01$ considered significant after Bonferroni correction for multiple comparisons.

Nonparametric related samples (Wilcoxon signed rank) testing across groups indicated a significant difference in the degree to which pain-relevant and irrelevant AUs were elicited, both for frequency and intensity (test-statistics 6.4 & 12.5, respectively, $p<0.001$). Specifically, as suggested in Figure 2-1, pain-relevant frequency and intensity scores were higher than their pain-irrelevant counterparts. Further, between-groups nonparametric independent testing showed that groups differed in the degree to which pain-relevant responses were higher than pain-irrelevant ones (test-statistic 8.6 & 9.2, respectively; $p<0.001$). Specifically, there was a greater increase in pain-relevant than irrelevant responses in AD patients, compared to HS.

Clinical pain assessor findings

Significant fixed effects (both for group and group*stimulus level) were found for comparisons of mPAINAD and FPS-R scores of HS and AD subjects (see Table 2-2, bottom). Average mPAINAD and FPS-R responses for individual stimulus intensities, and the results of their respective post-hoc Kruskal-Wallis pairwise comparisons are found in Figure 2-2. AD

Table 2-3 – Pearson correlations between FACS composite intensity scores (pain-relevant and irrelevant) and two clinical scales measuring pain behaviors and subjective pain ratings, respectively, for individual pressure stimuli (1-5kg). Correlations computed separately for healthy seniors (top) and Alzheimer's disease (bottom) subjects.

	1kg		**2kg**		**3kg**		**4kg**		**5kg**	
Healthy seniors	PR	PI	PR	PI	PR	PI	PR	PI	PR	PI
mPAINAD (n=33)	--	--	0.34	0.20	**0.67****	**0.62****	**0.67****	**0.62****	**0.75****	**0.61****
% mPAINAD face domain[1] (n=33)	--	--	0.28	0.17	0.30	0.30	0.30	0.30	**0.59****	0.41
FPS-R (n=33)	-0.02	0.03	0.20	0.003	0.32	0.18	0.32	0.18	0.29	0.23
Alzheimer's disease										
mPAINAD (n=35)	0.31	0.18	**0.59****	**0.49***	**0.69****	0.43	**0.70****	0.41	**0.78****	**0.51***
% mPAINAD face domain[1] (n=35)	0.40	0.21	**0.60****	0.34	**0.50***	0.32	**0.58****	0.41	**0.49***	0.32
FPS-R (n=17)	-0.02	0.21	0.12	0.23	0.10	0.01	0.30	0.24	0.26	0.08

kg – kilogram; PR – Pain-relevant; PI – Pain-irrelevant; mPAINAD – modified Pain Assessment in Advanced Dementia; FPS-R – Faces Pain Scale-Revised

[1] Indicates the percentage of the overall PAINAD score attributed to the facial expression domain

***p<0.01 two-tailed; **p<0.001, two-tailed**

subjects had greater mPAINAD scores than HS for 2-5kg pressure intensities (Chi-Sq. 12.4, 18.3, 16.03, and 18.3, respectively – $p<0.001$). AD patients able to reliably self-report subjective pain (n=17, all had MMSE>10) also rated low-level stimuli, 1-2kg, as more painful than HS (Chi-Sq = 8.7, 8.0 – $p<0.01$). Stimulus intensities 3-4kg were rated marginally more painful by AD subjects, though these results did not meet Bonferroni-corrected threshold (Chi-Sq.=3.6 & 3.5 – p=0.057, 0.06, respectively).

Correlating FACS and clinical pain assessors

As seen in Table 2-3, there were many significant positive correlations between AU intensity and mPAINAD scores, for multiple pressure intensities, in both HS and AD subjects. Notably, significant correlations were found for more pressure levels in AD subject mPAINAD scores (2-5kg in AD vs 3-5kg in HS) and were less likely to involve pain-irrelevant AU scores,

Table 2-4 – Top: results of linear regression analysis, within Alzheimer's disease subjects, between Mini-Mental State Examination (MMSE) scores and pain-relevant and irrelevant FACS scores across all pressure levels. Bottom: Pearson correlations between MMSE and pain-irrelevant indices for individual pressure levels.

Linear Regression	**R^2**	**F**	***p***
Pain-relevant			
Frequency	0.005	0.158	0.69
Intensity	0.005	0.151	0.70
Pain-irrelevant			
Frequency	0.067	2.37	0.13
Intensity	0.059	2.07	0.16

Pearson Correlation	**1kg**	**2kg**	**3kg**	**4kg**	**5kg**
Pain-irrelevant frequency	0.10	0.09	0.15	0.27	0.09
Pain-irrelevant intensity	0.10	0.06	0.09	0.25	0.10

compared to HS. Further, AD subject pain-relevant AU composite intensity scores significantly correlated with the percentage of each mPAINAD score attributable to facial expressions ('%mPAINAD face domain' in Table 2-3). In contrast, HS subjects' AU intensity scores (both pain-relevant and irrelevant) were generally weakly correlated with the facial domain of mPAINAD scores. Finally, there were no significant associations between pain-relevant and irrelevant intensity scores and FPS-R scores for either HS or AD subjects.

FACS and AD severity

There were no significant relationships found between AD severity (MMSE score) and the intensity or frequency with which AD patients showed pain-relevant or pain-irrelevant facial expressions (Table 2-4, top).

Interestingly, there was a magnitude difference in regression results between pain-relevant and irrelevant responses and MMSE scores of AD subjects. The 4kg pressure level was the likely cause; its coefficients for pain-irrelevant frequency and intensity were much higher

than other pressure levels (Table 2-4, bottom). However, these correlation coefficients did not reach significance

DISCUSSION

The purpose of this study was: first, to use the FACS to compare pain-relevant and irrelevant facial responses in AD patients versus HS; second, to examined the relationship between FACS-based findings and those of two validated clinical observational and subjective ratings scales for assessing pain in dementia; third, to assess whether pain-relevant or irrelevant facial responses vary as a function of AD severity.

FACS findings

Discrimination of pain-relevant and irrelevant responses yielded sets similar to those in prior research of pain facial responses[70,106,109]. In accordance with our first prediction, we found that AD subjects showed more frequent pain-relevant facial responses than HS. Additionally, AD subjects had greater increases in pain-relevant frequency and intensity with increasing pressure levels. While both frequency and intensity of pain-relevant responses were greater than pain-irrelevant ones in both groups, this increase occurred to a greater extent in AD subjects, compared to HS. We therefore conclude that augmented facial responses seen here in AD subjects are 'pain typical'[109], rather than non-specific increases in facial responsiveness.

Prior studies employing the FACS in the context of pain found similar results in AD or mixed groupings of dementia patients. Porter and colleagues[65] found overall increased facial responsiveness in AD relative to HS using venipuncture. In a group of AD, vascular, and mixed dementia patients, Kunz et al.[70,109] scored facial responses to various pressure levels and differentiated pain-relevant and irrelevant responses. They then found more frequent and intense pain-relevant responses in their dementia group compared to HS. In contrast to the latter three

studies, our findings conflict with work done by Lints-Martindale and colleagues[110]. Their study found no differences in facial response between AD and HS with electrical or pressure stimuli. It is likely that methodology, with respect to definitions of pain-relevance, account for this conflict. We and other authors[81,108,109,115,116] used a multi-step procedure to separate pain-relevant and irrelevant AU responses. Lints-Martindale and colleagues, on the other hand, examined overall facial activity and incorporated AUs not typically associated with pain into their analysis (e.g. eye movements)[106]. This may have prevented group differences from being detected.

FACS and clinical pain scales

We found in a prior analysis[67] that AD patients also showed increased overall pain behaviors compared to HS. By scoring subject responses with the vocal, facial, and body domains of the PAINAD we found greater degrees of overall pain behaviors in all but the lowest pressure level. We also calculated percentages of each score attributed to behavioral domains. Interestingly, while the vocal and bodily domains were greater in AD patients, compared to HS, for pressure levels 2-5kg, the facial domain was increased for only 2-3kg. In contrast, with the FACS we found more frequent and intense pain-relevant responses in AD over HS for all pressure levels. It is possible that a ceiling effect from the PAINAD's three-tiered scoring (0-2) as well as somewhat ambiguous/non-specific score descriptors[111] may have caused under-estimation of pain facial responses at higher stimulus levels (i.e. 4-5kg). Consistent with this notion, many have suggested utilizing experimental tools, such as the FACS, to improve facial descriptors in observational pain assessors like the PAINAD[81,109–111].

Scores between the FACS and mPAINAD were strongly correlated in HS and AD. Pain-relevant FACS scores in AD subjects were correlated for more pressure levels than HS (2-5kg vs 3-5kg). AD subjects also showed more correlations between FACS scores and the facial

component of the mPAINAD than HS, though these correlations were somewhat lower than for overall mPAINAD scores. Consistent with prediction 2, correlations between FACS indices and the mPAINAD, including its facial component, were more significant for pain-relevant AD responses. In contrast, HS mPAINAD scores were correlated with both pain-relevant and irrelevant responses. This finding indicates that pain-relevant indices obtained by FACS analysis do measure clinically observable facial behaviors in AD patients. Our results also imply that the PAINAD is more specific to pain-relevant vs irrelevant facial responses in AD patients. Indeed, in a previous study by Sheu et al.[111], PAINAD scores obtained from seniors experiencing motion-exacerbated pain significantly correlated with pain-relevant AUs.

Our FACS and PAINAD-related findings are consistent with FPS-R results in that AD subjects able to subjectively report pain had significantly (1-2kg) or marginally (3-4kg) higher pain ratings than HS. Higher subjective pain in AD vs HS subjects during experimental testing has been described in other recent studies[64,72]. However, in contrast with mPAINAD results, FACS indices did not correlate with FPS-R scores in HS or AD. The lack of a consistent correlation between subjective pain ratings and pain behaviors has been reported previously in young and elderly/demented subjects[81,108,110,117]. It was speculated that, while subjective pain ratings often involve top-down socio-cognitive influences, pain-related facial expressions are more reflexive and perhaps more resistant to these inhibitory processes[107,118]. Thus, subjective ratings and pain behaviors would reflect somewhat different aspects of pain. It may also be that examining the face alone is not sufficient to link subjective and expressive pain. One meta-analysis concluded that more 'global' measures of pain behaviors, such as those provided by the PAINAD or other observational scales, tend to improve measures of this relationship[117]. In

correlating our mPAINAD measures with FPS-R scores for HS and AD this would appear to be case (Table 2-5, top).

Table 2-5 – Top: Pearson correlations between modified Pain Assessment in Advanced Dementia (mPAINAD) and Faces Pain Scale-Revised (FPS-R) scores for Healthy Seniors and those Alzheimer's disease (AD) subjects able to self-report. Bottom: Pearson correlations between mPAINAD and Mini Mental State Examination scores of all AD subjects.

Pearson Correlations					
mPAINAD – FPS-R	**Stimulus level**				
	1kg	**2kg**	**3kg**	**4kg**	**5kg**
Healthy seniors (n=33)	--	**0.43***	**0.41***	**0.38***	**0.40***
Alzheimer's disease (n=17)	0.17	0.32	0.38	0.30	0.37
mPAINAD – MMSE	**Stimulus level**				
	1kg	**2kg**	**3kg**	**4kg**	**5kg**
Alzheimer's disease (n=35)	0.003	-0.14	0.04	-0.10	-0.16
***Two-tailed p<0.05**					

FACS and AD severity

Consistent with prediction 3, we found no significant association between pain-relevant or irrelevant FACS scores and MMSE score. This suggests the facial expression of pain does not vary as a function of AD severity. This finding is in agreement with a prior result, in which we found no differences in mPAINAD scores between mild/moderate (MMSE 11-23/CDR 0.5-2) and severe (MMSE<11/CDR 3) AD patients[67]. There are, unfortunately, few experimental studies examining pain behaviors in dementia that include severe patients[65,81,111,119,120]. The study by Porter and colleagues[65], described above, included a few AD patients with a CDR of 3. However, they did not report presence or absence of severity-dependent effects with respect to facial responsiveness, only autonomic response. Though data are scarce, some studies indicate

patients with very severe AD/dementia (MMSE<2) have fewer charted pain behaviors compared to less advanced patients[82,112]. Here, a fairly large number of subjects fit this very severe category (n=9). The lack of a significant association in AD subjects between FACS scores and MMSE (or mPAINAD scores with MMSE – supplemental Table 2-5, bottom) is inconsistent with the notion that very severe patients exhibit fewer acute pain behaviors, facial or otherwise.

Limitations

Here we found rather robust differences between pain-relevant facial expressions of AD and HS. Our determination of AU pain-relevance was made using somewhat lowered effect size threshold compared to prior work (see methods). Nevertheless, we found pain-relevant/irrelevant AUs consistent with prior studies[106,109]. Another limitation is the single modality nature of the study. It is possible that different results may have been obtained using alternate pain stimuli such as electrical shocks or nonprocedural modalities that elicit clinical (i.e. chronic or postoperative) pain. Also, though we performed repeated applications for each pressure level, results are based on a single pain-testing session. Testing responses over two sessions, on different days, would verify within and between subject consistency. Finally, as only 17 AD subjects passed self-report reliability testing, subjective pain differences reported here must be taken with some caution.

Implications and conclusions

Findings here have implications for improving detection and treatment of pain in AD, for example using facial expressions as a primary indicator. But do these findings truly reflect increased pain in patients? One alternative explanation would be that increased facial displays of pain in AD represent a cognitive-behavioral disinhibition. However, our finding of proportionally greater increase in pain-relevant vs. pain irrelevant facial expressions in AD

patients contradicts this notion. Further, in one fMRI study AD patients, compared to controls, had greater pain-related activation within and connectivity between several cortical and sub-cortical pain processing structures[72,121]. Healthy individuals who are more facially responsive to noxious stimuli also show greater activation in pain processing regions and less activity in cognitive control (prefrontal & striatal) regions, compared to "stoic" individuals[102]. These considerations strengthen the likelihood that behavioral results found here and in prior studies[64,65,67,70,72,109] represent increased acute pain processing in AD and suggests a more vigorous approach should be considered in treating pain in AD patients. They also indicate a potential mechanism for increased pain sensitivity in AD may be related to a dysfunctional relationship between structures involved in pain affect, behavior, and cognitive control.

Using the FACS to measure facial responses to varied levels of pressure we found that, regardless of severity, AD patients showed more frequent and intense facial expressions in comparison to HS. These increases were due to more pain-relevant, than irrelevant, facial responses. FACS-based results did not correlate with subjective pain ratings via the FPS-R for AD or HS. However globally scored pain-behaviors, via portions of the PAINAD, strongly correlated with pain-relevant FACS indices. Evidence of increased acute pain sensitivity in AD obtained with experimental tools therefore reflects behaviors identifiable using clinical observational pain scales.

CHAPTER 3

RESTING NEURAL CORRELATES OF ALTERED PAIN RESPONSES IN ALZHEIMER'S DISEASE

INTRODUCTION

Under detection or treatment of pain in patients with Alzheimer's disease (AD) can significantly reduce quality of life. Several experimental studies probing subjective ratings and behavioral indicators of pain have found evidence of equivalent or even intensified pain processing in AD patients, relative to healthy seniors (HS)[65,72,71,70,64,67]. On the other hand, autonomic responses tend to be diminished, particularly as AD worsens[62,65–67], suggesting perhaps reduced pain processing. Interpretation of these seemingly contradictory behavioral findings is challenging, but necessary to improve pain assessment and treatment for patients. Our understanding of pain in AD would benefit from examining various behavioral pain indicators in the context of brain function in patients compared to HS.

Pain involves a complex interplay between sensory, affective, and cognitive dimensions[17]; each of these dimensions has their neuroanatomical bases in specialized supraspinal pathways, the lateral and medial pain system. The lateral system, associated with pain intensity, localization, and quality, is centered in somatosensory thalamus, cortex, and posterior insula[122,123]. The medial system is associated with pain unpleasantness, behavioral motivation, and cognition[18,19]; it consists of brainstem (e.g. periaqueductal gray; PAG), limbic (medial temporal, anterior insula, anterior/mid cingulate) and prefrontal structures[20,124]; many medial pain structures are also central autonomic regulators[125]. Thus, physiologic, affective-behavioral, and cognitive processes are integrated into cohesive adaptational responses to noxious or otherwise salient stimuli.

Imaging and pathological studies have confirmed that, while lateral pain structures are preserved until late AD, medial pain structures are preferentially targeted by early AD pathology. This does not necessary translate to reduced activity in these structures during pain, however; the limited studies examining brain function of AD patients in the context of pain have found equivalent neural EEG responses or increased and prolonged pain-induced fMRI activation in medial pain structures, such as hypothalamus, dorsolateral prefrontal, middle cingulate, and sensorimotor cortices, compared to HS. AD patients also had greater temporal synchronicity (i.e. functional connectivity) between these same structures, as well as the PAG. It was suggested that impairment of memory-based contextual appraisal of noxious stimuli lead to increase vigilance and responsiveness to pain in AD patients, compared to controls. Thus, a confluence of dysfunctional pain-related memory and cognitive processes may lead to signs of greater sensitivity in AD. However, this mechanism does not account for the dissociation between increased pain behaviors and increasingly blunted autonomic responses as AD worsens. To that end, no studies have examined neural correlates of various pain indicators in patients with more advanced AD.

Recent work using rs-fMRI has shown baseline fluctuations and connectivity between somatosensory, prefrontal, and medial pain structures strongly influences tactile and/or pain perception, as well as autonomic reactivity[126–128]. Altered connectivity between these structures and their associated functional networks has been found in those with pain disorders as well as AD patients. These observations support the notion that altered pain responses in AD may be facilitated, in part, through abnormal resting connectivity among pain-related structures and networks. To test this hypothesis, we performed psychophysical pressure pain testing in AD patients and HS controls and subsequent rs-fMRI scanning. We then evaluated how group

connectivity differences were related to subjective and behavioral pain responses in AD and HS. Finally, within AD subjects, we investigated how connectivity was associated with autonomic responsiveness.

METHODS

Subjects

Twenty patients with diagnosed probable AD (mean age: 76.5 ± 8.6 SD; 14 Females) and twenty-four HS subjects (mean age: 75.1 ± 6.7 SD; 14 Females) participated in this study. HS subjects were recruited through senior newsletters and local AD support groups. HS included only if they had no current pain or history of subjective memory complaint. AD subjects were recruited through the outpatient Cognitive & Geriatric Neurology Clinic at Michigan State University. Diagnosis of probable AD was made by a geriatric neurologist (ACB) based on DSM-IV and NINCDS-ADRDA criteria. However, patient medical histories were scrutinized to ensure exclusion of those with clinical evidence of probable mixed, vascular, frontotemporal, or Lewy body dementia. General study exclusion included history of: Type II diabetes, history of stroke or transient ischemic attack, central or peripheral neuropathy, diagnosis of neurological (e.g. seizure disorder) or psychiatric disorders (e.g. major depression, schizophrenia) other than AD. All participants were screened for baseline pain via subject interview, chart review, or caregiver discussion as inclusion required abstinence from analgesics for 24 hours prior to study. We excluded individuals with current arthritic pain, those with a history of arthritis in distal forearms (the stimulus application region), and those taking daily arthritic pain medication. As our index of autonomic response was heart rate (HR), individuals taking beta-blocker medications were excluded unless temporary discontinuation for a period equal to three-half lives prior to study was agreed upon by their primary physician.

Once subjects passed screening they underwent neuropsychological testing, including completion of Mini-Mental State Examination (MMSE)[89], and Cornell Scale for Depression in Dementia (CSDD)[90], and Neuropsychiatric Inventory Questionnaire (NPI-Q)[91]. Mean scores for neuropsychological tests are found in Table 3-1. No subjects had a CSDD score indicative of probable depression (>12)[90].

Testing procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Michigan State University Internal Review Board. Written informed consent was obtained for all HS as well as AD subjects via named health care proxies identified as a power of attorney for health care or guardian. We obtained assent from all participants before behavioral testing and MRI scanning.

Table 3-1 – Demographics and neuropsychological scores for Healthy senior (HS) controls and Alzheimer's disease (AD) subjects.

	HS Controls (n=24)		AD group (n=20)		p-value
	Mean	SD	Mean	SD	
Age	75.1	6.7	76.5	8.6	0.54[a]
Gender (male | female)	16 | 8		14 | 6		0.81[b]
MMSE	29.1	1.0	15.3	7.6	<0.001
MMSE range	26 – 30		0 – 23		
CSDD	1.2	1.3	8.6	3.4	<0.001
NPI-Q	0.54	0.51	7.8	4.7	<0.001

SD: standard-deviation \| MMSE: Mini-mental state examination \| CSDD: Cornell scale for depression in dementia \| NPI-Q: Neuropsychiatric inventory-questionnaire

a – Independent samples t-test
b – Chi-square test

Procedures

Psychophysical testing

The study took place over two sessions. In the first, psychophysical testing took place. Mechanical pressures were applied to the volar surface of the distal forearm (2-5cm from the wrist) using a Force Dial FDK 20 Force Gauge (Wagner Instruments, Greenwich, CT), which allows accurate recording of pressure (kg/cm^2). The device, scaled in units of 'kg,' is fitted with a 1cm wide rubber disc to prevent skin abrasion. Subjects were seated, upright, during testing. Stimuli ranged from 1-5kg in intensity. Each intensity was repeated four times, between the right and left forearms, in a pseudorandom fashion with the order determined once for use in all subjects. Stimulus order was limited by the following rules: no intensity could occur more than twice, sequentially; any sequential intensity repetition could not occur on the same arm. Stimuli were applied at a rate of ~1kg/s to peak intensity. Pressures were held at peak intensity for 5s prior to an ~50s interstimulus interval. A single investigator (PAB) performed all pressure testing. Continuous video recording of psychophysical testing allowed for recording of autonomic and facial responses. Stimulus onset and offset was marked audibly.

A portable infrared monitor (ePulse2™–Impact Sports Technologies), which was attached just above the elbow, displayed HR throughout testing. Responses were video recorded and reviewed later for scoring. HR was recorded on a fixed time window of every five seconds. A given response was determined by subtracting the HR at stimulus onset (baseline) from the maximum response within 30s after offset, resulting in an overall positive or negative response. Interstimulus intervals allowed for return to resting HR.

After each stimulus application (<5s after) HS and AD subjects who could do so were asked to rate their subjective pain levels via the Faces Pain Scale-Revised (FPS-R)[95]. The FPS-R

is a subjective pain assessor validated for clinical use, including in cognitively impaired patients from mild to early severe status[84,96]. In the scale, each of the five faces after the first, which represents 'no pain,' indicates stepwise two-point increase in numerical value, for a maximum score a 10. AD subjects able to pass a 3-question quiz that is part of the FPS-R[97] were deemed reliable pain reporters (n=13).

Facial expressions were analyzed using the Facial Action Coding System (FACS)[105], which codes the frequency and intensity of facial expressions through analysis of 44 anatomically distinct Action Units (AUs). AU intensity (except 45 and 46, which have no intensity) was scored on a 5-point A-E scale. An 'A' designation represents a trace contraction, while E indicates maximum contraction. The study coder (JTH), who passed FACS qualification testing, was blinded to stimulus order and group designation. Frequency and intensity of AUs present in each 5s pressure trial were scored. For purposes of the current analysis we focus on intensity of facial responses.

Prior to determination of which AUs were pain-relevant, we performed necessary data reduction steps[106,108,109], such that AUs involving the same muscular group were combined (AUs 1&2, AUs 6&7, AUs 9&10, and 25-27). Procedures for determining which AUs were pain-relevant (i.e. more specific to pain) were utilized as in prior studies utilizing the FACS in the context of pain by ourselves[129] and others[81,108,109]. Briefly, AUs meeting a critical threshold of occurrence (at least 5%), separately in HS and AD, in "pain-segment" stimuli (here defined as 4&5kg to increase pain-specificity) were selected. Those AUs that further met a *Cohen's d* effect size threshold of d=0.35 in both HS and AD subjects were deemed "pain-relevant." AU4 (brow lowered) and AU6&7 (orbit tightened) met these criteria. Finally, composite intensity scores

were calculated by averaging pain-relevant AUs separately for each stimulus trial across subjects.

fMRI scanning

In a separate session, anatomical and rs-fMRI data were collected with a GE 3.0 Tesla Signa HDx MR scanner (GE Healthcare, Waukesha, WI) located in the Radiology Dept. of Michigan State University. Anatomical scanning involved collection of 180 T1 weighted sagittal volumetric images (TE = 3.8 ms, TR = 8.6ms, time of inversion = 831ms, flip angle = 8°, field of view = 25.6 cm×25.6 cm, matrix size = 256 × 256, slice thickness = 1mm, receiver bandwidth ± 20.8 kHz). Next, subjects underwent two 7-minute resting-state functional echo planar image scans under dimly lit conditions (TE = 27.7ms. TR = 2500ms, flip angle = 80º, field of view = 220mm, voxel size = 3.438x 3.438x3mm, matrix 64x64 voxels, 168 brain volumes via 36 contiguous axial 3mm slices). One AD subject was unable to complete the second resting-state scan. Subjects were instructed to hold still as much as possible, with their eyes open, and stay awake. Wakefulness was monitored during resting-state scanning through an MR compatible eye camera, which was attached to the radiofrequency head coil.

Statistical methods

Psychophysical analysis

Generalized linear modeling (GeLM: Generalized linear mixed modeling and generalized estimating equations) in SPSS ™ (Version 22.0, Armonk, NY: IBM Corp) determined impact of level-two effects (subject group – HS and AD) on level-one effects (FACS intensity composites and FPS-R scores, respectively), with subject and stimulus level as predictors. GeLM accounts for repeated measures (trials) and nuisance covariates (age, gender, and CSDD, NPI-Q severity). FPS-R data were each recoded into a set of five clustered scores to improve modeling of its non-

normal distribution. Significant 'group' (HS vs AD) or 'group*stimulus level' interaction effects ($p<0.05$) were followed-up with post-hoc nonparametric independent samples testing between groups under each stimulus level for FACS intensity composites and FPS-R scores. The exact test for each comparison was determined by SPSS based on their distributions. Post-hoc comparisons involved independent samples Mann-Whitney U testing.

We next examined whether AD subjects generally exhibited blunted autonomic responses with increased AD severity through GeLM (general linear mixed modeling) analysis of HR change between those with more mild/moderate disease (MMSE 11-23) and those with more severe disease (MMSE <11). To do so, HR changes were recoded to reflect no change (0), positive response (+1), or negative response (-1). GeLM was performed as described above, though with age and gender included as nuisance variables. A significant result was followed-up with testing differences at individual pressure levels between the aforementioned subgroups with Mann-Whitney U post-hoc testing. The latter testing occurred on non-recoded HR data.

fMRI analysis

Individual subject fMRI standard pre-processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Steps included: removal of the first two volumes due to enhanced longitudinal magnetization in the first few scans; brain extraction[130]; motion correction[131]; spatial smoothing (FWHM 5mm); and highpass temporal filtering (sigma 100s). Individual subject functional scans were nonlinearly registered to a standard MNI space through their structural scan[131–134]. Results of non-linear registration of anatomical and functional images were manually checked against the standard MNI152 template (see Figure 3-1). Single subject independent component analysis (ICA)-based exploratory data analysis was then carried out using

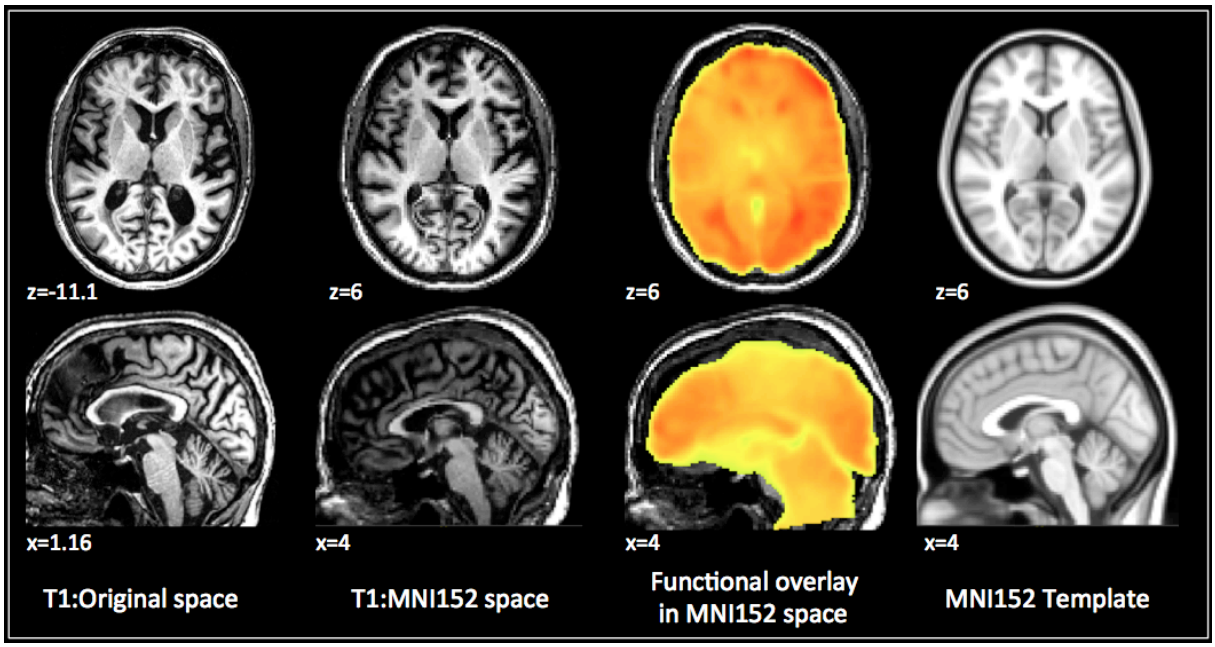

Figure 3-1 – Representative results of FSL's FNIRT non-linear registration of anatomical and functional images to MNI152 standard space for a severe AD subject.

MELODIC[135] (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.13, in to investigate possible artifacts or activation. Resulting components for each subject were then visually inspected to sort out those likely representing neuronal signal versus artifact in accordance with Kelly and colleagues[136]. Artifactual components were then regressed out of subject functional time series, resulting in denoised datasets.

We then computed voxelwise regressors to remove effects of regional atrophy on connectivity measures. For each subject, individual anatomical T1 scans, already in MNI space, were segmented using FSL's FAST[137] to obtain gray matter (GM) partitions. Individual Jacobian maps were then calculated using warp coefficients produced during nonlinear registration of subject anatomical scans. The GM partitions produced by FAST were then modulated by the Jacobian maps to reflect areas of local expansion or contraction of atrophied regions. Individual

Jacobian modulated GM partitions were then merged into a single 4D file to be used as a voxelwise nuisance regressor in group analyses.

To examine the resting neural correlates of our behavioral measures three General Linear Models (GLMs) were created, one for testing associations of resting-connectivity with each behavior measure (facial, subjective, and autonomic). All GLMs included regressors of interest (behavioral measures) and non-interest (age, gender, voxelwise atrophy measures). Non-voxelwise covariates were demeaned prior to GLM entry. The first GLM was tested associations between resting connectivity and FACS-based pain-relevant intensity response differences, at the 5kg pressure level, between AD (n=20) and HS (n=24). The second examined correlations between connectivity and FPS-R scores, at the 2kg level, for AD (n=13) and HS (n=24). The final GLM tested, within the AD group, associations between connectivity HR responses at the 5kg level. The most intense pressure level was chosen for FACS and HR-related GLMs to better ensure that connectivity associations pertained to at least moderately painful pressures (as per average subjective ratings for both groups). The 2kg pressure level was chosen for the FPS-R GLM as it was rated as significantly more painful by AD subjects able to self-report. Once all GLMs were created, further steps were taken prior to group analyses.

To examine group-level effects, we first carried out Group-level ICA (GICA), again using MELODIC[135]. Three GICAs were calculated, one for each GLM. This occurred to better ensure that connectivity differences reflected RSNs actually present in subjects included in each GLM. GICA-1 (for further facial expression analyses) included all scanned subjects. GICA-2 (for FPS-R analysis) included all HS and 13 AD subjects. Finally, GICA-3 included all 20 AD subjects. Using Principal Component Analysis with automatic estimation of dimensional number and variance normalization, individual functional scans were temporally concatenated and

decomposed into independent spatial maps characteristic of the entire study sample[135,138,139]. During the process, non-brain voxels were masked. Estimated component maps were thresholded ($p > 0.5$) by fitting a mixture model to the histogram of intensity values[135]. This process resulted in 28 components for GICA-1, 41 for GICA-2, and 28 for GICA-3. Visual comparison to "canonical" resting-state networks (RSNs)[140] indicated 24, 30, and 23 components, for GICAs 1-3, respectively, were neuronally plausible.

Spatial maps from each GICA were then used to generate subject-specific versions of the spatial maps, and associated time series, using dual regression[141]. For each subject, all estimated component spatial maps were regressed (as spatial regressors) into the subject's 4D space-time dataset, resulting in subject-specific time series, one per group-level spatial map. Next, individual subject time series were regressed (as temporal regressors) back into a single 4D dataset, resulting in a set of subject-specific spatial maps, again one per group-level spatial map.

Further analysis was limited to those components whose spatial distributions closely matched RSNs or structures associated with aspects of pain and/or autonomic regulation (i.e. salience, default mode, executive/fronto-parietal, somatomotor, and limbic RSNs or related structures – visualized in Figures 3-3 to 3-5 for GICAs 1-3, respectively). Each GICA's selected components were subjected to nonparametric permutation-based testing via FSL's randomise[142,143] tool (10,000 permutations), with threshold free cluster enhancement (TFCE)[144]. GLMs were incorporated appropriately in order to test the relationship between connectivity of selected components and behavioral measures. We addressed multiple hypothesis testing by controlling family-wise error (FWE) with a threshold of $p<0.05$.

To examine connectivity differences strictly between RSNs of interest we tested how temporal relationships between them varied in association with the GLMs outlined above. To do

so, we implemented the FSLNets package (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets) in Matlab (http://mathworks.com/products/matlab/). For each network of interest: individual subject time courses were extracted and normalized to their standard deviations; time courses for artifactual components, and those not of interest, were regressed out of each subject's time course; subject-level partial correlation matrices (L1-regularized, lambda=10), which are thought to better represent "direct" connections better than full correlations, were produced, based on the residual time series of component; each matrix's r-coefficients were z-transformed and subjected to autocorrelation correction; finally, individual subject correlation matrices were averaged to form group a matrix. The group-level matrix was then pre-masked with a t-value > 8, preventing connections that were not strong on average across all subjects from being tested. Nonparametric permutation testing on networks of interest occurred via randomise and GLM integration as outlined above (though with 5000 permutations). Multiple comparison control occurred through FWE correction, threshold of $p<0.05$.

RESULTS

Psychophysical testing results

In testing HS and AD subject differences in pain-relevant FACS composite score intensity we found no significant main effect of group (generalized estimating equations: Wald Chi-Square=0.187; p=0.66), but did find a significant group*stimulus level interaction (Wald Chi-Square=60.8; $p<0.001$). As indicated in Figure 3-2A, post-hoc Mann-Whitney U testing indicated that AD subjects showed more intense pain-relevant facial responses at 3-5kg pressure levels (Standardized Test Statistic, STS=4.2, 4.2, 2.2; $p<0.001$ and 0.027). Intensity of AD responses at 1-2kg pressure levels were marginally greater than HS (STS=1.9, 1.9, and 2.2; p=0.057, 0.056, respectively).

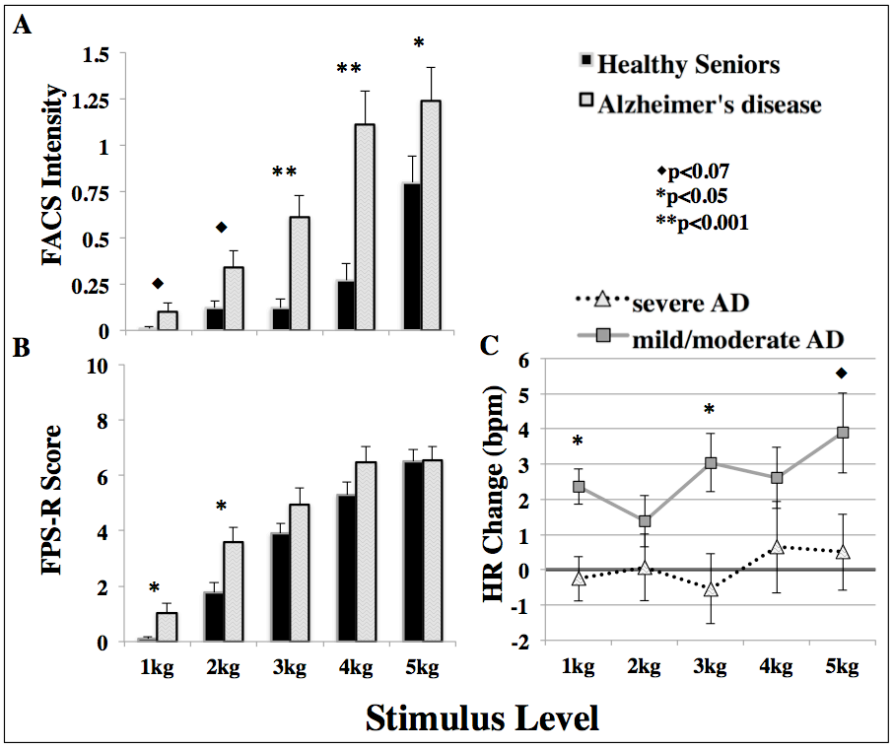

Figure 3-2 – Measured pain responses across individual pressure stimulus levels. A) Average composite intensity scores of pain-relevant Action Units for Healthy senior (HS) and all Alzheimer's disease (AD) subjects; B) Average Faces Pain Scale-Revised scores of all HS and those AD subjects who could reliably self-report; C) Average heart rate (HR) responses of AD subgroups.

Table 3-2 – Resting state networks whose connectivity significantly correlated with behavioral measures between (FACS, FPS-R) and within (HR changes) groups.

IC #	RSN	Statistical Connectivity Contrast	P_{FWE}	#Vox	MNI coord. X	Y	Z	Hemi	Cluster Location
GICA1 - FACS 5kg: AD vs HS									
IC 1	PCC & Precuneus (pDMN)	Interaction HS<AD	0.013	74	6	-38	20	R/L	PCC
		Interaction HS<AD	0.044	7	-6	-58	8	L	Rsp
IC 3	Mesial Temporal	Interaction HS < AD	0.006	40	-2	38	-12	R/L	pACC
		Interaction HS<AD	0.017	11	42	-10	-24	R	Fusiform
IC14	Premotor	Interaction HS<AD	0.031	7	-22	62	-12	L	Frontal Pole
GICA2 - FPS-R 2kg: AD vs HS									
IC 3	pDMN	Interaction HS < AD	0.005	399	18	-70	32	R	Precuneus
		Interaction HS < AD	0.005	204	10	-34	20	R/L	Posterior cingulate cortex
		Interaction HS < AD	0.005	103	42	-50	36	R	Intraparietal lobule/angular gyrus
IC 9	Mesial Temporal	Interaction HS > AD	<0.001	35	34	18	-32	R	Temporal pole
IC 13	Ventral Somatomotor	Interaction HS < AD	0.038	5	-42	-14	20	L	Secondary somatosensory cortex
GICA3 - HR 5kg: AD only									
IC 1	pDMN	Pos HR effect	0.002	328	6	-62	32	R/L	PCC/Precuneus
IC 14	Ventral Salience 2	Neg HR effect	0.001	261	-2	42	-16	R/L	OFC/pACC
		Neg HR effect	0.001	75	-30	22	-8	L	aIns
		Neg HR effect	0.009	5	-42	-6	-4	L	pINS

IC: Independent Component | RSN: Resting State Network | #Vox: Number of voxels within the cluster | MNI Coordinate: cluster maximum in mm | Hemi: Hemisphere (L: Left or R: Right) | GICA: Group ICA designation | FACS: Facial Action Coding System | FPS-R: Faces Pain Scale – Revised | AD: Alzheimer's disease | HS: Healthy Seniors | Rsp: Retrosplenial cortex | pACC: Pregenual Anterior Cingulate Cortex | PCC: Posterior cingulate cortex | pDMN: posterior DMN component | Neg/Pos HR: Negative or Positive Heart Rate effect | OFC: Orbitofrontal Cortex | PAG: Periaqueductal gray | aINS: anterior insula | pINS: posterior insula

Subjective pain ratings were also greater in AD subjects, compared to HS. We found both significant group and group*stimulus level effects (generalized linear mixed modeling: F=12.3 & 733.3; p<0.001). Post-hoc Mann-Whitney U testing found significantly greater pain ratings by AD subjects for 1-2kg pressure levels (Figure 3-2B).

Advanced AD patients showed blunted HR responses, compared to more mild patients. GeLM testing (generalized linear mixed modeling) found significant group and group*stimulus level interaction effects (F=5.7 & 801.9; p=0.004, <0.001, respectively). As indicated by Figure 3-2C, post hoc Mann-Whitney U testing found significantly lower HR responses in advanced AD subjects, compared with mild/moderate AD subjects, for stimulus levels 1 & 3kg, and marginally so at 5kg (STS: 2.7, 2.3, 1.9; p=0.005, 0.019, and 0.056, respectively).

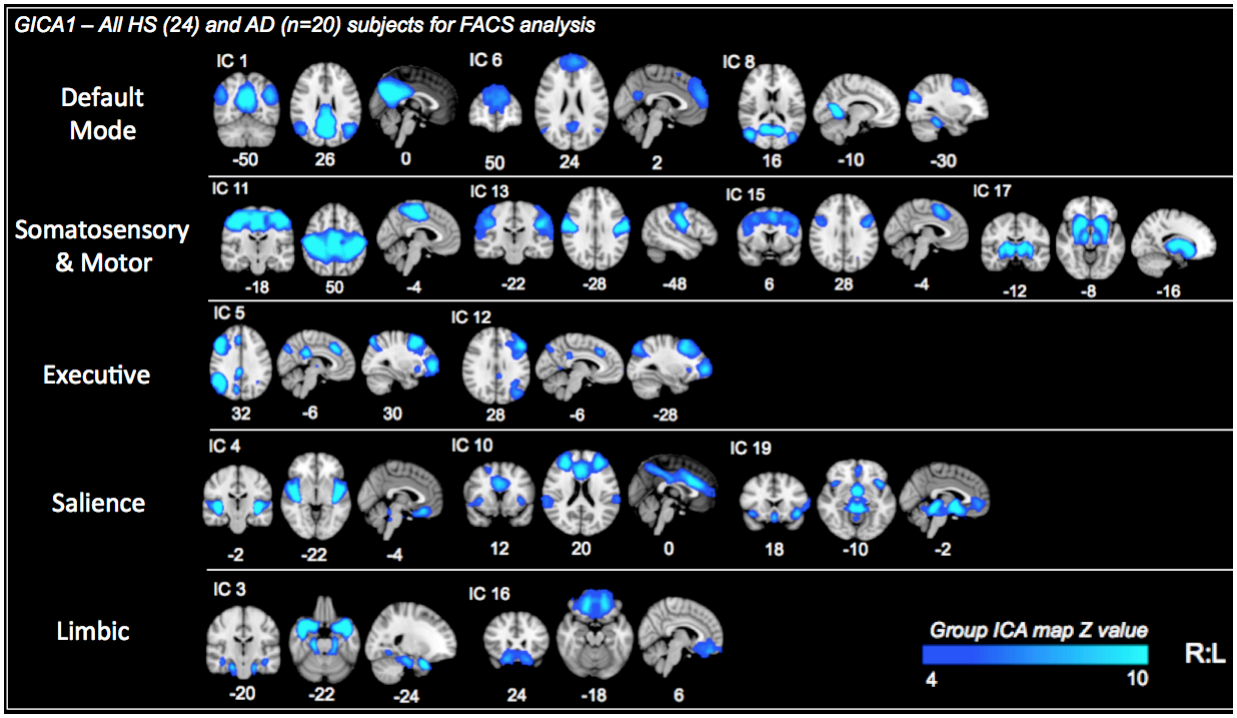

Figure 3-3 – Independent Components (ICs) of interest from Group ICA #1 (GICA1). These ICs were used in permutational tests exploring relationships between connectivity and facial pain responses across all subjects. MNI coordinates are below each IC.

Functional connectivity results

Fourteen ICs from GICA1 (visualized in Figure 3-3) were entered into dual-regression/randomise and FSLNets analyses to examine how group differences in connectivity were related to intensity of pain-relevant facial responsiveness (i.e. FACS intensity scores) at the 5kg level. In so doing, we found a significant interaction effect of diagnosis (HS vs AD) and facial response intensity within the posterior DMN (pDMN, GICA1 – IC 1), at a cluster in the posterior cingulate cortex (PCC), and between a mesial temporal network (GICA1 – IC 3) and a cluster in the pregenual anterior cingulate cortex (pACC; clusters reached $p<0.05$, FWE corrected; Table 3-2; results visualized in Figure 3-6A). As indicated in the top of Figure 3-6B, HS facial response intensity was negatively correlated with connectivity within the pDMN while AD patients tended toward the opposite relationship. Likewise, connectivity between the mesial temporal network and the pACC was positively correlated with facial response intensity of AD patients, but negatively correlated with HS responses. In contrast, connectivity between posterior and ventral DMN (pDMN & vDMN; GICA1 – ICs 1 & 8, respectively; Figure 3-6C) was marginally associated with HS facial responses and somewhat negatively associated with AD responses ($p=0.08$, FWE; Figure 3-6D).

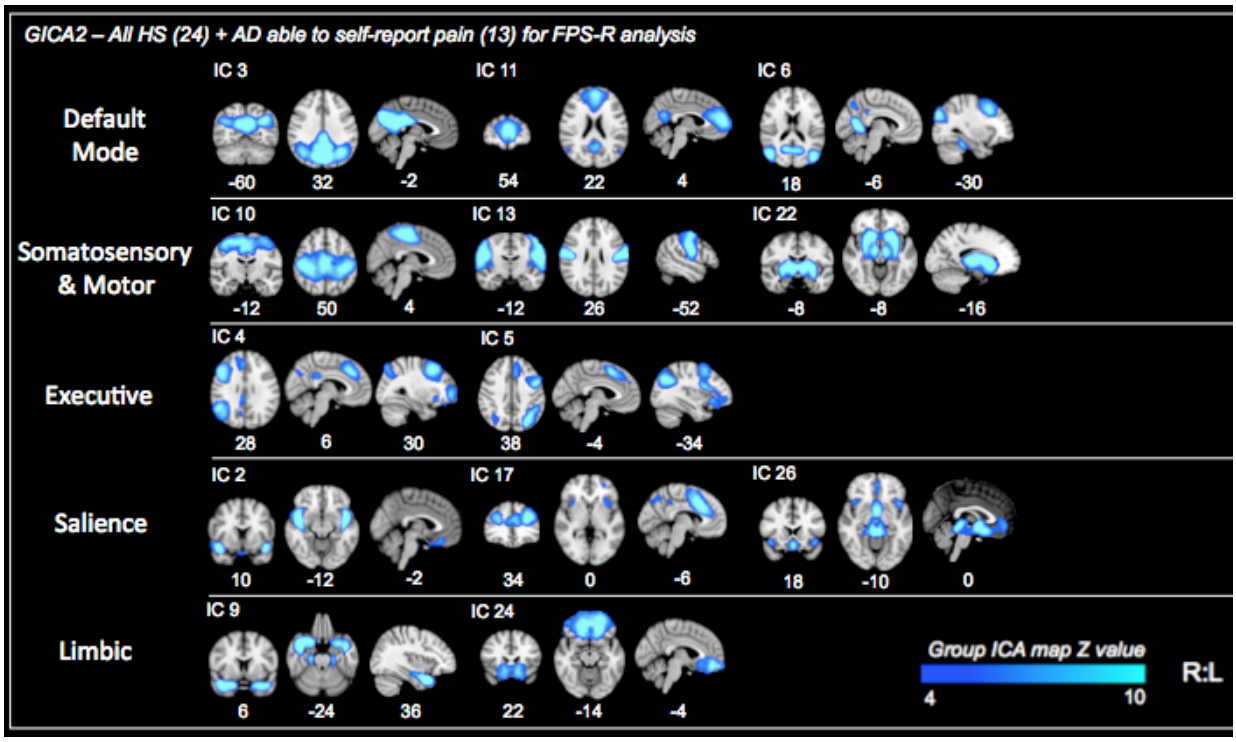

Figure 3-4 – Independent Components (ICs) of interest from Group ICA #2 (GICA2). These ICs were used in permutational tests exploring relationships between connectivity and subjective pain ratings in subjects able to self-report. MNI coordinates are below each IC.

Fourteen ICs from GICA2 were selected for further analysis of the relationship between connectivity and subjective pain-rating differences (FPS-R scores) between groups via dual-regression/randomise and FSLNets analyses (Figure 3-4). Among these ICs, three showed significant connectivity interactions for diagnosis and subjective pain ratings ($p<0.05$, FWE corrected, Table 3-2). Within the pDMN (GICA2 – IC 1) clusters in the PCC, precuneus, and intraparietal lobule/angular gyrus (Table 3-2; Figure 3-7A, top) were positively correlated with pain ratings of AD subjects but negatively correlated with pain ratings of HS (Figure 3-7B, top). Connectivity within a ventral somatomotor network (GICA2 – IC 13; Table 3-2; Figure 3-7A, bottom), specifically at a cluster located in the left secondary somatosensory cortex (S2) also showed an interaction with diagnosis and subjective pain ratings; greater subjective pain ratings in AD subjects were positively correlated while in HS there was a weak opposite relationship

(Figure 3-7B, bottom). In contrast, connectivity within a mesial temporal network (GICA2 – IC 9; a right temporal pole cluster) was negatively associated with pain ratings in AD subjects, compared to a weak positive relationship in HS subjects (Table 3-2). Between network connectivity pertaining to the mesial temporal network and ventral salience network (vSN; GICA2 – ICs 9 & 2; Figure 3-7C) also showed an interaction effect for diagnosis and subjective pain ratings. As visualized in Figure 3-7D, connectivity strength was positively correlated with FPS-R scores in AD subjects, but negatively correlated with FPS-R scores in HS.

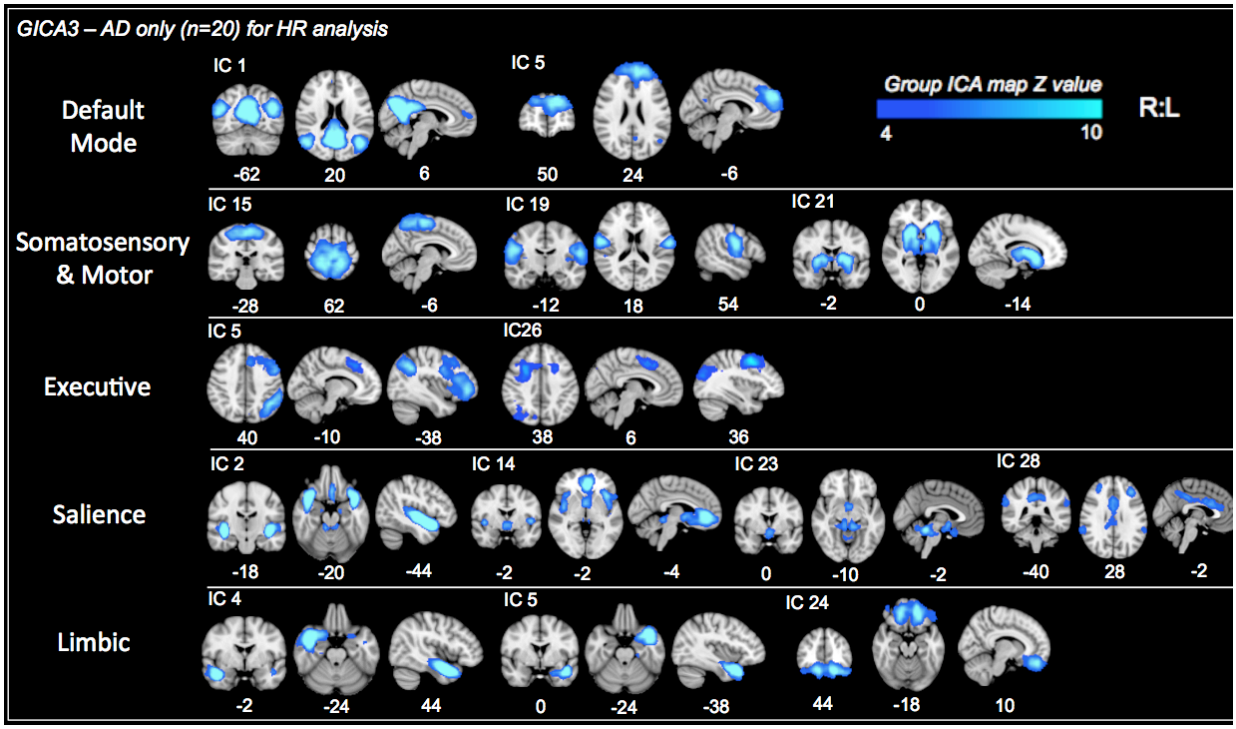

Figure 3-5 – Independent Components (ICs) of interest from Group ICA #3 (GICA3). These ICs were used in permutational tests exploring relationships between connectivity and autonomic pain responses in AD subjects. MNI coordinates are below each IC.

Thirteen IC networks from GICA3 underwent dual-regression/randomise and FSLNets analyses to probe the relationship between connectivity and autonomic responses (HR), specifically of AD patients (Figure 3-5). Two networks showed significant correlations with patient autonomic response: connectivity within the pDMN (GICA3 – IC 1), specifically at a

cluster located in the PCC/precuneus, positively correlated with HR responses of AD patients ($p<0.05$, FWE corrected; Table 3-2; Figure 3-8A); however, connectivity within the vSN (GICA3 – IC 14), specifically at the ventromedial prefrontal cortex (vmPFC) & pACC, left anterior insula (aINS), and left posterior insula (pINS) was negatively correlated with HR responses of patients (Figure 3-8B). No significant between network connectivity relationships were found for HR changes.

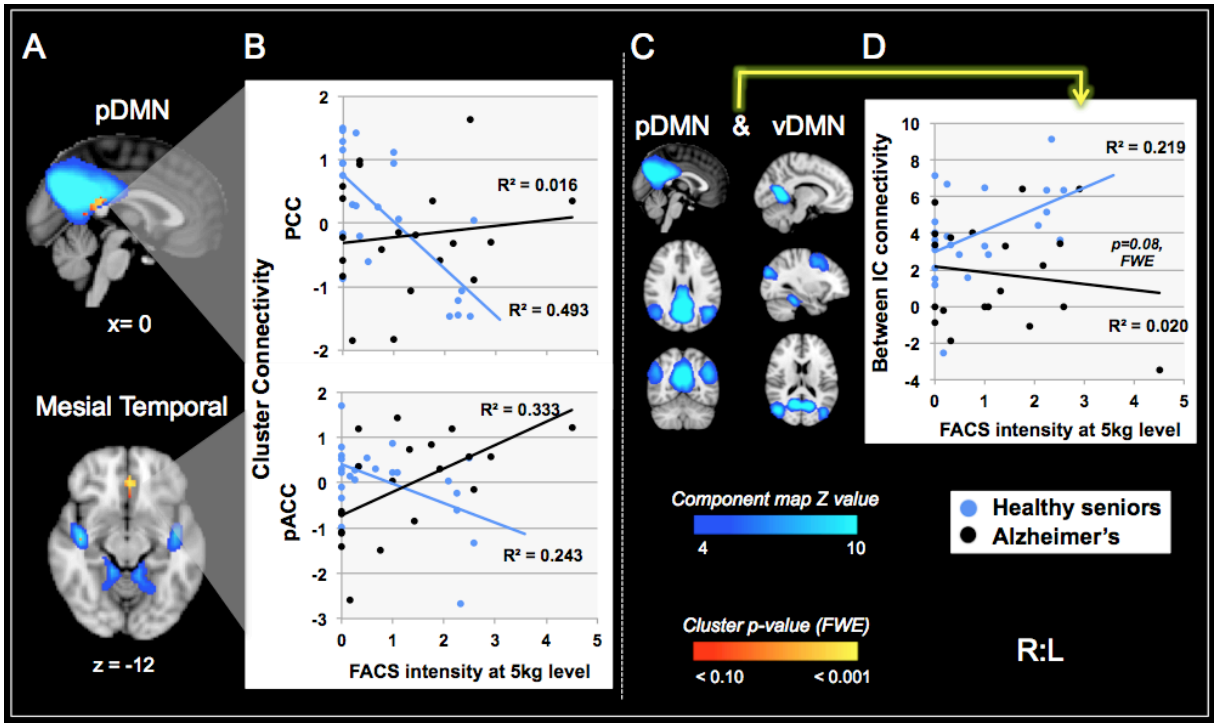

Figure 3-6 – Group connectivity differences associated with pain-relevant facial response (FACS) intensity at the 5kg pressure. Resting-state networks (from GICA1) presented in blue, while significant clusters are red-yellow: A) Connectivity within the posterior DMN (Top, posterior cingulate, PCC, cluster) and between a Mesial Temporal network and the pACC (bottom) showed significant interaction effects for diagnosis and FACS intensity; B) Visualization of these interactions, with beta-values (corrected for age, sex, and depression scores) of PCC (top) and pACC (bottom) clusters plotted against FACS intensity scores; C) Connectivity between posterior DMN and ventral DMN showed a marginally significant interaction effect for diagnosis and FACS intensity; D) Visualization of the interaction, with relative connectivity strengths (Z-transformed partial correlations) plotted against FACS intensity scores. Unless otherwise indicated, results are significant at $p<0.05$, FWE corrected. For visualization purposes, clusters are thresholded at $p<0.1$, FWE. FACS – Facial Action Coding System; PCC – posterior cingulate; pACC – pregenual anterior cingulate.

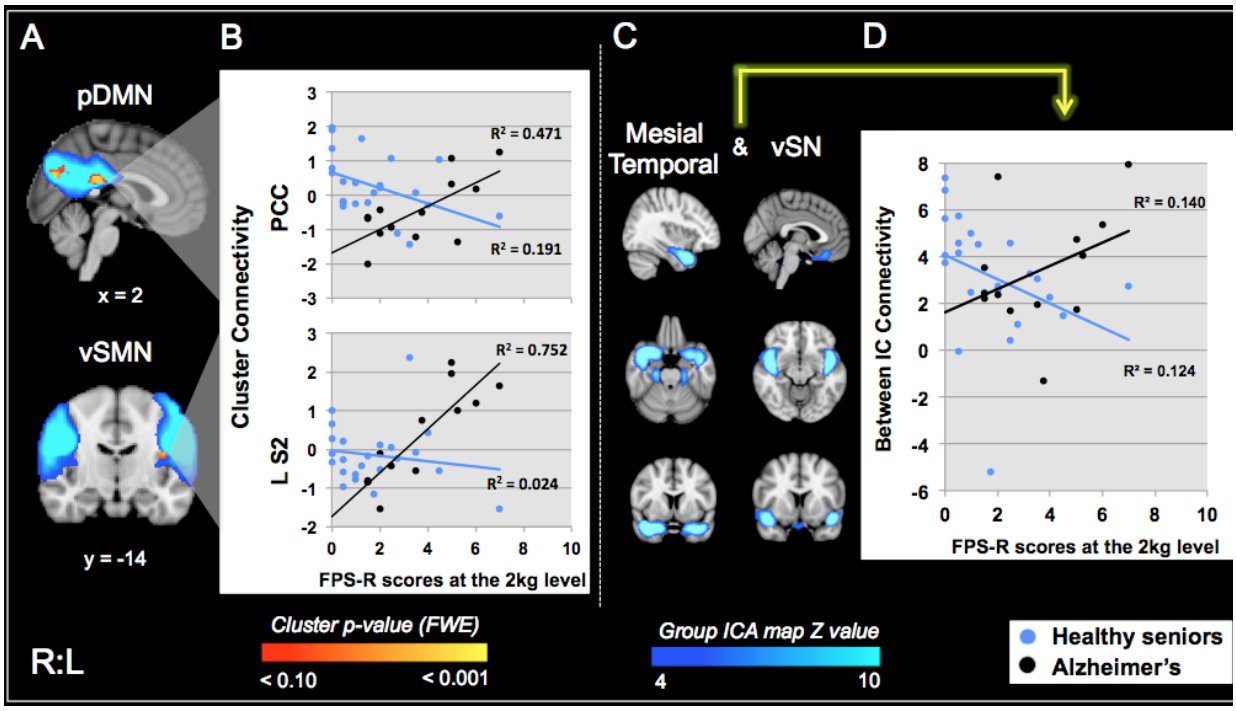

Figure 3-7 - Group connectivity differences associated with subjective pain ratings (FPS-R scores) at the 2kg pressure level. Resting-state networks (from GICA2) presented in blue, while significant clusters are red-yellow. A) Connectivity within the posterior DMN (top, in PCC and precuneus clusters) and the ventral SMN (bottom, S2 cluster) showed a significant interaction effect of diagnosis and FPS-R scores; B) Visualization of the interactions, with beta-values (corrected for age, sex, and depression scores) of the PCC (top) and S2 (bottom) clusters plotted against individual FPS-R scores; C) Connectivity between Mesial Temporal and ventral SN also showed a significant interaction between diagnosis and FPS-R scores; D) Visualization of the interaction, with relative connectivity strengths (Z-transformed partial correlations) plotted against FPS-R scores. Unless otherwise indicated, results are significant at $p<0.05$, FWE corrected. For visualization purposes, clusters are thresholded at $p<0.1$, FWE. FPS-R – Faces Pain Scale-Revised; SMN – somatomotor network; S2 – secondary somatosensory cortex; PCC – posterior cingulate; SN – salience network.

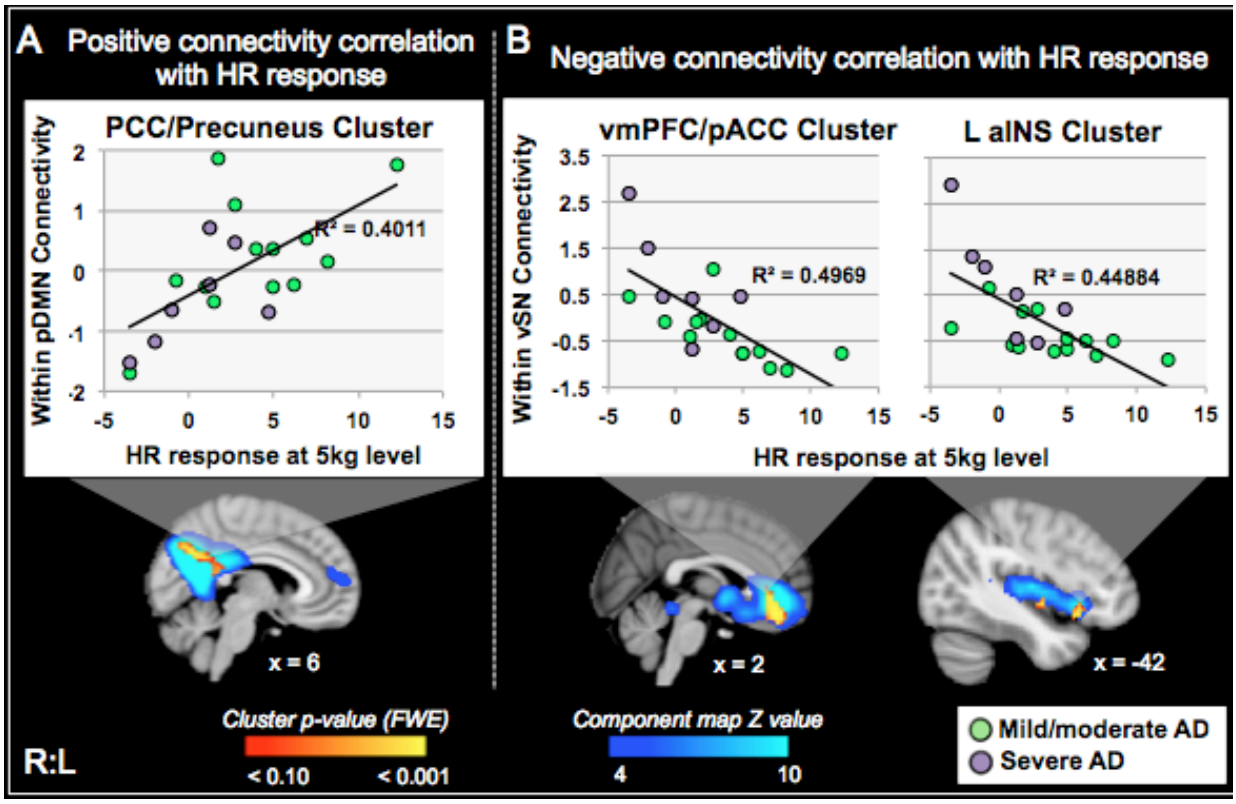

Figure 3-8 – Network connectivity associated with autonomic pain response (HR) within the AD group at the 5kg pressure level. A) Connectivity within the posterior DMN (bottom, PCC/precuneus cluster) positively correlated with autonomic responses in patients. This relationship is visualized at the top, where beta-values (corrected for age and sex) are plotted against autonomic responses of patients; B) Connectivity within a ventral SN (bottom left - vmPFC/pACC,bottom right - left anterior and posterior insular clusters) negatively correlated with autonomic responses in patients. This relationship for the vmPFC/pACC cluster is visualized at the top, where beta-values (corrected for age and sex) are plotted against autonomic responses of patients. Mild/moderate AD = MMSE 11-23. Severe AD = MMSE <11. All clusters reached $p<0.05$, FWE corrected. For visualization purposes clusters are thresholded at $p<0.1$ (FWE). PCC – posterior cingulate; vmPFC – ventromedial prefrontal cortex; pACC – pregenual anterior cingulate; aINS – anterior insula.

Discussion

There is mounting support for the notion that pain sensitivity in AD is increased, compared to HS. However, our understanding of the neural mechanisms underlying these findings is limited. Here, we first replicated prior studies' findings of greater pain-related facial response intensity and subjective ratings of pain in AD patients, relative to controls[70,109,129], as well as diminished autonomic responses seen with increasing AD severity[62,66,67]. Then, after

controlling for age, gender, depression scores, and regional atrophy we found that connectivity related to default mode, salience, somatomotor, and limbic-related networks was significantly associated with the altered pain responses seen in AD patients.

Prior studies have found greater pain-related facial expressions and pain ratings in AD patients[64,72,109,129], which we confirmed in our scanned sample. Connectivity between a mesial temporal network and a cluster in the pACC correlated positively with facial expression intensity in AD subjects, but negatively so in HS. The same relationship was found for between network connectivity of the mesial temporal network and vSN for subjective pain ratings. In contrast, connectivity within the mesial temporal network was negatively correlated with pain ratings in AD patients. These findings suggest altered affective processing with respect to heightened pain responses in AD. Mesial temporal structures, such as the parahippocampal gyrus and temporal poles are implicated in pain-related anxiety & threat appraisal and socioemotional regulation, respectively[145–147]. These structures are among the earliest to be affected by AD[57,148]. The pACC is part of the affective segment of the cingulate cortex and is also implicated in top-down pain modulation[149–152]. The vSN was comprised of insular and orbitofrontal cortices, which are implicated in emotion, anxiety, and directing attention to salient internal or external events[27,153–155]. Connectivity of the SN has been found increased, at least transiently, in AD[85,156]. Greater connectivity of the mesial temporal network to the pACC and vSN, along with and reduced connectivity within the mesial temporal network, suggests that the increased emotional display and subjective ratings of pain in AD are related to enhanced pain affect/anxiety and reduced affective regulation. Indeed, emotional disturbances are common in AD patients and are correlated with dysfunction in salience and affect associated structures[157–160]. The facial expression of pain is a means of communicating an unpleasant subjective state between

individuals[118]. It seems logical, then, that in AD patients aberrant connectivity among networks associated with socioemotional regulation and/or affect was found.

Though not considered a typical network affected by AD, altered function and connectivity of somatomotor structures have been reported in patients[85,86,121,161]. Here we found a relationship between SMN connectivity and subjective pain ratings. Specifically, there was a positive correlation between pain ratings in AD patients and connectivity within a ventral SMN at a cluster located at S2, whereas HS exhibited the opposite relationship. S2 is associated primarily with the sensory component of pain, specifically its intensity and discriminative aspects[19]. Thus, a propensity toward increased pain intensity may contribute to heightened pain sensitivity in patients as well. That altered behavioral and subjective pain in AD involves enhanced sensory/affective experience is consistent with findings of a prior study by Cole and colleagues[72]. They found that AD patients, compared to HS, exhibited greater amplitude and duration of pain-induced activity in regions associated with the somatomotor and affective dimensions of pain. Healthy individuals who are more facially expressive during pain also exhibit greater affective and somatomotor-related activation than stoic/less pain-sensitive individuals[40,102].

Our findings also suggest altered DMN dynamics play a role in greater behavioral and subjective pain responses in patients. Many studies have found functional connectivity alterations within the DMN of AD patients[162,163,86,164,85]. However, there is spatial and temporal variation in this disruption[161] and DMN-related connectivity alterations may include increased connectivity patterns[86], even in advanced AD[166]. We found that connectivity within the pDMN was negatively correlated with HS facial pain response intensity, whereas the opposite occurred in AD patients. A similar pattern occurred within the pDMN for subjective pain ratings. The

DMN is typically thought of as a "task negative" network implicated in internally directed attention and autobiographical memory retrieval[167–169]. However, in the context of pain DMN-related function is more complex. For example, the DMN is activated during periods of high pain, whereas it is deactivated during low pain or tactile somatosensation[170–172]; activation of the DMN during highly experimental pain was proposed to represent increased internal mentation directed toward threatening (painful) stimuli[171,172]. Thus, our DMN-related results suggest an increased propensity of AD patients, compared with HS, to attend to and inwardly evaluate stimuli perceived as noxious. Consistent with this notion, greater connectivity within the pDMN predicted increased pain sensitivity in chronic low back pain patients[173].

We also found that connectivity between the pDMN and vDMN was positively correlated with HS facial pain responsiveness, but not those of AD patients. Functional disconnect between pDMN and vDMN components in AD patients has been reported in a number of studies[174–176]. The vDMN, constituted by retrosplenial, parahippocampal, and posterior-inferior parietal cortices is implicated in episodic memory retrieval[177] and construction of mental scenes for future-oriented thought[178]. Thus, heightened sensitivity to pain in AD may, in part, relate to a predisposition for impaired integration of self-referential (pDMN) with memory-based contextual appraisal (vDMN) mechanisms. Impaired integration in this regard, coupled with the propensity toward greater sensory and affective pain experience as discussed above, could thus contribute to greater inwardly focused attention with respect to pain. The end result of these resting connectivity changes could be greater subjective and behavioral manifestations of pain in patients. This idea is consistent with interpretation Cole and colleagues of greater pain induced activation in AD patients[72].

Confounding understanding of pain in AD is the tendency for patients, particularly those who are more impaired, to show blunted autonomic pain responses compared to controls[65,62,66,70,67]. The result is a curious dissociation between behavioral/subjective and autonomic pain indicators in patients[67]. Indeed, in our scanned sample we confirmed reduced HR responses for low, mid, and high level pressure stimuli in more advanced AD patients (MMSE<10), compared to more mild/moderate patients (MMSE 11-23). As in facial and subjective pain analyses, connectivity within the pDMN was again significantly correlated with altered pain responses in AD patients. Specifically, a cluster spanning the PCC & precuneus was positively correlated with HR responsiveness in patients. In contrast, within-network connectivity for the vSN (containing insula, pACC, vmPFC, PAG, and hypothalamus) was negatively correlated HR responses of patients, specifically at clusters located in the left vmPFC, aINS, and pINS.

A major putative role for the DMN is passive environmental/sensory monitoring and preparation for environmental demands[179,180]. These processes actually require a very alert brain. It is logical then that alpha EEG power, an index of subjective arousal and cortical excitability, is associated with DMN activity at rest[181–183]. In AD patients, delta and theta power, which are associated with sympathetic suppression[184–187], increasingly predominate during wakefulness as the disease progresses [reviewed in 188,189]. Interestingly, Benedetti and colleagues found the degree of relative delta power both increased with AD severity and was negatively correlated with autonomic responses to pain in patients and controls. These findings suggest that impaired resting cortical arousal, in accordance with reduced DMN function, would be associated with blunted autonomic responses in more advanced AD. The positive association of within-pDMN connectivity and HR responses of AD patients, found here, supports this notion.

The negative correlation within the vSN for clusters in the vmPFC, and left aINS & pINS are particularly compelling findings. Each of these structures are part of the central autonomic network (CAN)[27,31]. Along with many other CAN/vSN structures, the vmPFC and insular cortices are affected early and progressively by AD[58,59,190]. Left insular cortex and vmPFC are both implicated in states of reduced sympathetic tone[191–196]. Interestingly, AD pathology in the vmPFC was predicted by Chu and colleagues[58] to contribute to autonomic disturbances in AD; insular pathology has been proposed to contribute to autonomic morbidity and mortality in AD patients[197,198]. Blunted HR responsiveness in more advanced AD would therefore appear, in part, to be related to hyperactive parasympathetic and hypoactive sympathetic cortical influence mediated by the vmPFC and left insular cortices.

Prior studies have found that, despite a tendency toward blunted autonomic responses, behavioral pain responses are increased in patients, relative to HS[65,70,129]. Interactions between nociceptive and autonomic systems are widespread, allowing for cohesive cognitive, affective, and somatomotor responses to internal or external environmental demands[26,27]. This makes the autonomic-behavioral dissociation seen in AD a somewhat paradoxical finding. According to Damasio's "Somatic Marker" hypothesis[199], the vmPFC, insula, and other structures within the vSN (e.g., hypothalamus, and PAG) perform important interoceptive functions (i.e. monitoring the body's internal physiological condition) and in so doing generate feelings and emotion[199,200,32,201,202]. Our results here suggest this dissociation reflects dysfunction at the neural interface of affect, salience, interoception and autonomic regulation. The vmPFC and insular cortices are prime candidates in mediating this dysfunction[27,154,200,203–205]. Though low to moderate levels of noxious stimuli induces greater degrees of affective, sensory, and attentional processing in patients[72,121], the signal may not be able to override the high threshold posed by a

"hyperactive" parasympathetic cortical influence mediated by the vmPFC and left insular cortex. This could result in an increased noxious stimulus threshold necessary for 'quasi-normal' autonomic pain responses[62,67]. At the same time, a propensity toward greater salience, emotional processing, and inwardly focused attention toward pain, in conjunction with impaired contextual appraisal of pain, could lead to greater subjective and behavioral signs of pain in patients, regardless of autonomic-related dysfunction.

These findings are limited by their correlational nature. Future work examining the relationship between pain-induced activations and pre-stimulus neural activity would provide a better estimation of how RSN connectivity contributes to greater pain sensitivity in AD. Also, a more comprehensive examination of autonomic function across AD severity in the context of pain, perhaps using hear-rate variability measures, would help to verify the sympathetic/parasympathetic balance with respect to pain in AD. Some caution is necessitated in directly comparing results from facial responses and subjective pain ratings as they pertained to different pressure levels (5kg for facial responses and 2kg for subjective report). Here the 2kg level was examined for subjective pain ratings as no higher stimulus level lead to significantly higher pain ratings in patients. Nevertheless, it is encouraging that several RSNs (i.e. the pDMN, mesial temporal, and vSNs) showed significant relationships across multiple types of measured pain responses.

Examining our results as a whole we found that: first, increased facial pain expression intensity and subjective pain in patients, compared to controls, is associated with altered connectivity patterns in networks and structures associated with memory, socioemotional regulation, affect, and internally focused attention. Second, blunted autonomic responses in more advanced patients, compared to those with less severe disease, was correlated with increasingly

dysfunctional resting connectivity within structures and networks associated with cortical parasympathetic influence and interoception. Overall, these findings represent an additional step in understanding the neural mechanisms underlying altered pain in AD. They also underscore the necessity of appropriate assessment and treatment of pain in patients with AD, regardless of severity.

CHAPTER 4

LOOSE ENDS

WHAT FAILED, AND WHY?

Connectivity analyses

Initially, experimental design with respect to the rs-fMRI component of this research involved a region of interest (ROI) and seed-based correlation analysis (SCA) approach to examining the relationship between connectivity and altered pain responses in AD. One fundamental advantage of SCA over ICA its ease of implementation: one simply picks an ROI, whether it is anatomically or functionally defined, extract the BOLD timecourse for each subject, and run correlation analyses on those time courses to obtain R-coefficients. It is also more intuitively understandable as a method, and more easily interpreted than ICA. SCA is a relatively 'old' method of functional connectivity analysis in fMRI as well; Biswal and colleagues[206] found positive correlations between time courses of primary motor and premotor cortices in the mid 1990s using seeds derived from a finger tapping paradigm.

Initial attempts at examining connectivity differences between HS and AD utilized an anatomical SCA approach, using Freesurfer[207], which reliably and automatically parcellates and segments surface and subcortical bran structures into defined atlases. This allows one to obtain fine grain structural information about cortical regions as well as subcortical gray and white matter structures[208,209]. However, SCA is a hypothesis-driven approach for assessing functional connectivity; one must have *a prior* rationale for choosing a given ROI-based seed from a specific atlas or published coordinates. The RSN one subsequently observes is then constrained by the anatomical extent of the seed. One also runs into problems with anatomical variability between subjects if using native subject space, or issues of normalization of the BOLD

functional data. In the present study these issues were frustrating with respect to defining ROIs for the insula: while the insula has well-defined gross anatomical structure (two long posterior gyri and three short anterior gyri, with a sulcus separating anterior from posterior)[210], there are a number of different functional specializations along its anterior-posterior and dorsal-ventral aspects related to pain, interoceptive/autonomic function, and cognitive control/salience function[32,211–213].

Frustration with anatomically based placement and definition of seeds led to exploration of *functionally* defined seeds. What is often done is to take a significant cluster from an activation paradigm (e.g. from pain stimuli) and utilize that cluster as your seed, but with a resting-state timecourse. Since the present research did not involve an activation task, I elected to utilize a recent addition to the Freesurfer package originated by Yeo and colleagues[214]. After scanning 1,000 subjects with rs-fMRI these authors applied >1,000 spherical seeds, in an even distribution, throughout subject brains. They then applied clustering techniques to model key cortical RSNs in both a coarse (7 RSNs) and fine-grained (17 RSNs) manner and validated it by comparing spatial distributions of the various RSNs to activations induced by simple stimulus paradigms. These models are visualized on the Freesurfer 'fsaverage' brain in Figure 4-1A&B The coarse model consisted of the DMN, somatomotor, salience, dorsal attention, limbic, frontoparietal (executive), and visual networks. The finer, 17-RSN model broke the majority of coarse RSNs into subcomponents – for example, the somatomotor network was separated into dorsal and ventral distributions, similar to what I found using ICA. Since their original publication, this group has applied this technique to the striatum[215], cerebellum[216], and thalamus[217]. By using various Freesurfer commands, one could take these parcellations and apply them to native subject space prior to performing correlation analyses and regression techniques

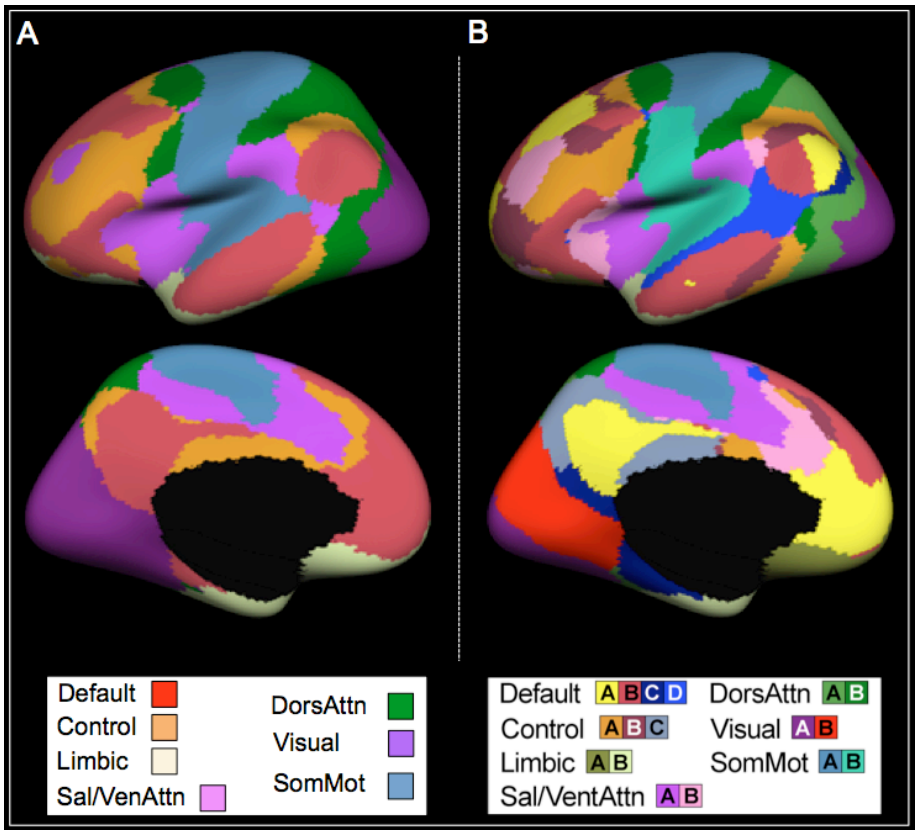

Figure 4-1 – Inflated surface view and associated network designations of the cortical parcellations by Yeo et al. Parcellations are viewed on the Freesurfer fsaverage brain. A) 7 Network model; B) 17 Network model.

against behavioral covariates (vis-à-vis FSLNets).

Here, again, one runs into a common pitfall of SCA – how to pick which network model to use? Each component of the 7-RSN model covered large swaths of cortex, which could potentially drown out subtle network differences associated with altered pain behaviors. However, the 17-RSN model, which could involve a parcellation of up to 15 distinct seeds often suffered from issues related to multiple comparison corrections, particularly when examining between-network connectivity at a pairwise level.

A final issue pertains to the very use of the model in subjects with such drastic neurological dysfunction, as is found in AD. As of this writing, the Yeo-based RSN models have

only been used in groups of healthy individuals and those with schizophrenia/psychotic bipolar disorder[218]. In this article the authors completely re-ran their clustering pipeline across all subjects and discovered that the spatial distribution of the RSNs were altered through inclusion of the patients; not to a large degree, but enough so that they used the disease-specific maps. It seems likely then that in AD – particularly in advanced AD – alterations would become severe enough to seriously confound results. This and previously discussed limitations of SCA ultimately lead me to utilization of ICA-based methods. Ultimately, I believe a combination of data and hypothesis-driven methods are an optimal means of deriving results. For example, using ICA to identify RSNs, performing group analyses using those RSNs (e.g. dual-regression and Randomise through FSL), and then using those RSNs or resultant clusters as seeds to narrow down connectivity differences between groups to specific 'nodes' within the network.

However, a robust behavioral dataset that is to be regressed against connectivity indices is also incredibly important for associating RSN integrity with behavioral measures. I believe this was another reason why I had difficulty with using the SCA approach. Not having a large number of subjects in both HS and AD groups score 'zero' on PAINAD measurements would have been helpful in this regard. Though the overall group averages yielded highly significant differences, this effect was weakened by the necessities of the GLM and regression – namely, performing mean-centering through the use of group-pooled means. In the end, the blame rests firmly on the shoulders of my experimental design: the pain modality must be robust enough to elicit consistent responses and the pain measurement should be sensitive enough to pick up occasionally subtle differences in response. The former problem would, in part, have been ameliorated by incorporating subject specific psychophysics of just noticeable, weak, moderate pain, etc. – though, of course, doing this even in the context of mild AD is problematic and doing

so with severe patients is unfathomable. However, quantitative testing results by Jensen-Dahm et al.[64] suggest that repeating measurements over separate days does ensure some consistency even in AD subjects. The ability of the FACS to score subtle facial responses is likely why it performed much better than the PAINAD at yielding connectivity associations (use of the PAINAD yielded none).

WHAT I'D DO DIFFERENTLY

There are several aspects of study design and execution that, given the chance, I would alter. First, I believe I spent too much time, initially, on recruitment of more advanced patients rather than pursuing mAD subjects as well. Though I found no differences in PAINAD or FACS-based measures of pain in mAD versus sAD, this may have been because of statistical power issues. Higher numbers of mAD may have allowed for a better differentiation of behavioral changes and likely would have made subjective pain rating findings more robust; particularly for mid level pressures (3&4kg), which were only marginally different between groups.

I also would possibly alter my pain induction paradigm. More robust responses, at lower pressure levels, may have resulted from application of pressure to alternative locations where there is likely less between subject variation in adiposity and muscle mass, such as the thenar eminence or volar phalanx. Pressure modalities have inherent clinical relevance, particularly to geriatric pain, due to musculoskeletal qualities. Indeed, while little change in superficial pain stimulation is evident in aging populations, modalities affecting deep tissue (i.e. ischemic and pressure stimuli) yield greater pain sensitivity in older adults[87,219,220]. However, alternative or complementary pain induction modalities may have been preferable as well. A recurrent issue in the study was a lack of responsiveness to pressure stimuli in both HS and AD groups. One possible reason for this was the time it took to reach peak levels of pressure with the manual

algometer. Such "ramping up" effects could allow for cognitive modulatory processes in HS to inhibit behavioral responses to pain stimuli. In contrast, modalities with more immediate nociceptive effects, such as electric or laser stimuli, may have elicited greater responses in subjects. With this in mind, pilot work, perhaps with young volunteers, examining the robustness of responses in comparing pressure to other modalities would have been a good idea.

Finally, I believe I would have been more productive and efficient with my time had I consulted with CSTAT during the design of my experiments. Because of the non-normal distribution of the various pain measures adequate statistical modeling was often difficult to achieve. This could have been prevented, somewhat, through early and regular discussions with statisticians to ensure an optimal experimental design.

FUTURE DIRECTIONS

Pain in AD

Many questions remain regarding the degree to which pain is altered in AD and the neural underpinnings of that alteration. First, much of the evidence showing enhanced sensitivity to pain comes from studies (including this one) where noxious pressure was used[64,71,72]. In contrast, examinations of laser, cold pressor, and heat pain have found no differences in sensitivity between HS and AD[63,64]. As previously mentioned, older adults generally show greater sensitivity to modalities involving deep-tissue pain induction, compared to more superficial stimuli. Thus it would be interesting to further examine this relationship in the context of AD by testing multiple stimulus modalities. In so doing, performing more in-depth examination of pain threshold and tolerance in AD, using as simplistic self-report measures as possible, in conjunction with examination of pain behaviors, would allow validation of the present study's finding that greater pain behaviors represent greater pain perception.

Further investigation of the pain-autonomic relationship in AD would also be beneficial. Several studies have examined general autonomic function in AD and have found evidence of sympathovagal dysfunction[221–225]. For example, one study found impaired sympathetic responsiveness to orthostatic challenge with advancing AD[226]. Thus, a more thorough examination of autonomic function across the spectrum of AD severity, with emphasis in relating those results to pain-related responses, would be very helpful in further understanding of the altered pain-autonomic relationship in AD. A very helpful measure in this regard would be heart rate variability (HRV), which can parse out relative sympathetic and parasympathetic influences on cardiac activity[227]. Given the autonomic findings here, one would expect that greater AD severity is associated with an increased high: low HRV frequency ratio (i.e. greater parasympathetic influence) with respect to pain response. One would expect that individuals who show greater impairment to orthostatic challenge would also show a more blunted HR response to noxious stimulation.

As discussed in Chapter 3, revisiting activation-based fMRI investigation of pain in AD would be the best way to further understanding of the neural mechanisms underlying altered pain in AD. Design of such a study would likely have to involve only mild/moderate patients to avoid motion confounds. However, considering that pain-induction fMRI studies have been performed on patients with disorders of consciousness (minimally conscious/vegetative state)[228,229] it would be unfortunate to completely dismiss the participation of sAD patients. It would be particularly interesting to further examine the rest-stimulus interaction[230], i.e. the relationship between resting-state connectivity and subsequent induced activation, with respect to pain in AD. One could also envision the use of simultaneous recording of autonomic measures (i.e. HRV) to further probe the pain-autonomic relationship in AD.

One could also incorporate structural measures of the brain into examination of the AD-pain relationship. Options include gray matter thickness, volume, or surface area measurements provided by Freesurfer-based parcellations and segmentations. One could also perform voxel-based morphometry analysis, for example using FSL, or even white matter analyses using diffusion tensor imaging. The results of an initial effort to examine structural correlates of altered pain responses are visualized in Figure 4-2. Here I extracted various cortical measures related to a core set of structures[31] in the central autonomic network (right aINS, left dorsal pINS, left amygdala, and the middle cingulate cortex; MCC) of scanned AD subjects; I then performed partial correlation analysis of each subject's metric with their average 3kg HR responses, with age and gender as nuisance covariates. Partial correlations that were fully or marginally significant were found for right aINS volume (R=0.44; p=0.069) and right MCC thickness (R= -0.51; p=0.03). Further investigation in this regard will require inclusion of vmPFC measures, determination of which ROIs to use (i.e. functional or structural atlas-based), and examination of relationships between structural measures and behavior between HS and AD groups.

Self-referential processing (SRP)

Neurologists have known for some time that patients with AD often present with impaired self-awareness. The most well-known involves a lack of insight whereby they will either deny impairments or overestimate their abilities[231]. The loss of the ability to self-recognize in a mirror or in recent photographs, first described in the early 1960s, is a less common and less well-known impairment of the self in AD, most frequently occurring in sAD, [232]. During mirror testing, affected sAD patients: do not spontaneously use a mirror in an appropriate manner (e.g. no self-grooming, they may instead talk to their reflection); fail to investigate conspicuous markings during "dot" testing; or cannot identify themselves in the mirror[232–234]. Likewise,

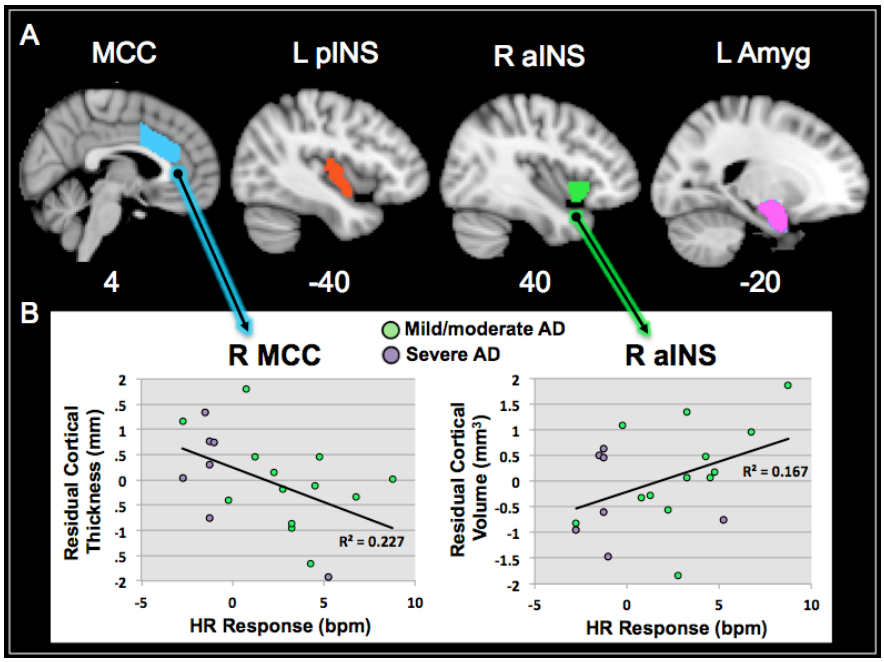

Figure 4-2 – Select cortical and subcortical regions of interest (ROIs), with respect to autonomic regulation, whose structural measures (thickness and/or volume) were extracted with Freesurfer. A) Spatial location of ROIs with MNI x-coordinates; B) Residual structural values (with age and gender effects regressed out) of two ROIs whose structural metrics were significantly (R MCC, left, p=0.03) or marginally (R aINS, right, p=0.069) correlated with HR changes plotted against HR changes of AD subjects. MCC: Middle cingulate cortex; L pINS: Left Posterior Insula; R aINS: Right Anterior Insula; L Amyg: Left Amygdala

affected sAD patients were not able to self-recognize when presented with video recordings of their faces or with recent polaroid photographs[233,235]. In contrast, patients with mild and moderate impairments are nearly always able to self-identify in front of a mirror and in recent photographs, despite having forgotten them being taken minutes earlier[236,237].

Biringer and colleagues[234] describe the issue, thusly: "Our approach to the phenomenon is from the perspective that self-recognition reflects the presence of a well-integrated sense of self – a facet of a more general self-concept." This assertion is "mirrored" in contemporary thought as well by Craig[238]: "...being able to recognize oneself in a mirror requires the capacity for emotional identification with the actions and displays seen in the mirror...this can only be provided by a functional, emotionally valid neural representation of the self." Given the above

findings, it would appear that, at least from a behavioral perspective, sAD patients no longer retain a fully "integrated sense of self," an assertion backed up by their inability to self-recognize across three modalities (mirror, video, and photographic). However, as described above, neuroimaging can tell us quite a bit about neural processes related to referencing the self, which is what occurs in mirror, video, and photographic self-recognition testing. The question arises, then, how would these findings relate to analogous neuroimaging studies?

Thus far, the only imaging experiments in which AD subjects were studied from the perspective of SRP was to investigate loss of insight into their cognitive impairments, perspective taking (imagining another's views on a task), or appraisal of the self-relatedness of various adjectives. FDG-PET studies have found that lack of insight is associated with hypometabolism of orbitofrontal cortex, medial temporal lobes, and the temporoparietal junction[239,240]. These findings are in sync with regions known to be involved in processing self-related stimuli (see above). Studies incorporating other self-referential stimuli (e.g. facial stimuli) that do not require any judgment by the subject might elucidate SRP deficits in sAD subjects. However, no such studies have been published as of yet.

Importantly, SRP studies have been conducted on a population base with even greater cognitive deficits than sAD, and their results *may* be informative on how to interpret the prior studies and theory related to the self and sAD. Specifically, studies of patients with disorders of consciousness, such as minimally conscious state (MCS) and vegetative state (VS), have investigated residual processing of several types of stimuli that reference the self. One recent study compared activity evoked by hearing one's own name vs. familiar names and unfamiliar names[241]. While only MCS subjects showed robust activity in SMA and pACC, both MCS and VS subjects yielded activity in the MCC in response to the self-specific condition (their name).

The authors further demonstrated a strikingly strong correlation (r=0.8) between activity in the MCC and scores on a putative behavioral marker for the degree of consciousness in non-communicative patients, the Coma Recovery Scale-Revised. These results indicate that, despite not being able to behaviorally indicate self-awareness, that self-referential processing may occur in severely impaired populations like those in a VS. Of course, these results may not strictly apply to sAD, as AD selectively targets areas such as the MCC/ACC and patients with disorders of consciousness are highly heterogeneous with respect to etiology. Further, the study did not find activity in the aIns, which is proposed to be a primary hub in processing self stimuli [4]. Regardless, the above study shows that compromised self-awareness leads to disruptions in processing of self-related stimuli and gives credence to the hypothesis that, in sAD, impaired self-recognition indicates impairment of structures that are responsible for processing self-referential stimuli, namely the ACC, aINS and PAG.

An alternative to the above hypothesis, put forth by Mograbi and colleagues[231], is that the self in AD is 'petrified' due to loss of medial temporal structures. Lost self-recognition, then, would not solely be due to impairments of the ACC and aIns. Instead, impairments in memory prevent updating of personal information, such as the mental representation of one's aging face or increasing cognitive impairment. Of course, these two hypotheses may not be mutually exclusive, as the ability to put a stimulus into self-referential context may be a necessary step to activate SRP regions like the ACC and aIns.

That the neural processing of affective pain and the self are highly related may seem odd at first glance. However, pain affect and SRP show extensive overlap of brain structures that process them,including ACC and aIns, medial prefrontal, premotor, and medial temporal regions

(hippocampus and amygdala). Structural overlap even continues subcortically with the PAG[201,242], showing just how deeply pain affect and the self are related, neuroanatomically.

Affective pain and self-processing also may share a functional relationship, likely influenced by their extensive structural overlap. The ACC, aINS, and PAG are posited to be part of a network of regions responsible for interoceptive (body-based self) processing – as opposed to exteroceptive (environment-based self) processing[201,242,243]. Effectively, then, processing of affective pain and self-stimuli must both be considered parts of overall interoceptive processing. Evidence for this relationship includes a recent study that showed increased interoceptive (body-based) sensitivity was associated with a lower pain threshold and a lower pain tolerance[244].

In performing interoceptive processing, the ACC, aINS, and PAG effectively allow for discrimination of self vs. familiar or other. Regions of the brain that then *integrate* intero/extero processing (e.g. mPFC) may utilize this discrimination to distinguish one's body/self from the external environment/other bodies. It is logical then, that empathy, or, 'feeling someone else's pain,' activates these same interoceptive/affective pain/self-processing regions, but not sensory regions[245].

How this may all relate to AD comes down to brain structures affected by the disease, of which interoceptive structures like ACC, aIns, and PAG, as well as medial prefrontal integrative structures are no exception. Given the extensive damage done to the ACC, insula, and PAG by the disease process, evidence presented here and in prior studies suggesting altered pain perception, decreased autonomic responses, and losses in self-recognition, it is reasonable to propose that AD leads to a disorder of interceptive processing and intero/extero integration. Impaired processing of interoception and intero/extero integration would alter normal behavioral and affective/autonomic responsiveness to noxious stimuli and self-referential stimuli.

To test the potential alteration of SRP in AD, all patients were examined the ability to recognize themselves under various conditions. Included were photographic recognition tasks and mirror and dot testing. Subject responses were scored on an 8-point scale (Table 4-1). HS were not behaviorally tested for their recognition abilities. However, to examine neural correlates of SRP, all scanned subjects underwent a passive viewing task whereby they were shown pictures of themselves (taken by me), pictures of familiar faces, and pictures of unfamiliar (sex, race, and age matched) faces. Future data analysis will thus involve examination of: the relationship between AD progression and self-recognition scores; differences in SRP activations and effective connectivity between HS and AD; differences in activations and connectivity in

Table 4-1 – Scoring of AD self-recognition testing by session type

Session Type	**Scoring**	
1. Spontaneous Mirror use	0 – no reaction 1 – speaks to reflection 2 – active interest (investigates by moving head)	3 – active interest (touches mirror) 4 – appropriate use (touches hair or rearranges clothes)
2. Forehead-dot testing (red dot & clear dot)	0 – no reaction to mark 1 – declares something about/investigates mark	
3. Hand-dot testing (control)	0 – no reaction to mark 1 – declares something about/investigates mark	
4. Mirror self-identification	0 – incorrect response/no response 1 – correct response (“me” or says name)	
5. Use of reflected space (control)	0 – incorrect response/no response 1 – correct response (“me” or says name)	
6. Self-photograph	0 – incorrect response/no selection 1 – correct selection (“me” or says name)	
7. Familiar photographs	0 – incorrect selection/no selection 1 – correct selection (“me” or says name)	
8. Self-photo within group of non-familiars	0 – incorrect selection/no selection 1 – correct selection (“me” or says name)	

mAD versus sAD patients.

Future work should also include examination of interoceptive function in AD patients, particularly with respect to patient pain-related responses and autonomic function. An initial step in this direction has been taken thus far by having a subset of HS and mAD patients perform a commonly utilized interoceptive sensitivity task[244]. This heartbeat-tracking task, developed by Schandry and colleagues[246], involves comparing the number of perceived and counted heartbeats by a subject with the actual number of heartbeats (detected, for example, with EKG). The task is performed over four timed segments of varying duration and an average score is calculated. Piloted testing of this task revealed that it was often very difficult to perform, even for HS. However, interoceptive awareness has been found to become somewhat impaired in the elderly[247]. Preliminary results for each group are plotted in Figure 4-3. Here, one sees only slight differences in the average interoceptive sensitivity score between groups (HS (n=24): 0.63±0.22 STD; AD (n=13): 0.58±0.22 STD; independent samples t-test – p=0.47 – n.s.)

Further examination of interoceptive function in AD will require pursuit of additional tasks or assessments, as it may be difficult to discern the degree to which cognitive impairment, rather than interoceptive dysfunction, is to blame for potential group differences between HS & AD subjects. One potential avenue involves visceral sensation assessments, including esophageal, gastric, or rectal distension testing[248]. Importantly, interoceptive sensitivity tasks can be performed during fMRI scanning, allowing for examination of how AD may lead to interoceptive dysfunction and, by extension, a heightened sensitivity to pain.

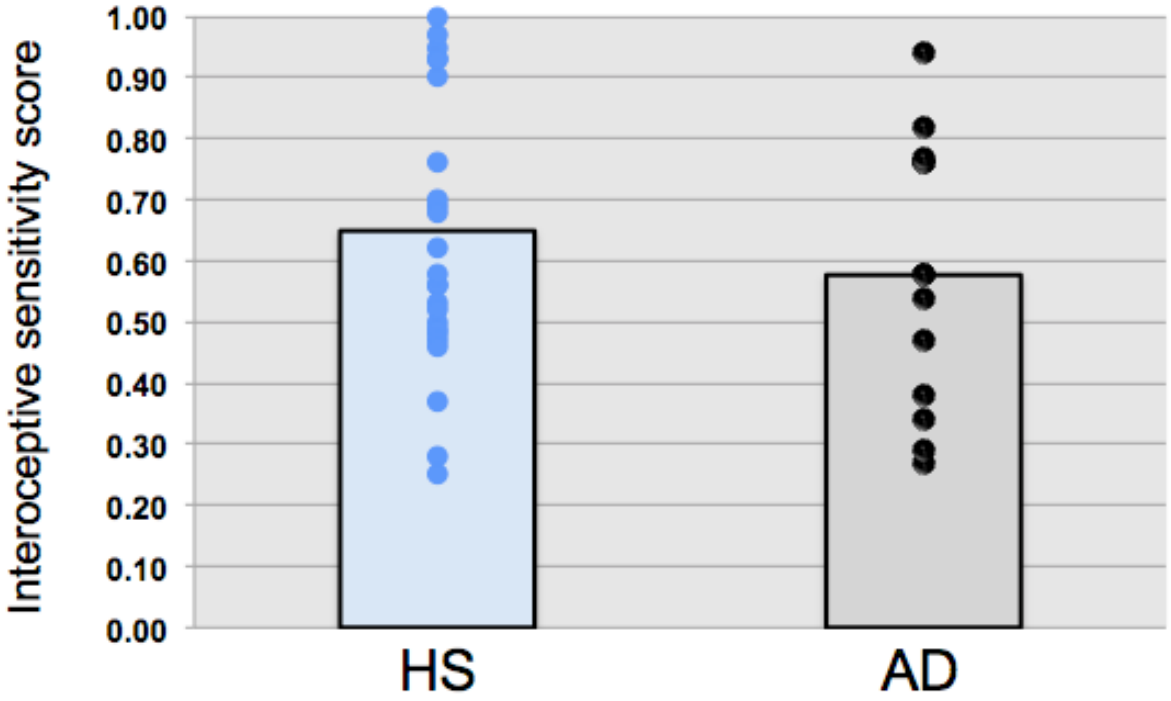

Figure 4-3 – Interoceptive sensitivity scores across groups, as tested by the heartbeat detection task. Bars indicate average group scores while dots indicate single subject scores.

CONCLUSIONS

Properly assessing and treating pain in patients with AD is a medical priority, particularly from the perspective of improving quality of life and reducing behavioral and psychological comorbidities[reviewed in10]. Doing so is complicated, however, as patients often cannot self-report and assessment relies on inferring pain from potentially ambiguous behaviors. As a result, AD patients are vulnerable to both under and overtreatment of pain, which can lead to unnecessary suffering, medical complications, and reduced quality of life. Completion of this study was a next step in helping to reduce this vulnerability.

The experiments discussed in this dissertation were meant to address various gaps in the literature. First, behaviorally based studies of pain in AD often focus on measuring one kind of pain indicator (e.g. self-report) and have yielded inconsistent results. Second, few studies have included patients with advanced disease. Third, our understanding of the neural underpinnings of altered pain in AD is limited. In this study I characterized various pain responses across the spectrum of AD, in comparison with HS controls, and probed the association of functional connectivity, via rs-fMRI, with measured pain responses. I thus examined subjective, behavioral, and autonomic responses to varying levels of pressure stimuli in AD and HS participants and scanned a subset of those subjects to relate altered pain responses to changes in RSN connectivity.

Not only did mild/moderate AD patients rate low to mid-level stimuli as more painful than HS, they also showed greater degrees of pain behaviors than controls, regardless of severity. Increases in pain behaviors were found first with respect to global behaviors as measured by the PAINAD, a clinical pain scale. Greater pain-relevant frequency and intensity of facial responses

were also found in AD subjects, versus controls, using an experimental tool, the FACS. In contrast, autonomic responses were blunted with advancing AD. Greater facial and subjective pain responses in patients were correlated with group connectivity differences of multiple RSNs, including mesial temporal, DMN, somatomotor, and vSN. Blunted autonomic responses within the AD group were associated with connectivity changes in the pDMN and vSN, specifically within the left vmPFC. Thus, altered pain responses in AD are associated with altered function of RSNs involved in attention and internal mentation, affect, somatosensation, and interoception. These findings provide further evidence and an improved understanding of the neural basis for heightened pain sensitivity in patients with AD. However, due to the correlational nature of the fMRI work presented here, several avenues of further investigation remain.

First, examining RSN connectivity associations with altered pain responses of AD patients with ICA, the data-driven technique utilized here, provides a kind of "bird's eye" view of connectivity. Further study should examine specific nodes within given RSNs elicited by ICA to examine whether interactions between certain structures may be driving connectivity changes associated with altered pain responses in AD patients. Revisiting pain-induction in the context of fMRI to examine the rest-pain stimulus interaction in AD would also be incredibly advantageous. Second, one could examine the brain structural changes associated with altered pain responses in AD. Third, along with blunted autonomic responses, sAD patients often have difficulty with self-recognition. This suggests altered SRP in AD, implicating dysfunction in many of the same structures and networks investigated here in the context of pain. Further work should involve investigation of altered SRP in AD and probe for potential relationships between SRP and pain in AD. Finally, I hypothesized that altered pain and SRP in AD may both be manifestations of dysfunctional interoception. Further work should therefore also include

behavioral and neuroimaging explorations of interoceptive function in AD, particularly with respect to its connection to altered pain and SRP.

Altered pain in AD presents a complex problem spanning basic neuroscience understanding of pain, functional brain networks, the pathology of the disease, and the clinical aspects of pain assessment and treatment in a challenging patient population. As such, the implications of this study are broad. Though further study is needed to obtain a more complete picture of how and why AD affects pain, the clinical implications are clear; these findings truly emphasize the necessity to improve pain assessment and treatment strategies for a vulnerable patient population set to expand greatly in the coming decades.

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