



THE BALDWIN-WALLACE COLLEGE

JOURNAL OF RESEARCH AND CREATIVE STUDIES

Volume 3, Issue 1
September 2010

TABLE OF CONTENTS

U.S. Stinger Missile Support for the Mujahedin in Afghanistan – 1986.
James Nichols Pages 1-7.

President George W. Bush's Rejection of the Kyoto Protocol.
Ellen K. Mackall Pages 8-14.

The efficacy of ellagic acid in attenuating neurophysiological and cognitive-behavioral symptoms associated with infusion of amyloid-beta (A β) peptide fragments in adult rats.
Gina N. Wilson, G. Andrew Mickley, and Kathryn M. Matera Pages 15-30.

U.S. Stinger Missile Support for the Mujahedin in Afghanistan - 1986

James Nichols

International Studies Program, Baldwin-Wallace College, 275 Eastland Rd., Berea, OH 44017.

President Ronald Reagan had assimilated an apocalyptic view of the Soviet Union and by 1986 had begun a policy of 'rolling back' what he saw as Soviet gains in developing countries. 'Rollback' was the Reagan Doctrine. His NSC advisors convinced him that negotiations would not work and that coercive diplomacy was the only viable policy. However, the concept of Mutual Assured Destruction (MAD) still applied, making face-to-face confrontation with the Soviets a grave mistake. But

the policy in Afghanistan was spearheaded by congressional actors. The White House decided to support the congressional decision to support the mujahedin with Stinger Missiles with the purpose of defeating the Red Army, as opposed to bleeding them in a Vietnamese-like quagmire.

Key words: Ronald Reagan, Afghanistan, Stinger Missiles, Soviet Union, Rollback, Charlie Wilson,

Assessing the decision to arm the mujahedin in 1986 with Stinger Missiles requires a scholarly guide because it involved multiple factors and multiple players. An application of Eugene Wittkopf's analysis of the factors that account for American foreign policy will demonstrate this decision's unique aspects to U.S. history and the country's policymaking process. Discussed here are the individual actors, their roles and the institutions in which they operated, the historical and geopolitical context of 1986, and the societal and external factors that contributed to arming the mujahedin. This paper will show that this foreign policy decision was unorthodox, although its impact on U.S.-Soviet relations cannot be underestimated because it contributed directly to the Soviet Union's disintegration, ending the Cold War. The issue also has been revisited numerous times since the attacks of September 11, 2001, as the same characters who perpetrated those crimes were also involved in the Soviet-Afghan War. This case will also show a shift in U.S. foreign policy that began in the Carter administration from idealism to realism to essentialism. In the grand scheme of U.S. foreign policy, this case has provided a precedent for covert action that was largely seen as a success and has influenced other covert actions since.

By 1986, the global order had taken its first substantial steps away from the strict bipolarity seen in the decades following World War II. With the Soviet Union mired in the Afghan war and its economy showing signs of weakness, the United States was increasingly accepted as the world's great power in this period. Wittkopf aptly describes this period as 'bipolycentric' – "The concept emphasizes the continued military superiority of the United States and the Soviet Union at this time and continuing reliance of the weaker alliance partners on their respective superpower patrons for security" (Wittkopf 150). U.S. President Ronald Reagan (Republican) inherited a U.S. economy recovering from "stagflation" – stagnation *and* inflation – while the Soviet

Union had begun its descent toward disintegration. This paper will provide an explanation into how Afghanistan played a role for the narratives of both great powers.

Afghanistan has been an on-going conflict for decades with indirect intervention by both the US and USSR in the country's civil conflict. The direct Soviet intervention in April 1978 marked a turning point in their Afghan policy, though their involvement in Afghanistan extends back much further than that. The Communist coup of 1978 ousted Afghanistan's ruler, Sadar Muhammad Daoud, who ironically was killed by the same Soviet-supported group that backed his takeover five years earlier (MacEachin 2). The communists deposed Daoud when he became a liability for Soviet aspirations in Afghanistan. Instead of strengthening a relationship with the Soviets, Daoud began to reduce Afghanistan's dependency on the Soviet Union; aggressively cutting ties with Moscow-allied political and military factions. (3) The April 1978 Communist coup thus should be seen as "a halt and prospective reversal of what had been a deterioration of the long-standing Soviet investment in Afghanistan." (3) Internal fissures in the new Afghan regime caused the Soviet Union to step up their military involvement in the form of military advisors and assisting the Afghan army in combat operations (4) and eventually lead to the Soviet invasion and occupation in 1979. That occupation further increased (by 1985 an estimated 120,000 Soviet troops were in the Afghan theater (Rubin)) and set the stage for increased U.S. intervention and the ultimate Stinger Missile decision in 1986.

President Reagan set the stern rhetorical tone toward the Soviet Union and the rest of the world following a Carter administration based on a platform of human rights. It is important to note, though, that even the Carter administration saw the Soviet invasion of Afghanistan "as an example of the kind of unacceptable Soviet international behavior that made friendly relations with Moscow all but impossible and signaled the end of

the period of détente” (Alexiev). Systematically, Reagan laid out his policy commonly referred to as ‘Rollback’ to reverse what he saw as Soviet gains in developing countries (Wittkopf 175). Specifically, the National Security Decision Direction 75 enunciated the Reagan Doctrine as weakening and, where possible, undermining the existing links between (Soviet Third World allies) and the Soviet Union (111). From Reagan’s first days in the White House, the rhetorical assault began:

“The specter of Marxist-Leninist controlled governments with ideological and political loyalties to the Soviet Union poses a direct challenge to which we must respond. They are the focus of evil in the modern world” (Curtis Volume 1).

As Steven W. Hook and John Spanier describe, there were two tactical purposes for the harsh denunciations of the Soviet Union. First, Reagan sought to mobilize American public opinion after the détente years and second, he wanted to send the Soviets a message that “Vietnam Syndrome” was a thing of the past. But despite the harsh words, Richard Pipes, a member of Reagan’s National Security Council and Director of East European and Soviet Affairs, noted that Reagan thought he could negotiate with the Soviets. In an interview, Pipes said, “(Reagan) had a rather benign view of human beings. He was a very kindly man and attributed kind motives to others” (Curtis, Volume 1). Nevertheless, “Reagan’s foreign policy was basically a return to the containment policy of the immediate post-World War II years” (Hook 170) as practiced by Secretary of State John Foster Dulles under President Dwight D. Eisenhower. For example, Reagan expanded U.S. military forces because he was “especially worried about the state of U.S. nuclear forces” (170). At the same time, the Reagan Administration turned up the rhetoric to win domestic support for aiding the mujahedin against the Soviets. At a press conference, Reagan dedicated the launch of the Columbia Space Shuttle:

“Just as the Columbia we think represents man’s finest aspirations in the field of science and technology, so too does the struggle of the Afghan people represent man’s highest aspirations for freedom” (Curtis Volume 2).

Such rhetoric might convince one to expect utterly massive amounts of aid to be donated to the Afghan cause, but “the reality of American assistance... both militarily and politically, was considerably less impressive than the rhetoric” (Alexiev). A reason for this disparity was a concept called the casualty hypothesis – the widely accepted notion that “Americans’

tolerance for war is limited by the number of casualties suffered by its soldiers” (Wittkopf 257) – did not apply here because no U.S. troops were deployed for this mission.

The rhetorical onslaught should be seen as policymakers relating to the political culture of American society. Painting the Soviet Union as ‘evil’ was socially acceptable because “Americans and their leaders generally share the notion that the United States is set apart from others” (Wittkopf 243). The idea of American exceptionalism has pervaded U.S. culture since its independence, as it was “explicitly founded on particular sets of values... Because it is set apart, the reasoning continues, the U.S. has special responsibilities and obligations toward others... because the U.S. itself is unique” (243). The aforementioned rhetoric toward the Soviet Union should not be seen along party lines. As we will see in a discussion of congressional actors, many Democrats and Republicans used essentially the same apocalyptic terminology to describe the USSR. In fact, as Wittkopf describes preserving one’s powerbase, the rhetoric here may be seen as “at the extreme, (where) theater substitutes for rational policy choice” (242).

The other major societal element at play is the use of civil religion to mobilize American public sentiment against the Soviet Union. Civil religion as described in Wittkopf’s text is “religiosity (that) provides much of the glue that knits together the nation’s predominantly Christian culture around a single theme or cause without violating the constitutional division of church and state” (245). Reagan used civil religion in the 1980 presidential campaign, and later to galvanize public opinion against the Soviet Union. Conservatives successfully enlisted several high-profile ministers such as James Robinson and Paul Weyrich to criticize the Soviet Union’s atheist image (Curtis Volume 2).

There are several factors that explain how the Reagan Administration arrived at this view of the U.S. and U.S.-Soviet relations. One is how Reagan interpreted the role of the presidency. According to Article II in the Constitution, the president takes an oath to

“preserve, protect and defend the Constitution of the United States... In practical terms, this means protecting the physical security of the United States “against all enemies, foreign and domestic.” Protecting the principles of the Declaration and the Constitution are meaningless without regime preservation and defending the nation” (Friedman).

It is up to the president to determine the limits on his obligation of this charge. Reagan, with an apocalyptic view of the Soviet Union, adopted a \$3 trillion defense

program, which included funds and troops for Afghanistan. However, the majority of these funds were allocated toward the Strategic Defense Initiative, which was commonly referred to as “Star Wars” (Hook 173). The intercontinental ballistic missiles (ICBMs) that were developed in this program were so strong that they gave rise to a doctrine called Mutual Assured Destruction. This doctrine suggests that the deployment of such weapons will destroy both parties involved. The Reagan Administration, then, needed to find alternative ways to coerce the Soviet Union. The Stinger Missile was an ideal weapon for the mujahedin in Afghanistan. A Stinger Missile is a shoulder-fired rocket weighing thirty-four pounds and is fired like a shotgun, but with less recoil (Cordovez 194). Only five feet in length, the “Stinger is portable and was thus ideal for the Afghan environment” (194). They also have an infrared homing device and easily targeted the MI-24 and MI-25 assault helicopters deployed by the Soviets with brutal force in 1984 and 1985 (194). The Afghan conflict was protracted with most mujahedin resistance fighters using AK-47s in guerilla warfare. Therefore it was not a difficult transition for guerillas to add Stinger Missiles to their tactics without taking the fighters out of their element. With the USSR mired in Afghanistan, covert action through the CIA proved to be a strong engagement, but not rising to the nuclear level. Stinger Missiles were both logistically plausible and also fit the Administration’s view of how the Soviet Union should be approached in Afghanistan.

Many of the key players that shaped the Administration’s policy process toward Afghanistan were in Reagan’s National Security Council (NSC), which “began as a cabinet-style approach and ended in a more White House-centered structure” (Wittkopf 344). In the beginning, Reagan originally wanted his secretary of state, George Schultz, to be his “primary adviser... chief formulator and spokesman for his foreign policy” (345). However, personal conflicts and internecine warfare left the NSC in a state of flux throughout the period of 1982-1987. An indicator of Reagan’s shift in perspective on the NSC in the pre-1986 period was his selection of William Clark to be national security advisor. Although the secretary of state maintained its responsibility for the “overall direction, coordination and supervision of the interdepartmental activities incident to foreign policy formulation,” (345) Clark’s job description covered essentially the same tasks: “developing, coordinating, and monitoring national security policy” (346). This overlap in responsibility led to a natural conflict between the two positions and allowed each adviser to jockey for position in the White House. Clark’s conservative disposition led him into squabbles with pragmatists like Chief of Staff James Baker and prompted Clark’s departure in 1983. Such internal fissures characterized the Reagan NSC and aided ambitious congressional actors to work in conjunction with intelligence community actors rather than institutionalized bureaucratic ones.

The constitutional allocation of power to Congress is another important topic to address when assessing the policy process, especially in comparison to the president’s role as commander-in-chief. Article I of the Constitution grants more extensive responsibilities to Congress, including “general legislative power, the ‘power of the purse,’ the powers to declare war, to raise and support armies, to provide and maintain a navy, to make rules for the government... and to organize, arm, discipline and call forth the militia” (Wittkopf 414). In this case, the ‘power of the purse’ proved to be the greatest responsibility undertaken by Congress. In light of the recent Iran-Contra ‘cover-up,’ Congress was far more assertive in the appropriation of funds and arms. The Contra scandal was seen as an example of the executive branch overstepping its bounds, giving NSC members too much power. However, it is important to note that “Congress has considerable power and authority in foreign policy making *if it chooses* to act” (415). If it does choose to act, it finds itself confronted by the “invitation to struggle” – “the congressional setting that permits many congressional actors to have a voice in foreign policy” (415). Wittkopf’s analysis of Congress’ role in foreign policymaking emphasizes recognition that “many actors comprise the institution” (415). He explains that an array of elements can be considered foreign policy actors, ranging from political parties as a whole to individual members of Congress who become foreign policy activists and entrepreneurs (415). These actors can combine in a number of different ways on a given issue. Wittkopf warns, however, that “this is one reason why Congress has a difficult time asserting itself on foreign policy: different actors may advocate different policies, making it difficult for any one to succeed” (415). In addition, the Constitution structures foreign policy to be largely the purview of the President, with ‘advice and consent’ powers granted to the Senate for confirmation of treaties and appointments.

Beginning in 1984, the U.S. Congress, whose “support for the Afghan cause was unanimous and truly bipartisan, became increasingly concerned about the seemingly ineffective assistance to the resistance” (Alexiev). It was in this period that Congress introduced a resolution mandating a large increase in U.S. assistance that included making the supply of effective weapons (Stinger Missiles and Sidewinders) possible (Alexiev). In fact, the chairman of the Senate Select Committee on Intelligence, Malcolm Wallop (R-ID) indicated the State Department and CIA strongly opposed the initiative (Alexiev). However, once the resolution passed both houses of Congress, President Reagan signed a national security directive that backed the Afghan resistance “by all means available” (Alexiev). Congressman Charlie Wilson (D-TX) also played an integral role in coordinating the allocation of funds to the mujahedin as a member of the powerful House Defense Appropriations subcommittee (Winthrop). He became

enthralled by the mujahedin while abusing government privileges to travel the world first class with former beauty queens (Coll 91). When asked his view of the conflict in 2008 in an interview with Rolling Stone magazine, he said,

“When they (the mujahedin) were fighting the Soviets, there were only two sides — the right side and the wrong side — and they were all on the right side. They were all passionately involved in expelling the Soviet Union from their country.”

He persuaded his colleagues to contribute unused funds to the CIA mission to arm the mujahedin and was so effective that Afghan funds rose to \$1 billion a year (Winthrop). For his joint work with the CIA, Wilson was conferred the highest award (Honored Colleague Award) by the CIA, being the first civilian to receive the award (Winthrop). Republican Senator Orrin Hatch of Utah also contributed towards facilitating the delivery of the Stinger Missiles. A strong supporter of arming the Afghans, Hatch went to then-President Zia Ul-Haq of Pakistan and convinced him to formally make this request to President Reagan (Cordovez 195). He also got five members of the Senate and House intelligence committees to travel with him to China and Pakistan to strengthen support for the Stinger decision (195).

Congressional policymakers coordinated their efforts with the intelligence community for implementation of the policy to fund the mujahedin. The Reagan administration was adamant about exorcising the “ghost of Vietnam” (Wittkopf 111), but was acutely aware of the domestic wariness of American casualties in foreign conflicts. The decision to “unleash the CIA” reenergized its capacity for covert action. From 1947 to 2004 the Director of Central Intelligence (DCI) was responsible for managing the entire intelligence community, and was also director of the CIA (390). William Casey was the DCI from 1981 to 1987 and subscribed to the findings of *The Terror Network*, a book written by Claire Sterling that claimed the Soviet Union was the source of all international terrorism (Curtis Volume 2). Despite objections from Melvin Goodman, the Head of Soviet Affairs at the CIA, who said that “a lot of (the book) was made up,” (Curtis) Casey moved forward with “wide latitude” in conducting numerous covert actions around the globe (Wittkopf 111). However, the diversity in opinion in the intelligence community also was a potential constraint on the implementation of the policy. One senior intelligence official said in 1985, “A weapon like this could force the Soviets to become more indiscriminate in their use of force. They could begin much more bombing. It could change the equation radically” (Alexiev). But Milton Bearden, a CIA Field Officer in Afghanistan from 1985 to 1989 recalled of Casey,

“Afghanistan seemed to be possibly one of the keys, and so he tapped me one day to go. He said, ‘I want you to go out to Afghanistan... and I will give you whatever you need to win.’ He said, ‘I want you to go there and win. So he gave me the Stinger Missiles and a billion dollars’” (Curtis).

This marked a shift in the U.S. stance toward the Afghan war. While some in the Reagan White House recommended bleeding the Soviet Union in a Vietnam-like quagmire, the strategy shifted toward defeating them, despite not deploying ground troops. In the specific terms of the Reagan Doctrine, the U.S. would now “undermine the existing links between Soviet Third World allies and the Soviet Union” (Wittkopf 111).

The intelligence community is the most important part of the foreign policy bureaucracy in this case. However, there are other elements of the bureaucracy that played a role, though much less significant. By 1986, the U.S. had constructed “an elaborate complex of organizations and instruments through which to engage in the world” (367). These organizations and instruments engaged in diplomatic relations, international organizations, economic and military aid and sales, military deployments which gave the U.S. the ability to strike anywhere in the world, and direct trade and investments. The Departments of State, Defense, and the Joint Chiefs of Staff all work in conjunction with the intelligence communities in the implementation of foreign policy. The Defense Reorganization Act of 1986, popularly known as the *Goldwater-Nichols Act*, did not directly affect this case because this legislation pertained only to command of military forces (383). Again, there were no U.S. troops deployed to Afghanistan in the 1970s and 1980s. However, “some of the Joint Chiefs of Staff feared accountability problems and proliferation of the technology (Stinger Missiles) to Third World countries” because this would break the embargo on “Made-in-America” arms (Rubin). With regards to the other elements of the foreign policy bureaucracy, Ronald Reagan was particularly resistant to allowing the governmental bureaucracies to dictate the decisions of his administration. “You know,” he said, “one of the hardest things in a government this size is to know that down there, underneath, is that permanent structure that’s resisting everything you’re doing” (Wittkopf 464). His famous line, “Government is not the solution, it is the problem” (481) has become one of the most popular campaign themes across party lines ever since. Therefore, it is not surprising that Reagan tried to “control the bureaucracy by infiltrating it with political operatives” (481).

The final relevant source for this case is the external factor. Clearly the most important external actor

here is the Soviet Union. Mikhail Gorbachev, the General Secretary of the Communist Party from 1985-1991, began his policies of *glasnost* (openness) and *perestroika* (radical economic reform), which “required new thinking in the Soviet Union’s policy toward its former Eastern European satellite” (Wittkopf 151). Furthermore, “Gorbachev repudiated the Brezhnev Doctrine in favor of the ‘Sinatra Doctrine,’ which decreed that satellite states would be permitted to ‘do it their way’” (151) is this really what it was called? This is the first time I’ve heard of a ‘Sinatra Doctrine’ double-check your source to make sure it is characterized accurately.... However, a level of respect remained between the two nuclear powers due to the concept of Mutual Assured Destruction (MAD) – a doctrine that described a full-scale nuclear attack between two opposing sides that would effectively destroy both the attacker and the defender (94). Such a scenario sparked several U.S. presidents to push for Strategic Arms Limitations Treaties (SALT) and Strategic Arms Reduction Talks (START) (100), which are still issues for the Obama administration to address.

Pakistan was also extremely important to the U.S. effort to arm the mujahedin with Stinger Missiles. The leadership in Pakistan saw two clear reasons to help the Americans aid the mujahedin in Afghanistan. First, the Pakistanis were operating a covert nuclear reactor that was discovered by former State Department employee Richard Barlow, who was hired by the CIA’s Office of Scientific and Weapons Research (Levy). Barlow found that the tools and equipment present at the plant were dual-use components and could be used for nuclear weapons or other non-nuclear purposes (Levy). However, the large number of oscilloscopes convinced Barlow that the Pakistanis were more focused on weapons (Hersh). This discovery happened simultaneously with the forming of a relationship with Pakistan’s Inter-Service Intelligence (ISI) and the CIA. The U.S. depended heavily on the ISI to physically deliver the Stinger Missiles to the mujahedin. “Upon arrival at the port of Karachi or the Islamabad airport, the ISI would transport the weapons to depots near Rawalpindi or Quetta, and hence on the Afghan border” (Rubin). The second reason U.S. intervention in Afghanistan was in Pakistan’s interest was that Pakistan did not want Afghanistan to become a Soviet-dominated state. Religion played a major role in how these parties coordinated themselves. Communism’s ties to atheism angered all Islamist groups and affected the broader cultural dynamic of the region. “The ISI refused to recognize any Afghan resistance group that was not religiously based” (Rubin), which tends to explain the rise of the Taliban after the Soviets withdrew. Pakistan also did not want to be sandwiched by pro-Soviet countries.

“India, the greatest possible diplomatic check to Washington’s escalating

relationship with Islamabad, removed herself from any position of influence because its unabashed pro-Soviet policy eviscerated any American fear of antagonizing India. The State Department considered India a lost cause” (Rubin).

The degree to which the U.S. outsourced this mission is notable because of a concept called ‘blowback’ – the unintended consequences of U.S. policy abroad. Blowback is common when combat missions are outsourced to foreign fighters as in cases like these. The U.S. paid little attention to missiles potentially falling into the wrong hands in the 1990s, which later had consequences in the 2001 U.S. campaign against the Taliban (Rubin). This is due to the degree of latitude the U.S. gave to the Pakistani ISI to identify fighters and distribute arms. “Even at the height of American involvement in Afghanistan, very few CIA operatives were allowed into the field” (Rubin). While fear of the use of these same Stinger missiles was evident, this was nullified somewhat by a covert campaign in the 1990s “to purchase or otherwise recover surplus Stinger Missiles still in the hands of the mujahedin factions” (Rubin). Not all were recovered, though. While conducting research in Afghanistan in 1997, Peter L. Bergen passed some Arab fighters – “Some of them were carrying Stinger (American anti-aircraft) missiles” (Bergen 241).

Because the distribution of Stinger missiles was largely out of U.S. hands, anyone who claimed a religious affiliation to the Islamists could obtain arms. This includes the Arab mujahedin who came to Afghanistan to aid the Afghans in the jihad against the Soviets. Abdullah Anas, General Commander of Afghan Arabs in Northern Afghanistan from 1984 to 1989 said, “I saw the fatwa, the order, saying that every Muslim has a duty to help the Afghans to liberate their land” (Curtis Volume 2). This fatwa came from Abdullah Azzam, a Palestinian member of the Muslim Brotherhood “who was the critical force both ideologically and organizationally for the recruitment of Muslims from around the world to engage in the Afghan struggle against the Soviets (Bergen 24). Azzam was the mentor of one Saudi who also volunteered. Osama bin Muhammad bin Laden, the current leader of al Qaeda, arrived in 1984 to start the Services Office that would place “Arab volunteers either with relief organizations... or with the Afghan factions fighting the Soviets on the frontlines” (24). This work was “at best a cursory role” (Rubin), though. In fact, the CIA’s Milton Bearden described,

“(t)he relationship between the Afghans and the Internationalists was like a varsity team to the scrubs. The Afghans fought their own war and outsiders of

any stripe were kept on the sidelines...
Bin Laden sat out the 'big one'" (Rubin).

Bearden flatly rejects the notion that the CIA trained, recruited or otherwise used the Arab volunteers who fought in Afghanistan, which has become a common criticism of U.S. foreign policy since September 11, 2001. The Afghans, he explains, did not need "fighters from outside their culture... (the idea) was deeply flawed and ignored basic historical and cultural facts" (Rubin). They may not have needed foreign mercenaries, but the beginnings of jihadist ideology fomented the migration of these foreign fighters into various conflicts throughout the Muslim world, for example Afghanistan, Bosnia, Egypt and other theaters.

There is little doubt that the Stinger missiles helped the mujahedin jihad. The debate lies over whether the Soviets would have been driven out without them. The Pentagon called Stingers "the war's decisive weapon" (Jentlesone). Not up for debate is the historical record of Soviet withdrawal. In 1988, the Soviets made concessions at the Geneva Accords, which were "an unprecedented reversal of policy. Never before had the Red Army retraced its footsteps and withdrawn from a country it had invaded" (Jentlesone). The Soviet decision to withdraw was heavily influenced by "the ability of the Afghan mujahidin to continue and increase their military opposition" (Jentlesone). The final withdrawal of Soviet troops occurred in 1989 with two distinct groups claiming victory. As Milton Bearden describes, "I thought we (the U.S.) won, because I was part of it. I'm sure that the Afghan Arabs thought, 'we won'" (Curtis Volume 2). In any event, the total disintegration of the Soviet Union followed quickly after their withdrawal from Afghanistan.

It is this precise moment when America became the world's unipolar power. One might even say the Stinger missile decision was the lynchpin, however unlikely, that solidified American global supremacy, and formed the world order where America's leadership became essential to meet global challenges. Congress' assertive role in directing assistance was the critical element that moved the policy process forward, despite objections from the State Department and elements of the CIA. The secretive nature of the decision, in spite of the public overtures praising the mujahedin and condemning the Soviet Union, makes this case purely unique to the history of U.S. foreign policy. The case describes a scenario where one great superpower wanted to engage another, but had to do so in a tempered manner due to the MAD principle. There most likely will not be another case that follows this same pattern due to the rapid "flattening" or globalizing world. In other words, it is highly unlikely that the global system will be dominated for such a long period of time by only two superpowers. However, the case does indicate a strong ability on behalf of the U.S. to be assertive in its position as a global leader when it sees fit. Wittkopf

agrees that such assertiveness will be necessary to confront the challenges ahead, though "old solutions may no longer fit new realities.

REFERENCES

- Alexiev, Alexander. 1988. *The United States and the War in Afghanistan*. The Rand Corporation.
- Bergen, Peter. 2006. *The Osama bin Laden I Know: An Oral History of al Qaeda's Leader*. New York, NY: Free Press.
- Coll, Steven. 2004. *Ghost Wars: The Secret History of the CIA, Afghanistan, and Bin Laden, from the Soviet Invasion to September 10, 2001*. Penguin.
- Cordovez, Diego & Selig, Harrison, Selig S. 1995. *Out of Afghanistan: The Inside Story of the Soviet Withdrawal*. Oxford University Press US.
- Curtis, Adam. 2004. *The Power of Nightmares: The Rise of the Politics of Fear*. British Broadcasting Company,
- Friedman, George. 2009. *Torture and the U.S. Intelligence Failure*. Stratfor.
- Hersh, Seymour M. 1993. *On the Nuclear Edge*. The New Yorker.
http://www.newyorker.com/archive/1993/03/29/1993_03_29_056_TNY_CARDS_000363214
- Hook, Steven W. & Spanier, John. 2007. *American Foreign Policy Since World War II*. Washington D.C.:Congressional Quarterly Press.
- Jentlesone, Bruce W. 1991. *The Reagan Administration and Coercive diplomacy: Restraining More Than Remaking Governments*. Political Science Quarterly 106.
- Levy, Adrian and Scott-Clark, Cathy. 2007 *The Man Who Knew Too Much*. The Guardian.
<http://www.guardian.co.uk/world/2007/oct/13/usa.pakistan>
- MacEachin, Doug, and Nolan, Janne E. 2005. *The Soviet Invasion of Afghanistan in 1979: Failure of Intelligence or of the Policy Process?* Institute for the Study of Diplomacy, Georgetown University. Working Group Report, No. 111.

- Rollingstone, The. 2008. *Charlie Wilson: Osama bin Laden Not Trained by CIA*.
<http://www.rollingstone.com/blogs/caprilounge/2008/04/charlie-wilson-osama-bin-laden.php>
- Rubin, Michael. 2002. *Who is Responsible for the Taliban?* Middle East Review for International Affairs, Volume 6, No. 1.
- Winthrop, Lynn. 2003. *During Book Signing, Wilson Recalls Efforts to Arm Afghans*. *The Lufkin Daily News*.
- Wittkopf, Eugene and Jones, Christopher M. and Kegley Jr., Charles W. 2008. *American Foreign Policy – Patterns and Process*. Seventh Edition. Thompson Wadsworth Press.

Address correspondence to: James Nichols
18 Somerset Drive, Rocky River, Ohio 44116;
Email: nichols9286@yahoo.com

Received: 15 May 2009
Revised: 15 January 2010
Accepted: 8 February 2010.

Copyright © 2010 by James Nichols and Baldwin-Wallace College

President George W. Bush's Rejection of the Kyoto Protocol

Ellen K. Mackall

Department of Political Science, Baldwin-Wallace College, 275 Eastland Rd., Berea, OH 44017

Many factors came into play in George W. Bush's foreign policy decision to remove the signature of the United States from the Kyoto Protocol to the United Nations Framework Convention on Climate Change. Individual factors such as connections to the oil industry, skepticism about climate change, and leadership style were very influential; societal factors such as interest groups and corporations were important as well, despite a significant gap in public opinion. State level factors were less influential, as Bush did not heed the policy of the EPA director at the time. System level factors in foreign policy-making were important insofar as

President Bush had the power to go against world opinion and other external pressures due to a degree of US hegemony. Finally, in this case, role factors mainly demonstrate the dissemination of policy, as Bush was the main foreign policy creator. This case of foreign policy decision-making has set the stage for future climate change negotiations in terms of mandatory emissions reductions.

Key words: Kyoto Protocol, UN FCCC, foreign policy, climate change, George W. Bush, EPA

"Since the beginning of the Industrial Revolution, the combustion of coal, oil, and natural gas and other human activities have increased carbon dioxide concentrations in the Earth's atmosphere by about thirty-five percent" (Payne & Payne, 2008, p. 361). This increase in carbon dioxide in the atmosphere has led to what is commonly referred to as climate change or global warming. Climate change is clearly a problem that affects not only those who pollute, but also countries that have much lower carbon dioxide emissions levels. The consequences of climate change could easily range from the flooding of low-lying coastal regions to the disruption of agricultural patterns to hundreds of thousands of environmental refugees (Payne & Payne); it is thus not surprising that international efforts have been underway for the past decade and a half to halt this great potential danger. Research continues to indicate that carbon dioxide and other greenhouse gases are contributing substantially to climate change; population and economic growth could increase enough that carbon dioxide concentrations could reach up to 250 percent more than in preindustrial times (Payne & Payne). Although this problem can affect all people in all countries, issues such as climate change are difficult to regulate, as are other examples of Hardin's tragedy of the commons (Steurer, 2003).

The Kyoto Protocol of 1997 was the first international attempt to set binding limits on greenhouse gas emissions in order to combat climate change. It is part of the United Nations Framework Convention on Climate Change (FCCC), a treaty that was presented at the so-called "Earth Summit" in Rio de Janeiro in June 1992. It called for industrialized, or Annex I, countries to return emissions to their 1990 levels by 2000, but did not require any binding commitments. The United States

(US) was the first industrialized country to ratify the FCCC in October 1992 (Payne & Payne, 2008). However, because no mandatory targets were created by the FCCC, the Kyoto Protocol was proposed in 1997 to further fight climate change. Under Kyoto, countries were allowed to choose varied reduction goals, but overall, Annex I countries would have to cut emissions of three greenhouse gases (including carbon dioxide) by 5.2% on the basis of 1990 emissions levels by 2008-2012 (Steurer, 2003). The US target was a reduction of 7% from 1990 rates (Payne & Payne). However, to become binding, the Kyoto Protocol had to be ratified by at least fifty-five countries which would account for at least fifty-five percent of 1990 emissions from Annex I countries (Payne & Payne).

Because of the high level of carbon dioxide emissions in the US — estimated at 24% of global emissions and 36% of emissions from industrialized countries in 2002 (Steurer, 2003, p. 349) — its participation in Kyoto would have had a particularly great effect on worldwide efforts against climate change. But Congress had problems with Kyoto from the start. For example, Kyoto did not address the American concern of developing countries being required to reduce emissions: the Republican-dominated Senate was also concerned that the efforts of developed countries would simply be diluted by bigger developing countries increasing their fossil fuel use and emissions (Payne & Payne, 2008). The US also favored some market-friendly emissions trading systems that would encourage greater efficiency than regulatory approaches, such as a cap-and-trade approach that allows businesses to buy and sell pollution permits to meet pollution caps (Payne & Payne).

Despite these imperfections that Senators found, President Clinton gave his support to the treaty and its mandatory emissions cuts. While it was officially signed by Vice President Al Gore in November 1998, "Gore's signature was nothing other than a symbolic gesture of the Clinton administration's climate policy intention" (Steurer, 2003, p. 346). While Clinton was committed to the legally binding restrictions, the Senate, required to ratify the treaty, was far less in support of the Kyoto Protocol. In fact, it was never actually given to the Senate for ratification, as the Byrd-Hagel resolution (discussed below) made the legislative branch's position clearly anti-Kyoto (Payne & Payne, 2008).

Kyoto's future began to look a bit brighter during the 2000 election season. In his campaign, George W. Bush announced his commitment to regulating carbon dioxide emissions as a pollutant. This went even beyond what Gore was promising, which were mere voluntary cuts (Agrawala & Andersen, 2001). However once Bush was elected, the Kyoto Protocol "languished without support on Capitol Hill until March 2001, when President Bush's EPA administrator, Christine Todd Whitman, stated Kyoto was 'dead' (from an American standpoint) because the president had 'no interest in implementing the treaty'" (Wittkopf et al., 2008, p. 420). Bush's retreat from the Kyoto Protocol was officially announced on March 28, 2001 (Steurer, 2003), on the basis of two main factors. First, it would hurt the US economy and slow growth; Bush believed that signing onto Kyoto would have cost the US up to \$400 billion and would have lost 4.9 million jobs (Daynes & Sussman, 2005). Second, the US would be unfairly burdened while developing countries like India and China would be excluded under Kyoto's provisions (Hook & Spanier, 2010). Bush also believed that enforcing mandatory compliance would be impossible, and that no treaty was better than a flawed one (Hook & Spanier).

It is also notable that with the rejection of Kyoto, "Bush disputed the scientific evidence linking greenhouse gases to global warming" (Hook & Spanier, 2010, p. 268). When Bush announced the US exit from the treaty via the removal of its signature, the protocol had been approved by eighty-three other countries, despite the fact that the US was producing around one-quarter of the world's greenhouse gases (Hook & Spanier). It is clear that the rejection of Kyoto is symbolic of environmental policy in this era of the Bush II administration. Yet, even though international agreements are the norm for many environmental topics such as climate change, this case reflects the American penchant for a realist approach to various areas of foreign policy. US interests are favored over international opinion, as the potential burdens Kyoto would place on the US economy are frequently cited; the power of America, while not military in this case, remains important compared to other concerns.

In the handling of this case and the decision-making processes and factors by the Bush administration, some aspects are more influential than others. Societal and individual factors played the largest role in this case, while state level factors were fairly important as well. External factors were only important insofar as the configuration of the international system allowed the US to set its own environmental policy course, and role factors were minimal. These findings generally agree with analyses of the case, as it is evident that the US acted alone among advanced industrialized countries in rejecting Kyoto, and that individual factors strongly shaped President Bush's policy agenda in this area. Societal factors are mentioned less often, but public opinion and interest groups are clear elements in the decision of this case.

As with many presidents and their policies, individual factors can be a major factor on foreign policy decision-making. However, in this case, individual characteristics and aspects of the background of not only President Bush, but also of other key actors in this case, played a large role. First there were the "business-friendly" views of the Bush administration, which at the time were perceived as less than compatible with a regulatory approach to climate change (Payne & Payne, 2008). Yet more important are the various connections to the oil industry that various actors in the case have. President Bush himself worked in the oil industry before running for governor of Texas, but he also surrounded himself with advisors with similar backgrounds (Payne & Payne). Vice President Dick Cheney is the former CEO of global oil giant Halliburton, where he made more than \$30 million (Lisowski, 2002). National Security Advisor Condoleezza Rice was on Chevron Oil's board of directors from 1991-1993, and Commerce Secretary Don Evans had ties to the oil industry as well.

In addition, some members of the Bush administration received significant campaign contributions from the automotive industry; Energy Secretary Spencer Abraham received the most of those. Bush's Chief of Staff Andrew Card had also been the President and CEO of the American Automobile Manufacturers and then the chief lobbyist for General Motors (Lisowski, 2002), where his main responsibility was to lobby the Clinton administration against Kyoto (Agrawala & Andersen, 2001). Clearly, these backgrounds had a great impact as to the type of energy policy the administration preferred and on the solutions that would be found to be in the national interest.

To focus more on personal characteristics of President Bush himself, he had a strong tendency to be skeptical about climate change, despite much evidence to the contrary. It was later found that his administration intentionally tried to manipulate public debate on the scientific evidence behind climate change, after the Kyoto rejection as well. For example, the *Climate Action*

Report 2002 says that human actions contribute to global warming and warns of its consequences, yet does not call for US action to reduce emissions (Payne & Payne, 2008); Bush dismissed the report anyway, and the administration took out the entire global warming section of the 2002 EPA report on air pollution. "Indeed, the administration seems to have responded to conservative criticisms primarily by suppressing information about the science of global warming (Payne & Payne, p. 376). Such actions have often been funded or spurred on by groups funded by large oil companies (Payne & Payne).

President Bush's leadership style can also be seen to be an individual factor in his rejection of the Kyoto Protocol. He is characterized as an active-positive president, although that distinction becomes much clearer after his response to the terrorist attacks of September 11, 2001 (Wittkopf et al., 2008). Thus it would seem that his model of leadership is more pertinent for his case; he followed a corporate or CEO model of leadership in which he delegated responsibility but had primary control like a CEO would (Wittkopf et al.). Bush was certainly the primary decision-maker in this case, as will later be evident by the surprised reaction of other actors at the policy announcement.

Using his model of leadership, Bush did take the duties of his role seriously, though this had less of an overall impact on the decision-making of the case as a whole and more demonstrates who made which decision. First, Bush, as the primary creator of American foreign policy, made this policy reversal decision with limited help from others; he did, however, consult his Cabinet. "[T]he White House said a cabinet-level review had concluded that Mr. Bush's original promise had been a mistake inconsistent with the broader goal of increasing domestic energy production" (Jehl & Revkin, 2001).

President Bush also created an energy task force in January 2001 to help him create the national energy policy to shape future decision-making in areas such as climate change. This group was to focus mainly on additional oil and gas production to address the energy supply, with drilling in Alaska's arctic wildlife preserves as a possibility (Lisowski, 2002). Personal biases were evident here again, as the task force headed by Vice President Cheney "consulted heavily with oil and gas and nuclear energy executives, but met with producers of energy from wind, solar, and geothermal sources only once" (Lisowski, p. 112). In the final report produced by the task force that would become the official energy policy, the main focus was the need to increase fossil fuel and nuclear energy, to be reached in part by drilling in Alaska. This was also linked with the current California energy crisis at the time to gain legitimacy—even after it had passed, it had paved the way for their energy policy based on oil and gas

production instead of what were seen as costly alternative sources (Lisowski).

But in terms of Bush's actual policy to reject Kyoto, this came from President Bush himself to a great extent, first in the form of a letter to prominent Republican Senators Chuck Hagel (R-Nebraska), Jesse Helms (R-North Carolina), Larry Craig (R-Idaho), and Pat Roberts (R-Kansas), who were opposed to Kyoto because of potential harm to the economy and because it would allow United States energy policy to be essentially governed by an international treaty; Hagel had asked Bush to clarify his stance on the issue, and his well-known letter resulted (Jehl & Revkin, 2001). In it, Bush discusses his opposition to Kyoto because of the exclusion of developing countries such as China and India, around eighty percent of the world, and because of harm to the US economy. He proposes instead "a multipollutant strategy to require power plants to reduce emissions of sulfur dioxide, nitrogen oxides, and mercury...[he did not believe, though] that the government should impose on power plants mandatory emissions reductions for carbon dioxide, which is not a 'pollutant' under the Clean Air Act" (Bush, 2001, p. 391). He reiterated that reducing carbon dioxide emissions would raise electricity prices due to a shift from coal to natural gas amidst rising energy prices and an energy shortage. All of these facts were "especially true given the incomplete state of scientific knowledge of the cause of, and solutions to, global climate change" (Bush, p. 391).

Bush's resistance to allowing others into the decision-making process was evident by the definitive language he used in this letter that surprised many. In fact, some of the language "remained in the letter even after meetings early in the week at which Secretary of State Colin L. Powell and other foreign affairs experts suggested changes" (Revkin, 2001, p. 1). Bush also retained his skeptical statements on climate change, despite environmental groups and even some officials in the chemical industry believing that scientific consensus was stronger than ever. He kept his letter to the Senators as he preferred it, and it thus "lacked the usual nuances and qualifications required to sustain diplomatic discussions, regardless of a country's stance on the substance of a treaty" (Revkin, p. 2). As will be further examined below, President Bush in this case tended to disregard international opinion and the potential diplomatic consequences of his policy reversal. Overall, he strongly adhered to his role as primary foreign policymaker, rarely allowing others to be part of the major decision-making process.

However, in the build-up to Bush's reversal of policy on the Kyoto Protocol, many state level factors played an important role. The Byrd-Hagel Resolution passed by the Senate during Clinton's second term, for instance, was vital in shaping Bush's policy. This

resolution passed 95-0 in 1997 opposing US acceptance of climate change commitments that would hurt the US economy or exclude the developing world (Payne & Payne, 2008). The bill was sponsored by Democrat Robert Byrd from West Virginia, an important coal-mining state, and Chuck Hagel. Under the Byrd-Hagel Resolution, any future protocol would need to have a detailed explanation of regulatory action needed for implementation, as well as a financial analysis of the costs to the US economy (Payne & Payne). Even sympathetic Democrats supported this resolution, not wanting to anger supporters in labor groups, and knowing that Kyoto was essentially a lost cause; ideological agreement with the resolution as well as other factors likely played a role in its passage as well. Regardless, Senators were well aware that the Senate could not muster a two-thirds majority to ratify Kyoto to bring it into effect in the United States (Agrawala & Andersen, 2001).

There were also Senate and House committees and hearings that made the viewpoint of Congress quite clear. The Kyoto Protocol was discussed in committees as diverse as Science, International Relations, Energy and Natural Resources, Small Business, Commerce, and Government Reform and Oversight, along with various subcommittees (Steurer, 2003). Looking at the often-biased titles of the hearings, the Senate's view becomes apparent. In the Committee on Small Business, the title was *The Kyoto Protocol: the Undermining of American Prosperity — the Science*. In the Subcommittee on National Economic Growth, Natural Resources, and Regulatory Affairs, the hearing was titled *The Kyoto Protocol: is the Clinton-Gore Administration Selling Out Americans?* (Steurer). It was clear that the Senate was not favorable toward the Kyoto Protocol, at least not at the end of the Clinton administration and into the beginning of Bush's first term. In fact, Steurer (p. 346) even states that "the Bush administration's decision was nothing else than the final step of the administrative branch of a series of retreating steps, made by the legislative branch of the government in previous years." This does not, however, account for the sudden nature and force with which Bush reversed his previous views on carbon emissions reductions.

The reversal was clearly a shock to many, but was particularly surprising to those in the legislative branch. When the policy reversal was announced, several Congress members had been preparing bills to regulate carbon dioxide emissions from power plants; moderate Republicans like Senator Jim Jeffords of Vermont had been preparing to join Democrats in a bipartisan effort to reduce emissions in power plants over the next six years ("Reverses Course," 2001). The bills were still planned to be introduced, but the members of Congress recognized that with the sudden shift in policy, there was little hope of bills on this topic

succeeding (Jahl & Revkin, 2001). Amid a background of international protests, the Senate Foreign Relations Committee soon voted unanimously to call on Bush to develop ideas for a binding climate treaty; even Republicans demanded US reductions in emissions by August 2001 (Steurer, 2003).

Another area of the governmental structure that was shocked and affected by Bush's rejection of Kyoto was the Environmental Protection Agency, primarily its director, Christine Whitman. As recently as ten days before the announcement of Bush's policy reversal, Whitman was describing campaign promises in the area of climate change as though they were policy (Jehl & Revkin, 2001). She was advocating a new regulatory approach that would include carbon dioxide, and there were even reports that the administration might announce a plan to regulate power plant emissions (Payne & Payne, 2008). Whitman did not really support Bush's change in position; indeed, Cheney and Abraham had been more instrumental in decision-making on climate change than Whitman had been (Jehl & Revkin). In fact, the decision was somewhat of a rebuke of Whitman (Jehl & Revkin): "In a confidential memorandum to President Bush...she had explicitly recommended that the administration avoid being seen as opposed to the Kyoto Protocol" (Jehl, 2001, p. 1). This was clearly not followed, and Whitman was not given specifics as to the new position on climate change either (Jehl). Whitman had urged Bush to seem engaged on climate change; instead he proclaimed the treaty dead (Henneberger, 2001). Greg Wetstone of the Natural Resources Defense Council said that Whitman had "probably suffered one of the most immediate and embarrassing eviscerations of a new cabinet secretary ever" (Henneberger, p. 1). Two weeks after Bush announced his domestic plan to regulate emissions, Whitman officially announced that Kyoto was "dead" (Payne & Payne, 2008). She claimed that Bush was right about the energy crisis and other related factors (Henneberger), but the damage had been done. Whitman eventually resigned in 2003, around the same time that the Bush administration modified reports from the EPA on climate change to replace sections with text written by the American Petroleum Institute (Mercier, 2006). There were struggles between Bush and the EPA, particularly Whitman, but Bush's role was obviously dominant in this case over that of the government, considering that the Senate was in favor by that time of emissions regulation.

Societal factors played a much larger role in the case of Bush's rejection of Kyoto than the role of state level factors. A major player here was interest groups. "The pressure to make the decision came in part from lobbyists for coal companies and utilities dependent on coal" (Jehl & Revkin, 2001, p. 1); coal producers would shoulder much of the burden of an agreement like Kyoto,

so they pressured Bush to change his mind on the treaty. They also argued that converting coal-burning plants to natural gas to follow the treaty would cause a spike in gas prices and hurt consumers ("Reverses Course," 2001).

Companies within American society often had an impact on President Bush's climate change policy as well. Exxon Mobil, for instance, distributed \$2.9 million to thirty-nine groups that denied evidence of climate change (Payne & Payne, 2008). The company also pressured Bush to remove an outspoken key figure from the Intergovernmental Panel on Climate Change, Dr. Robert Watson, chief scientist for the World Bank and world-renowned atmospheric scientist (Payne & Payne). More direct evidence for Exxon Mobil's policy shaping emerged later: Bush thanked them for active involvement in forming climate change policy and sought advice on policy options. There was even a briefing note for Under Secretary of State Paula Dobriansky revealing that Bush "rejected Kyoto in part based on input" from an industry group largely funded by Exxon Mobil (Payne & Payne, p. 378). In fact, Borger and Macalister (2001, p. 3) went so far as to say that "Exxon's executives appear to hold sway over a man who once dreamed of rivaling their success but failed as an oil man and had to settle this year for becoming president of the United States."

There were other corporations and industry associations driven by concern for potential economic loss from Kyoto as well, such as the American Chamber of Commerce, several labor unions such as the United Mine Workers of America, and even farmers' associations against increased food prices that could result from higher oil prices (Steurer, 2003). Most anti-Kyoto actors were part of the Global Climate Coalition (GCC), which is made up of more than 6 million companies and corporations from all sectors (Steurer). The GCC frequently questions the science of climate change to justify their rejection of binding regulations and the unjust exemption of developing countries. They favor realistic targets instead of binding ones, and long-term actions to be voluntary only (Steurer). The GCC has a remarkable coincidence of interests with the Bush administration, and was able to affect policy in various ways such as contributing to IPCC documents, attending FCCC Conference of Parties negotiations, and commenting on proposed legislation (Steurer). They even held a thirteen million dollar advertising campaign before the Kyoto Protocol talks (Agrawala & Andersen, 2001). There exists a loose business coalition that is pro-Kyoto, the Pew Center on Global Climate Change, but they had little political influence in this era (Steurer).

There was also a major gap between public opinion and the views of the administration on this issue. Essentially, "public opinion did not back the President's retreat from Kyoto" (Steurer, 2003, p. 349). In August 2000, seventy-two percent of Americans believed in the

theory of global warming, and eighty-five percent saw climate change as a very or somewhat serious problem in September 2000. The month after Bush rejected the Kyoto Protocol, sixty-one percent of Americans thought America should join Kyoto, and a majority explicitly disagreed with Bush's decision to withdraw his support from Kyoto (Steurer). Bush subsequently tried to settle these unexpectedly large waves of resentment in the American public with rhetoric and small changes to the energy plan (Steurer), but the public opinion gap remained. Societal factors seemed to sometimes be extremely influential in Bush's decision-making, but in the case of public opinion, this was less so.

The final source category affecting the policy making process in this case is the international or system level category. The setup of the international system is a major factor here since the US was able to act alone in a hegemonic fashion. One could see the world power system as set up in a "uni-multipolar" fashion (Wittkopf et al., 2008, p. 155), with the US remaining a hegemon, but where other powers such as the European Union, discussed below, were gaining influence. The US is the only country even coming close to being a hegemon in the post-Cold War world, and frequently shows its ability for unilateral action and unilateral opposition to international action (Agrawala & Andersen, 2001). Yet, even while being unafraid to promote its own interests by "going it alone", the US is not a true hegemon, where it can always exercise leadership and expect others to follow. A Conservative member of the British Parliament, John Gunner, said that "the rest of the world doesn't need the US in quite the same way" that it did during the Cold War; there is now more freedom of action (Payne & Payne, 2008, p. 370). The complexities of the international system played a major role in allowing the US to unilaterally reject the Kyoto Protocol without most other major players following its lead.

Indeed, other rising powers such as the EU not only did not follow the lead of the US, they also pressured the US to join the Kyoto Protocol. Several former Prime Ministers and Presidents as well as European diplomatic delegations urged Bush to reconsider his decision; there were worries about the isolationistic course of the Bush administration, but their efforts were unsuccessful (Steurer, 2003). Angry European leaders not only believed that the US undermined the Kyoto Protocol, they also saw US efforts as trying to effectively kill the treaty by persuading others to walk away as well (Andrews, 2001).

The EU believed that Kyoto was vital to prevent a climate catastrophe, but the US response differed significantly: Under Secretary of State for Global Affairs Dobriansky stated, "We very much appreciate that others are reaching out to the United States and are thinking of ways of engaging us, but we do truly believe

that the protocol is fundamentally flawed. We will not be coming back to the protocol” (Andrews, 2001, p. 1). As was also evident from the strong language used in Bush’s letter, he was not looking for diplomatic discussions, but was generally ambivalent about international opinion on this matter. While the EU, and Tony Blair in particular, was an important diplomatic lobbyist favoring Kyoto in the “hot phase” of March-May 2001 (Steurer, 2003, p. 350), not all countries criticized the US decision. “Mr. Bush’s policy change was welcomed by Saudi Arabia, one of the treaty’s staunchest opponents” (Revkin, 2001, p. 1). However, overall international opinion was that the US should have accepted the Kyoto Protocol.

Once the US rejected Kyoto, the EU did take leadership and began to try to convince Japan and Russia to ratify it so that it could go into practice under the conditions previously set (Payne & Payne, 2008). Some thought that the US was trying to persuade Japan not to sign the treaty (Andrews, 2001), but Europe moved forward and was able to isolate the US on this issue—which many saw as a diplomatic setback for Bush (Payne & Payne, 2008). Cohen (2004, p. 83) describes the interpretation of US actions as such: “The White House’s decision to stick its finger in the eye of the international community, and to forsake the accord root and branch, is evidence of deep contempt for multilateral commitments and expert appraisal.” In this case, Bush acted on US interests, isolating America from the rest of the world. However, Kyoto went into effect in February 2005 without the US, after it was ratified by Russia. At that point, there were 168 countries and the EU party to the Protocol (Payne & Payne).

The final external actors affecting this case are various non-governmental organizations (NGOs) and intergovernmental organizations (IGOs) trying to sway the administration one way or the other, generally with little success. Not surprisingly, environmental NGOs supported Kyoto by trying to raise public concern and having the people apply pressure on the government, as well as lobbying in Washington and internationally (Steurer, 2003). However, their success in the US during the Bush administration was limited. Dan Weiss, former political director of the Sierra Club cynically said, “[A]nother day, another environmental standard wrecked. This is worse than under Reagan” (Henneberger, 2001, p. 2). IGOs were similarly unsuccessful in the US, the most prominent in this case being the Intergovernmental Panel on Climate Change (IPCC). Bush frequently questioned the validity of their science; they had little effect on his decision-making in regards to climate change despite their effect on others’ implementation of the protocol.

Overall, many factors came into play in Bush’s decision to reject the Kyoto Protocol in a reversal of his earlier policy. Individual factors such as his

administration’s connections to the oil industry and skepticism about climate change were very important in this case, as well as societal factors such as interest groups and corporations, though there was a major gap in public opinion. Governmental factors were also important, in terms of precedents set, but Bush did not generally abide by their recommendations or views in this case — such as the debacle with EPA director Whitman and resistance in the Senate. External factors were mainly important in that Bush could choose whatever plan of action he desired, as the US had some hegemonic powers; other countries strongly disapproved of his administration’s actions, but this did not change the decision. Finally, role factors mainly help explain here exactly how the policy was disseminated, in this case via a letter to Senators, as Bush was the main foreign policy creator. This case can help in understanding US foreign policy in general, as it set the stage for climate change negotiations in terms of mandatory emissions reductions; these actions will continue to affect the post-Kyoto framework being put into place now. This is not an issue that has been solved, though it remains unclear why exactly Bush took the step to un-sign the Protocol when it was not ratified by the Senate. One can speculate that perhaps he was asserting his priority on the economy over the environment, or that he wanted to differentiate himself from Clinton and his policies. Or perhaps he was concerned that he was losing Republican support early in his presidency. Sources do not indicate why Bush actually unsigned the treaty — an interesting aspect of this case.

Because this was not a crisis situation, the decision-making framework was built up over years, with the Byrd-Hagel Resolution and so on. However, the final decision to reverse the policy seems to have been done in secret and was a shock to many. This was characteristic of George W. Bush’s policies, along with US isolationism and unilateralism. The US seems to deal with its global leadership role here by effectively disregarding international opinion and focusing on its own interests. However, the situation for Bush changed drastically after September 11, 2001, when he became significantly more involved in global affairs (Hook & Spanier, 2010). After the terrorist attacks, climate change was not resurrected until Bush’s proposal of an alternative in February 2002 (Steurer, 2003). Various initiatives concerning climate change have been introduced federally since then, and states have also taken leadership roles in implementing emissions restrictions (Payne & Payne, 2008). In January 2007, nearly six years after rejecting the Kyoto Protocol, President Bush called global warming a “serious challenge” in his State of the Union address (Payne & Payne, p. 379). Overall, many factors came into play in Bush’s decision in 2001 to reject the Kyoto Protocol on

climate change, and it seems that some of these changed in time for a recognition of the problem near the end of his presidency. However, only time will tell what future climate change agreements will hold, and if they will be able to prevent serious changes in the Earth's climate.

REFERENCES

Agrawala, S., & Andersen, S. (2001). US climate policy: Evolution and future prospects. *Energy & Environment*, 12(2-3), 117-37.

Andrews, E.L. (2001, July 16). Frustrated Europeans set to battle US on climate. *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Borger, J., & Macalister, T. (2001, April 17). How the high priests of capitalism run roughshod over fears for planet. *The Guardian*. Retrieved April 29, 2009 from <http://www.guardian.co.uk>

Bush, G.W. (2001). President Bush's letter to three Senators explaining his rejection of the Kyoto Protocol. *Energy & Environment*, 12(4), 391-2.

Cohen, M. (2004). George W. Bush and the Environmental Protection Agency: A midterm appraisal. *Society and Natural Resources*, 17, 69-88.

Daynes, B.W., & Sussman, G. (2005). The "greenless" response to global warming. *Current History*, 438-43.

Henneberger, M. (2001, April 17). Despite appearances, Whitman says she and Bush agree on environment. *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Hook, S.W., & Spanier, J. (2010). *American Foreign Policy Since World War II* (18th ed.). Washington, DC: CQ Press.

Jehl, D. (2001, March 30). US stance on warming puts Whitman in tense spot. *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Jehl, D., & Revkin, A.C. (2001, March 14). Bush, in reversal, won't seek cut in emissions of carbon dioxide. *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Lisowski, M. (2002). Playing the two-level game: US president Bush's decision to repudiate the Kyoto Protocol. *Environmental Politics*, 11(4), 101-19.

Mercier, J. (2006). American hesitations to reduce greenhouse gas emissions: An institutional interpretation. *International Review of Administrative Sciences*, 72(1), 101-21.

Mr. Bush reverses course. (2001, March 15). *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Payne, R.A., & Payne, S. (2008). The Kyoto Protocol and beyond: The politics of climate change. In R.G. Carter (Ed.), *Contemporary Cases in U.S. Foreign Policy: From Terrorism to Trade* (pp. 357-89). Washington, DC: CQ Press.

Revkin, A.C. (2001, March 17). Bush's shift could doom air pact, some say. *New York Times*. Retrieved April 27, 2009 from <http://www.nytimes.com>

Reynolds, P. (2001, March 30). Kyoto: Why did the US pull out? *BBC News*. Retrieved April 27, 2009 from <http://news.bbc.co.uk>

Steurer, R. (2003). The US's retreat from the Kyoto Protocol: An account of a policy change and its implications for future climate policy. *European Environment*, 13, 344-60.

Wittkopf, E.R., Jones, C.M., & Kegley, C.W. (2008). *American Foreign Policy: Pattern and Process* (7th ed.). Belmont, CA: Thomson Wadsworth.

Acknowledgements: The author would like to thank Dr. Judy Krutky for her assistance with the writing of this case analysis.

Address correspondence to: Ellen Mackall, *Department of Political Science, Baldwin-Wallace College, 275 Eastland Rd., Berea, OH 44017*; Email: ellenkathleen@gmail.com

Received: 15 May 2009
Revised: 11 January 2010
Accepted: 3 June 2010

The efficacy of ellagic acid in attenuating neurophysiological and cognitive-behavioral symptoms associated with infusion of amyloid-beta (A β) peptide fragments in adult rats.

Gina N. Wilson,¹ G. Andrew Mickley,¹ & Kathryn M. Matera²

¹Neuroscience Program, Baldwin-Wallace College, 275 Eastland Rd., Berea, OH 44017; ²Chemistry Department, Elon University, Elon, NC 27244

Alzheimer's disease (AD) is a debilitating disorder that plagues our aging population. Three neurological events that contribute to AD progression include amyloid-beta (A β) aggregation, acetylcholine depletion, and oxidative stress. Walnut extract has been shown to inhibit actions of two sites on acetylcholinesterase (AChE) that are involved in A β aggregation and acetylcholine catabolism. Our previous *in vitro* Congo Red aggregation and kinetic assays specifically showed that ellagic and gallic acids (EA and GA), components of walnut extract, were responsible for these inhibitory actions. The current study employed those findings in an *in vivo* investigation with EA. Adult rats were infused, intracranially, with an A β or a control solution, followed three weeks later by control or EA treatments. Animals infused with A β followed by control solution had significantly impaired spatial working

and reference memory, which the EA treatments restored to normal. Group differences in initial measurements of brain AChE levels were not statistically significant. However, later *in vitro* assays using remaining brain tissue showed a marked decrease in brain AChE activity in the presence of EA. Analyses also showed a significant increase in oxidative damage by A β treatment when followed by control, but not EA, treatment. These findings demonstrate a possible benefit of EA treatment, but necessitate further research on the subject before it is applied to clinical settings.

Key words: ellagic acid (EA); acetylcholine (ACh); acetylcholinesterase (AChE); oxidation; reactive oxygen species (ROS); working memory; reference memory; amyloid-beta (A β); Alzheimer's disease (AD).

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that afflicts over 26.6 million people worldwide and the number is projected to soar to over 105 million by the year 2050 (Brookmeyer, Johnson, Ziegler-Graham & Arrighi, 2007). Symptoms span from mild cognitive impairment, in early stages, to the complete loss of language production and comprehension abilities, and coordinated motor skill, in the later stages of the disease (Zhang, Zhou, & Dani, 2004). Genetic inheritance has been recognized as the primary cause in about 5% of AD cases, but the cause is largely unknown for the majority of cases (Gassen & Annaert, 2003). However, there are commonalities in all cases of the disease and certain neuropathological features that are known to contribute to the characteristic cognitive decline and neurodegeneration (Muñoz-Ruiz et al., 2005). Specifically, a positive correlation has been established between symptomatic cognitive decline observed in AD and the neurophysiological features that include, but are not limited to, amyloid-beta (A β) plaques in the brain, oxidative damage, and acetylcholine (ACh) depletion (Stepanichev, Moiseeva, Lazareva, Onufriev, & Gulaeva, 2003; 2005; Chacón, Barría, Soto, & Inestrosa, 2003; Shin et al., 1997).

The neurotransmitter ACh is vital for learning and memory processes (Miguel-Hidalgo et al., 2002). For example, the muscarinic ACh receptors play a significant role in early stage memory acquisition and memory consolidation (Miranda et al., 2003; Power, Vazdarjanova, McGaugh, 2003). The cholinergic

system, additionally, is activated during tasks that explicitly require the maintenance of attention (Arnold, Burk, Hodgson, Sarter & Bruno, 2002). Behaviorally, attentional deficits are among the first underlying cognitive impairments in AD, lending to initial episodic and semantic memory problems that augment the development of even greater memory acquisition and consolidation impairments characteristic of AD (Perry, Watson & Hodges, 2000).

Breakdown of ACh occurs at a specific site on the acetylcholinesterase (AChE) enzyme, called the catalytic gorge (Miguel-Hidalgo et al., 2002; Zhang Zhang, Zhou, & Dani, 2004). However, AChE has a secondary function related to a physically distinct location called the peripheral anionic site (PAS). The PAS associates with A β peptides and can induce the aggregation of these peptides into abnormal A β plaques (Muñoz-Ruiz et al., 2005). There is also evidence that AChE is indeed an integral part of the plaques and accelerates the fibrillization of A β protein to form these plaques, *in vitro* and *in vivo* (Miguel-Hidalgo et al., 2002; Muñoz-Ruiz et al., 2005; Zhang Zhang, Zhou, & Dani, 2004).

Protein aggregation is a major feature of all AD cases. The A β protein is primarily found in extracellular senile plaque deposits and A β aggregation plays a critical role in the symptomatic progression of AD (Rajan, Illing, Bence & Kopito, 2001). Protein aggregation can result from a variety of endogenous factors including DNA or RNA mutations, protein denaturation, or abnormal secretase activity, among others (Rajan et al.,

2001). It has been strongly suggested that A β deposition is primarily linked to the main pathogenesis and cognitive decline in AD (Selkoe, 1996; Wang et al., 2006). The cholinergic deficits and release of reactive oxygen species (ROS) characteristic of AD, additionally, have been linked to the overabundance of A β peptides and aggregates. Even in the absence of a neurotoxic environment, very low concentrations of A β have been shown to inhibit cholinergic neurotransmitter function, further demonstrating a specific interaction of the cholinergic system and the A β protein.

Aside from A β aggregation and ACh depletion, oxidative damage (i.e., the aforementioned release of ROS) is process that causes the cognitive decline and cellular death associated with AD (Melo, Agostinho, & Oliveira, 2003). Fibril formation results in the release of ROS, which leads to oxidative stress in the brain. While the presence of ROS may increase with general aging, their presence in the AD brain is unregulated and dangerously high (Melo, Agostinho, & Oliveira, 2003; Frautschy et al., 2001). ROS, capable of damaging cells by attacking lipid membranes and other vital cellular moieties, are induced by the A β ₍₁₋₄₀₎ fragment and even more by abnormal A β ₍₁₋₄₀₎ plaques (Parks et al., 2001). Additionally, Lauderback et al. (1999) described the tendency of A β ₍₂₅₋₃₅₎ to inhibit sodium-dependent glutamate uptake *in vivo*. Since glutamate is the main excitatory neurotransmitter, an increase in extracellular levels of this neurotransmitter can lead to glutamate excitotoxicity and subsequent neuronal death (Lauderback et al., 1999). Glutamate excitotoxicity also increases intracellular ROS production, a direct cause of oxidative damage (Mattson, Goodman, Luo, Fu & Furukawa, 1997). In addition to oxidation via glutamate excitotoxicity, free radicals (or ROS) result from and associate with the A β plaques of AD. The distribution of senile plaques within the brain of AD sufferers is also positively correlated with the distribution of glutamatergic synapses (Mattson et al., 1992; 1997). Consequently, oxidative stress resulting from ROS production augments the problem of glutamate excitotoxicity, leading to more neuronal death and directly resulting in further neurodegeneration and symptomatic cognitive decline observed in AD (Harris et al, 1995).

Bastianetto, Brouillette & Quirion (2007) found that certain polyphenol antioxidants protected hippocampal cells against oxidative stress induced by A β peptides and plaques. Furthermore, antioxidants have long been associated with health benefits including, but not exclusive to, improved brain function (Shukitt-Hale, Cheng, Bielinski, & Joseph 2007; Ono & Yamada, 2006). Research has demonstrated that ROS activity can be diminished by administration of various antioxidants, especially from natural plant extracts (Bastianetto, Brouillette, & Quirion, 2007; Shukitt-Hale, Cheng, Bielinski, & Joseph., 2007; Ono & Yamada, 2006). However, this knowledge has yet to be fully

harnessed to create an AD treatment that utilizes the power of plant products and naturally found antioxidants.

Most of the drugs that have recently been on the market for AD treatment target breakdown of ACh by inhibiting the catalytic gorge of AChE. Such drugs include rivastigmine (3-(-1(dimethylamino)ethyl)phenyl ethyl(methyl) carbamate) and donepezil (Aricept: 2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one). Aricept is a popular AChE inhibitor currently on the market for treatment of moderate to severe AD (Giacobini, 2003). This method of blocking ACh breakdown can efficiently increase levels of this neurotransmitter in the brain and improve cognition for a limited period of time (months to years). However, in neglecting the other major features of the disease (i.e. plaques and oxidative damage), such drugs are imperfect in their abilities to fully treat the disease symptoms and halt cognitive decline.

Other drugs target the cleavage of the amyloid precursor protein (APP) to hinder formation of amyloidogenic fragments; Alzemed (3-amino-1-propane-sulfonic acid), currently in clinical trials, aims to hinder aggregation of the A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ fragments (Weinstock, 1999). Still, other drugs, such as tacrine (Namenda: 1-amino-3,5-dimethyl-adamantane), target the oxidative damage due to glutamate excitotoxicity, by inhibiting NMDA receptors (Lauderback et al., 1999; Weinstock, 1999). Each of these drugs only target one problem associated with AD. Surely, such a multifaceted disease requires a multifaceted, multilateral attack.

Namenda and Aricept are often co-administered and in combination can offer additional benefits for cognitive functioning even in severe cases of AD (Mohs & Carrillo, 2006). This combination is the most effective drug treatment thus far, but the disease still inevitably progresses and the ultimate fate of the patient is not changed, only postponed months to a maximum of two years. So, even though it is possible to administer some of these drugs together and benefit from their combined effects, it may not always be an option due to undesirable side effects of drug interactions, ineffective inhibition of molecular targets, or negligible combined effects on cognition (Weinstock, 1999).

Even though the potent effect current AD pharmaceuticals have on the brain is desirable, such potency creates a need to minimize the amounts introduced into a biological system due to certain side effects or overdose effects. For example, acetylcholinesterase inhibitors (e.g. Aricept) can cause muscle weakness, heart block, convulsions, and gastrointestinal problems. Likewise, NMDA antagonists that work to minimize glutamate excitotoxicity and oxidative damage have also been linked to cardiac failures, hypotension, vertigo, ataxia, increased rates of pneumonia, and aggressiveness (Rx List, 2007). For such reasons, it is important to consolidate the effects of Alzheimer's treatments and use components that carry the most benefit with the least risk; it would be ideal to

find one drug with actions at multiple targets. Finding drugs that can target more than one problem associated with the disease is imperative if truly significant advancements in the treatment of AD are to be gained. Improved treatments should ideally aim to simultaneously inhibit both sites of AChE while also targeting the oxidative damage that occurs as a result of general aging and the A β aggregates (Muñoz-Ruiz et al., 2005).

Preliminary *in vitro* data indicated that gallic and ellagic acids (GA and EA, respectively), found in black walnut (*Juglans nigra*) extract, could inhibit fibrillization and defibrillize preformed aggregates of A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ in the presence and absence of AChE, and inhibit AChE catalytic activity (N. Alexander, unpublished data, personal communication, 2005; Wilson, Marinis, Alexander & Matera, 2007). These studies have thus far determined that AChE catalytic activity can be inhibited by both GA and EA concentrations of 0.120-1.05 μ M in solution and that A β ₍₁₋₄₀₎ aggregation and A β ₍₁₋₄₂₎ aggregation can be inhibited and reversed at about 3.19 μ M in solution (Wilson et al., unpublished data).

Both GA and EA have powerful antioxidant properties (Frautshy et al., 2001). Additionally, since Frautshy et al. (2001) and Ono & Yamada (2006) have determined anti-fibrillogenic and anti-inflammatory potential of such antioxidants, GA and EA have become even more attractive candidates for Alzheimer's therapy. However, despite the potential therapeutic effects of these acids for Alzheimer's, their *in vivo* abilities to target aggregation of injected A β peptides, oxidative damage or possible alterations in AChE activity characteristic of the disease is unknown (Wilson et al., unpublished data). The ability of these chemicals to reduce, if not eliminate, memory impairments has not been investigated, nor has confirmatory *in vivo* work been done to fully elucidate the metabolic or physiological effects of these components.

Both acids have been determined to have low health risks and both are used in various sorts of biological research (Borges et al., 2007; Ono & Yamada, 2006; Gallic Acid, MSDS; Ellagic Acid, MSDS). Furthermore, the literature suggests that they are quickly metabolized and cleared from the system 4 hrs after subcutaneous injection (Borges et al., 2007).

The aim of this study was to determine the *in vivo* efficacy of EA, a dual-inhibitor of AChE and general antioxidant, in reducing the cognitive deficits, lipid peroxidation, senile plaque formation, and acetylcholine depletion associated with A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎. EA was chosen over GA for this *in vivo* study because it has a higher LD₅₀ value and is generally shown in the literature to have greater health benefits than gallic acid (Ellagic acid, MSDS; Gallic acid, MSDS; Roberts et al., 2007; Borges et al., 2007). EA supplements have also been safely administered to humans for their antioxidant properties. Neither supplement, in an oral form, has been especially promoted for memory enhancing

abilities in humans. A partial rationale for this lack of promotional support is due to the fact that neither has been thoroughly researched in human learning and memory paradigms and neither is FDA approved nor regulated. More importantly there is not a high enough concentration of EA or GA in an oral form for either to have a direct impact on the CNS (www.ellagicdirect.com, 2008).

We hypothesized that the EA agent would have significant benefits in restoring spatial working and reference memory capacity, reducing the extent of lipid peroxidation by ROS, and reducing AChE breakdown of ACh in animals infused, intracerebrally, with a mixture of A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎. This research aimed to increase the understanding of amyloidegenic A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ in the brain, both at a cellular and behavioral level. Furthermore, it assessed any therapeutic benefit of this antioxidant, AChE dual-inhibitor to slow, halt, or reverse the pathology and cognitive decline associated with the presence of A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ in the brain.

Materials and Methods

Subjects

This study used 11 male and 11 female Sprague-Dawley rats, 250-350g in weight and 3-6 months of age, randomly assigned to one of four groups (see Table 1). Animals were housed in individual plastic cages (44.45 cm long \times 21.59 cm wide \times 20.32cm deep) with corncob bedding (Bed o'cobbs, The Andersons Industrial Products, Maumee, OH). A 12-hr light-dark cycle (lights on at 0600 hr) was maintained, and temperature was kept within 23-26°C. Also, animals had free access to Purina Rat Chow (no. 5001, PMI Nutrition International, Brentwood, MO) and tap water throughout the duration of the study. All procedures were consistent with the guidelines set by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Baldwin-Wallace College Institutional Animal Care and Use Committee.

Chemicals

Ethanol (200 proof, absolute, anhydrous) was purchased from Pharmco-Aaper (Brookfield, CT). The A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ peptides (Ultra Pure, packaged in 0.1% NaOH) were purchased from rPeptide (Bogart, GA). All other chemicals were purchased from Sigma-Aldrich, Inc (St. Louis, MO).

Artificial cerebrospinal fluid (aCSF) was the vehicle for all intracerebral peptide injections. The aCSF vehicle was mixed according to Chacón et al. (2003): 130mM NaCl, 2.6mM KCl, 4.3mM MgCl₂, and 1.8mM CaCl₂. The aCSF was filtered through 0.22 μ m filter paper and then autoclaved for sterilization. Salts

present in the aCSF (NaCl, KCl, MgCl₂, CaCl₂) were of the highest purities available (greater than 96%).

Consistent with peptide mixtures of Stéphan, Laroche & Davis (2004), 10µg of each individual, sterile peptide was homogenized in 5µl of sterile aCSF vehicle. Then, the two peptide solutions were combined thoroughly in a 1:1 volumetric ratio so that each peptide fragment contributed 50% of total peptide concentration. The purity of these recombinant peptides was guaranteed to be consistently greater than 97% and the salt content made up 10% of the total weight (www.rpeptide.com, 2007).

EA powder (96% purity or greater, Sigma-Aldrich) was dissolved in sterile aCSF to an initial concentration of 60mM. The solution was then sterilized and after filtration via 0.22µm filter paper, was at a concentration of 4µM (verified via ultraviolet-visible spectroscopy).

Stereotaxic Surgery

Thirty minutes prior to surgery, male animals were injected with a sodium pentobarbital (60mg/ml; 1ml/kg; i.p.) and ketamine (20mg/ml; 1ml/kg; i.m.) cocktail. The dosing was altered for female rats as follows: sodium pentobarbital (60mg/ml; 1ml/kg; i.p.) and ketamine (20mg/ml; 0.75ml/kg; i.m.). Sodium pentobarbital salt and (±)-ketamine hydrochloride were both purchased from Sigma Aldrich and dissolved in sterile, physiological saline.

The surgical procedure was consistent with that described by Skinner (1971) and Wellman (1994) with appropriate modifications made for the implantation of cannulae into the brain instead of electrodes. The functioning guide cannulae was consistent with that used by Hilliard, Dillstone & Oliver (1968); in brief, it was made of dental amalgam with precisely drilled holes into which the stainless steel cannulae were placed. The guide cannulae protruded slightly out of the scalp and were visible post-surgically. The injection cannulae were inserted directly through the guide, without reopening the rat for administration of any treatment (peptide infusate, EA infusate, or aCSF control solution).

All cannulae were stainless steel. The implanted guide cannula was 21 gauge with an outside diameter of 0.0813cm and inside diameter of 0.0508cm. The injection cannulae were 26 gauge with an outside diameter of 0.0457cm and an inside diameter of 0.0254cm.

The surgical technique required for this study involved a standard stereotaxic, metal frame for small animals (David Kopf Instruments, Tujunga, CA, USA) equipped with ear bars and an incisor bar to properly position the animal. A heating pad, on low heat (approximately 37±3°C), was also placed under the animal during surgery to help maintain body temperature. This surgery was necessary for the implantation of the bilateral, chronic cannulae used for infusion of the Aβ peptides, EA, or control solutions.

Bilateral injection cannulae were placed at the following coordinates to deliver infusates bilaterally to the dorsal, CA1 field of the hippocampus: -3.5mm posterior to bregma, ±2mm lateral from the midline, and -2.7mm below the skull surface (Paxinos & Watson, 2007). The implanted guide cannulae, however, were placed 0.5mm above the target (-3.5mm posterior to bregma, ±2mm lateral from the midline, and -2.2mm below the skull surface), to avoid damaging target neural tissue (Paxinos & Watson, 2007; Wellman, 1994).

Bilateral holes were drilled in the skull with a trephine over the target coordinates of each brain hemisphere. The dura matter at the site of the cannula implantation was removed with a small wire pick. The cannula piece was secured with 4 appropriately fitted self/ tap Alan screws (00 x 3/16", Fine Science Tools Inc.) and additional dental amalgam. The perosteum was sealed over skull holes and the skin was sutured over the scalp incision (around cannulae protrusions). Marcaine (0.4cc; 0.25%) was also infused into the incision site at time of suturing. Sterile, tapered (26mm long needle) dry catgut thread chrom absorbable sutures from Chromend™ were used for this procedure (size 4/0).

Rats received appropriate post-operative care and twice daily health checks. Tylenol water (4.48mg/ml acetaminophen) was freely accessible to the animal for 3 days following surgery and marcaine was infused around incision/ cannula site once daily for 3 days following surgery (Mickley, Hoxha, Biada, Kenmuir & Bacik, 2006). These precautions were meant to provide sufficient analgesic effects so that the animals remained relatively comfortable and any pain, if not completely eliminated, was kept at a minimum (Mickley, Hoxha, Biada, Kenmuir, & Bacik, 2006).

Apparatus/ Equipment

The water maze used for behavioral analysis measured 64cm long x 46cm wide x 42cm deep. The platform was 8cm x 8cm, and the water level was set at 2cm above the platform. Water temperature was maintained at 26.5±1°C. Water was made opaque with addition of condensed milk to the pool, which virtually eliminated the ability of the rats to use visual cues within the maze. Every run was video recorded and Noldus (EthoVision® 3) software was used to analyze swim speeds, latency to find and mount the platform, time spent in the designated quadrants, and paths taken by animals (Morris, 1984).

Drug Injections and Behavioral Procedures

Forty-eight hours following the cannula implantation, animals were infused (i.c) with either the peptide solution or the control aCSF solution, initiating phase 1 of drug treatments (refer to Tables 1 & 2). Experimental animals were injected with 10µl of the Aβ peptide infusate into each of the bilateral CA1 areas of the hippocampus for a total of 20µg of peptide into the

brain. Control animals were infused with 10µl/hemisphere of aCSF. Infusions were completed in 2 stages (5µl/hemisphere infused at a time), separated by 2 hours, to avoid pumping too large of a volume into the brain at one time.

solutions at a rate of 4µl/min (Sipos et al., 2007; Zhang, Zhou, & Dani, 2004). Animals were allowed to roam freely in their home cage during all infusions.

Treatments of EA or aCSF (phase 2) began three weeks post-phase 1 infusions. EA or control (aCSF) treatment (phase 2; refer to Table 2) was given once daily (1700h) for seven days, via the intracerebral route made possible by chronic cannulation. Each single

Table 1: Group Treatments

| Group | Number of Subjects (N ¹) | Phase 1 Treatment: "Peptide" Infusion | Phase 2 Treatment: "Drug" Infusion |
|-----------|--------------------------------------|---------------------------------------|------------------------------------|
| Aβ+EA | 5 | 4µl peptide infusate | 2µl EA ³ |
| Aβ+aCSF | 6 | 4µl peptide infusate | 2µl aCSF |
| aCSF+EA | 5 | 4µl aCSF ² | 2µl EA |
| aCSF+aCSF | 5 | 4µl aCSF | 2µl aCSF |

¹ Both males and females were used for this study; equally distributed 2 males to 3 females or vice versa per group, or 3 males and 3 females for groups with an N of 6.

² aCSF=Artificial cerebrospinal fluid

³ EA=Ellagic acid solution (4µM; 4µl, i.c.)

Table 2: Timeline of Procedures

| Day 1 | Day 2 | Day 3 | | Days 24-27 | Day 28 | Day 29 | Day 30 |
|-----------------------------|----------|--|----------------------|---|--|----------------------------|---|
| Chronic Cannulation Surgery | Recovery | Phase 1 Treatment: Aβ peptide or aCSF ¹ Infusion | Aβ Incubation Period | Phase 2 Treatment: Commencement of Twice Daily Drug Infusions (aCSF or EA ²) | Drug Infusion 1, SWMT ³ , Drug Infusion 2 | Twice Daily Drug Infusions | Drug Infusion 1, RMT ⁴ , Drug Infusion 2 |

¹aCSF=Artificial cerebrospinal fluid

²EA=Ellagic acid (4µM; 4µl, i.c.)

³SWMT= Spatial Working Memory Test performed via Morris watermaze task. Animals were given eight 90s trials to find and mount a hidden platform, with an intertrial interval of 5min.

⁴RMT= Reference Memory Test performed via Morris watermaze task, except there was no platform for animals to find. This test consisted of only one 90s trial.

Thionin-stained olive oil was used to push the infusate through the line and into the brain. There was only minimal mixing of the two liquids; this was made apparent by the observation that the actual infusate solution turned a light blue. However, the olive oil remained largely behind the Aβ infusate, so any side effects due to the thionin-stained olive oil should have been negligible and inconsequential for the measures of this study. Also, concentration of the infusate was verified by ELISA.

For the infusion of all chemicals, an automatic pump was used (Kd Scientific, programmed to inject the

treatment consisted of 4µl per hemisphere of the specific treatment infusate. For the animals receiving EA, the 4µl dose of the 4µM initial concentration delivered 16 picomoles of EA into each hemisphere (48.3mg of EA per hemisphere; 96.6mg total in brain). This concentration was nearly 50 times more concentrated than the acid concentrations determined in the *in vitro* study (Wilson et al., unpublished data), but well under the LD₅₀ value for EA. The reason for increasing the injected concentration so greatly, compared to our *in vitro* study (data not reported; refer to Wilson et al.,

unpublished data), was simply to maximize potential observable effects of EA treatment on A β -infused and control animals while still considering the animals' health and well-being. The injection cannulae were left in place for 1min after the injection ended to ensure that the entire volume was delivered.

The spatial working memory (SWMT) of animals was tested in a water maze task on day 5 of the drug treatment (day 28 of the study; refer to Table 2 for timeline). The spatial working memory test consisted of eight trials during which the animal was allowed up to 90s to find the hidden platform. The water maze was divided into four quadrants: the platform was always located in the southwest quadrant and every animal started each trial in the northeast quadrant, facing away from the platform. Rats were placed in the opaque water and after finding and mounting the platform they were allowed to remain on it for 15s. If an animal did not find the platform in the allotted 90s, it was placed on the platform and allowed to remain there for 15s. The time between each subsequent trial, or intertrial interval, was 5min. During this intertrial interval, procedures were taken to make the rats comfortable. Each rat was placed in a warm, dry cage. After the eighth trial was completed, the animals were completely blow-dried and then returned to their home cages.

Forty-eight hours later, one reference memory test, or probe trial, was administered. During the reference memory test (RMT), the platform was removed and the pool was again split into four quadrants. The animal was again introduced into the opaque water in the northeast quadrant, facing away from the southwest quadrant, and given one 90s probe trial to swim in the water maze (Hoz, Moser, & Morris, 2005). The amount of time spent in each of the four quadrants was noted and the more time spent in the target (southwest) quadrant indicated better reference memory.

Tissue Preparation

Immediately following the reference memory test, animals were anesthetized with isoflurane and decapitated (Nakai et al., 2005). Following decapitation, a coronal section, containing the entire hippocampus, was dissected (cuts were made at -0.92 and -6.72mm posterior to bregma and included anterior hippocampus to the posterior hippocampal fissure) (Paxinos & Watson, 2007). The tissue was evenly and alternately divided for the thiobarbituric acid reactive species (TBARS) assay and AChE activity assay.

Assay for AChE Activity

A colorimetric assay, consistent with Lassiter et al. (2003), was used to measure AChE activity in tissue samples. Two sets of AChE assays were run: one that was an uninhibited assay (containing buffer, brain homogenate and reaction components) and the other that was an inhibited assay (containing EA, brain homogenate and reaction components). Fresh tissue

(2mg) was frozen at -70°C. The following day, tissue was homogenized in ice-cold sodium phosphate buffer (50 μ l, 0.5M; pH 8.0) containing 1% Triton X-10 (modified method from Lassiter et al., 2003). In a 96-well ELISA plate, (Corning Incorporated costar[®] 3590; 96 Well EIA/RIA Plate; flat bottom, high binding certified surface chemistry, polystyrene) ten microliters of brain homogenate was then added to 160 μ l dithionitrobenzoate (DTNB; initial concentration, 1.2mM), followed by the addition of either 10 μ l buffer (uninhibited assay) or 10 μ l of EA (4 μ M; inhibited assay). The solutions were then incubated in the ELISA plate at room temperature (24 \pm 1°C) for 10min, after which time 20 μ l of acetylcholine iodide was added (initial concentration, 80mM) and the 96-well plate was put into the Tecan Sunrise ELISA reader for immediate analysis. Light absorbance of the solution was measured at 412nm every 10s for 6min, by the ELISA reader. Between each kinetic cycle, the ELISA was programmed to shake the plate for a duration of 2s, on the lowest setting, with a settle time of 2s before the next absorbance reading was made.

Light absorption was a direct reflection of acetylcholine iodide (AChI) breakdown by brain AChE and subsequent reaction of the breakdown product, thiocholine (ChI) with DTNB to form the yellow product that absorbs light at 412nm. The increased absorbance over time reflected an increase in thiocholine concentration and thus a decrease in AChI concentration. The rate at which thiocholine was formed was directly related to the rate of AChE catalytic activity. Therefore, plotting absorbance versus time and obtaining the slope of the best fit line for samples in this assay served as a sufficient means for measuring and comparing AChE activity in these samples. Steeper slopes suggested greater AChE activity and reaction velocity (v_0).

Control solutions that lacked individual components necessary to observe the reaction via absorbance changes were also used to ensure the assay was working properly and that any increase in absorbance values over time was not attributable to something other than the activity of AChE in brain homogenates. Additionally, 200 μ l aliquots of DTNB, AChI, buffer and a few brain homogenate samples from each group were assessed individually by the ELISA reader to assess any variations over time. Due to the small amount of tissue available, control assays could not be run on every brain homogenate sample collected.

All experimental solutions in which a change in absorbance over time was necessary for analysis were run in triplicate. Data used in analyses were from the first 140s immediately following commencement of the reaction. Any data that had a minimally negative slope (less than or equal to 1×10^{-6}) were recorded as zero AChE activity.

TBARS Assay for lipid Peroxidation

A thiobarbituric acid (TBARS) assay, consistent with Leutner, Eckert, & Muller (2001), was used for the measurement of lipid peroxidation in tissue samples. Fresh brain tissue (10mg) was homogenized in 1ml of ice-cold Tris-HCl buffer (5mM; pH 7.4; $4\pm 2^\circ\text{C}$). Homogenates were then centrifuged at $17,500\times g$ for 10min. The supernate was collected and 1ml of 0.67% thiobarbituric acid [%w/v] was added (Leutner et al., 2001). The solution was then incubated in boiling water for 20min. After the incubation, light absorbance of the solution was measured at 532nm via a Perkins Elmer ultraviolet-visible spectrophotometer (UV-vis) and/or the ELISA reader (Lassiter et al., 2003; Leutner, Eckert & Muller, 2001; Garcia, Rodriguez-Malaver, & Peñaloza, 2005). The measured absorbance of the solution was directly related to the amount of oxidative damage in a solution. Specifically, following the TBARS analysis of other laboratories (Leutner, Eckert, & Muller, 2001), the absorbance values were used to calculate the concentration of malondialdehyde (MDA) in each sample; MDA is the major product of lipid peroxidation and thus, directly reflective of the presence and activity of ROS in a sample. The calculation of MDA concentration called for the use of Beer's law: $A = \epsilon bc$, where 'A' is the measured absorbance, 'b' is the path length the light travels through a given solution, 'c' is the concentration and ' ϵ ' is the molar absorptivity constant for MDA. The molar absorptivity constant used for MDA was $1.556 \times 10^5 \text{cm}^{-1}$ (Siciarz, Weinberger, Witz, Hiatt & Hegyi, 2001). The path length of the UV-vis was 1cm and the vertical path length for the ELISA in this experimental protocol was 0.89cm. The ELISA was used over the UV-vis in many cases because it requires much less sample (100-200 μl versus 1ml for the UV-vis). When sample was available, both the ELISA and UV-vis were run to ensure consistency of calculated concentrations via Beer's law (Siciarz, Weinberger, Witz, Hiatt & Hegyi, 2001).

Statistical Analyses

All statistical analyses were performed using SPSS software (Chicago, IL) and α was set at 0.05 for all measures. Since there were two main treatment phases (phase 1 and 2; see Tables 1 & 2), we performed a series of two-way analyses of variance (ANOVAs) to observe any main effects of the individual phase treatments or drug interactions of treatments within the 2 phases [Phase 1 treatments ($A\beta$ or aCSF) X Phase 2 treatments (EA or aCSF)]. If main effects were detected with the ANOVAs, subsequent Bonferroni *post hoc* comparisons were performed. Specific measures and additional statistical tests for each behavioral and chemical evaluation are listed separately in the following subsections.

Spatial working memory task

Data acquired from the spatial memory test allowed for the analysis of the following variables: time to

mount the hidden platform for each of the 8 trials, distance traveled, averages for the first four trials and the last four trials (first and last "trial sets"), and swim speeds (cm/s). In addition to the two-way ANOVAs performed for each variable, a repeated measures ANOVA (RM-ANOVA) was performed to analyze changes over time for the 8 individual trials [Condition x Trial (1-8)] and also for the animals' averages between the first and last trial sets [Condition x Trial Set (1-4, 5-8)].

Reference memory task

Data acquired from the reference memory test allowed for analysis of the following variables via two-way ANOVA: number of entrances to the target quadrant, time spent in the target quadrant and swim speeds (cm/s).

AChE Activity

Data from the uninhibited AChE assay of each animal's tissue samples (hippocampal and overlying cortical samples) were plotted as absorbance versus time and the slope of the best-fit line for the first 120s of the reaction was measured using *Microsoft Excel*TM. Data from each individual animal were taken and group averages were calculated. Additionally, these data were plotted for the inhibited portion of the AChE activity assay in which EA was added directly to brain homogenates for each subject in the same manner. Two-way ANOVAs were performed on slopes for each assay separately to test for main effects of the treatments given during Phases 1 and 2 and possible interactions on inhibited and uninhibited AChE activity levels. Additionally, since slope is a direct measure of enzymatic activity, the difference in slopes between the first uninhibited assay and the second inhibited assay were calculated, and percentage inhibition by *in vitro* EA addition, compared to the uninhibited assay, was also calculated. This was done to detect differences in tissue responsivity to the AChE inhibitor, EA, due to earlier *in vivo* treatments (phases 1 and 2). The percentage inhibition for both the cortical and hippocampal samples was calculated by dividing the slope difference between the two assays by the slope of the first, uninhibited assay and then multiplying by 100. Both sets of percentage data were analyzed via two-way ANOVAs [(Phase 1 Treatment ($A\beta$ or aCSF) X Phase 2 Treatment (EA or aCSF)] with subsequent Bonferroni *post hoc* comparisons. Percentage data passed Levene's test for equality of variances, Welch's and Brown-Forsyth's Robust tests for equality of means, and test for homogeneity of variances. Additionally, percentage data was largely within the 20-80% range, so data transformation (i.e. arcsine transformation) was not

needed prior to running the ANOVA (www.isogenic.info/index.html, 2006).

TBARS Assay for lipid peroxidation

Absorbance was recorded for each cortical and hippocampal sample at the 532nm wavelength. Beer's law ($A=\epsilon bc$) was then used to calculate the millimolar content of malondialdehyde (MDA) in each sample (Leutner, Eckert, & Muller, 2001). Data from each individual animal and each brain area were individually averaged and recorded. Additionally, a measure of total MDA was made by summing the average cortical and hippocampal MDA content values together for each individual animal. A two-way ANOVA (as described above) was performed on MDA measures taken.

A Pearson correlation was used to correlate hippocampal MDA content with the slope of hippocampal samples for the uninhibited AChE assay. MDA content was also correlated with the amount of time spent in the target quadrant during the reference memory test. Specifically, these correlations related (1) hippocampal ROS content with hippocampal AChE enzymatic activity and (2) hippocampal ROS content with reference memory test performance.

Results

Spatial working and reference memory

Some significant treatment effects in both spatial working and reference memory were elucidated. No significant differences were found for measures of spatial working memory by the two-way ANOVA [Phase 1 treatments ($A\beta$ or aCSF) X Phase 2 treatments (EA or aCSF)]. However, a RM-ANOVA revealed a significant decrease between the animal averages from the first four trials to the last four trials in all groups except the $A\beta$ +aCSF group [aCSF+aCSF: $F(1, 5)=10.240$, $p=0.024$; aCSF+EA: $F(1, 5)=9.430$, $p=0.028$; $A\beta$ +EA: $F(1, 4)=31.568$, $p=0.005$], possibly indicating a deficit in spatial working memory in the $A\beta$ +aCSF that was not demonstrated in the other experimental conditions. Figure 1 shows an overall decrease in test times from trail set 1 (trials 1-4) to set 2 (trials 5-8) in all groups, but this decrease is not statistically significant in the $A\beta$ +aCSF group. Data support a small, but significant, decrease in spatial working memory capabilities in those animals that received $A\beta$ not followed by EA treatment.

The reference memory test continued to draw out group differences in memory capabilities, as illustrated in Figure 2. A two-way ANOVA revealed significant phase 1 and 2 treatment effects, but no interaction effect. Significant differences were revealed for phase 1 ($A\beta$ versus aCSF) treatment [$F(1, 21)=9.646$, $p=0.006$] and phase 2 (EA versus aCSF) treatment [$F(1,21)=7.939$, $p=.012$]. As shown in Figure 2, phase 1 $A\beta$ treatment decreased the reference memory score (time in target quadrant), while phase 2 EA treatment increased the reference memory score with respect to

the animals' prior phase 1 treatment. *Post hoc* analyses showed that $A\beta$ +aCSF animals spent significantly less time in the target quadrant than all other groups. Significant differences between treatment groups were not found for swim speeds, number of entrances to the target quadrant or distance traveled. Data support that $A\beta$ treatment, when not followed by EA treatment, decreases reference memory capabilities.

AChE activity assays

While basal AChE activity measured from post-mortem tissue proved to have a large range (rate of AChI breakdown = 0.0001 – 0.0300 absorbance units/second) and varied greatly among animals, some differences were uncovered. It is important to mention that there were differences revealed by the two-way ANOVA for phase 1 $A\beta$ treatment, indicating higher basal AChE activity in $A\beta$ -infused animals versus aCSF-infused animals (phase 1) [$F(1,16)=10.105$, $p=0.008$] in cortical homogenates only, but *post hoc*s revealed no specific differences between the four experimental conditions. Data do not support that *in vivo* treatment with EA decrease AChE activity; if there was any decrease, it was not detectable via this *post mortem* assay of the brain tissue homogenates.

EA did prove to be a successful AChE inhibitor. The second inhibited assay (in which EA was added to all samples) showed that brain AChE (both in the hippocampal and cortical regions) was inhibited by *in vitro* exposure to EA as a decrease in AChE activity (slope) was qualitatively observed for each individual subject.

Most notably, measures of the percent inhibition of AChE activity by *in vitro* EA in the hippocampal samples were shown to be differentially affected by prior *in vivo* treatments. A two-way ANOVA revealed a significant main effect of phase 1 treatment ($A\beta$ versus aCSF) on the percentage of AChE inhibition observed in hippocampal samples following the addition of EA ($F(1,14) = 6.519$, $p=0.029$). As shown in Figure 3, tissue from rats that received $A\beta$ in phase 1 showed significantly increased percent inhibition of hippocampal AChE upon *in vitro* exposure to EA compared to rats given aCSF in phase 1, meaning that the tissue exposed to $A\beta$, *in vivo*, was more responsive to EA inhibition. There was no effect of *in vivo* EA treatment (phase 2) on tissue responsiveness to the EA added to the brain homogenates and no interaction effect. These data showed that EA is an effective inhibitor of brain AChE, but the specific effects of *in vivo* treatment could not be detected in *post mortem* analyses of the uninhibited brain homogenates (i.e., without the *in vitro* addition of EA). The data also showed that brain homogenates that were previously treated with $A\beta$, *in vivo*, had a greater inhibition response to subsequent EA addition *in vitro*.

TBARS Assay

As expected, A β -infused animals had higher ROS activity, as evidenced by increased MDA content. A main effect of A β treatment in phase 1 was demonstrated, with those rats that received an A β infusion showing significantly increased total MDA values compared to rats that received aCSF in phase 1 [F(1, 15) = 10.026, p=0.008]. As illustrated in Figure 4, a main effect for phase 1 treatment of A β or aCSF was only demonstrated for total MDA content and not for individual cortical or hippocampal samples. *Post hoc* comparisons showed that the A β +aCSF group had significantly higher total MDA concentrations than the aCSF+aCSF group and the aCSF+EA group (p<0.0475). Also, the aCSF+EA group was significantly less than the A β +aCSF group and its aCSF+aCSF counterpart (p<0.027). No main effect was observed for phase 2 EA treatment on total MDA concentrations. These data demonstrated the success of the A β infusion in producing increased ROS levels, but no overall effect of EA treatment on total MDA content (i.e., hippocampal plus cortical MDA content).

While total MDA concentrations did not reveal an effect of EA treatment, analysis of hippocampal MDA content did show some effects of phase 2 treatment (aCSF vs. EA). There was a significant effect of phase 2 treatment on hippocampal MDA concentrations [F=4.567, p=0.050], but no significant effect of phase 1 treatment and no interaction. As illustrated in Figure 4, micromolar MDA content in the hippocampal samples was increased in the A β +aCSF group compared to all other groups (p<0.037), shown by the Bonferroni comparisons. The data showed that animals receiving A β treatment that is not followed by an EA treatment have significantly increased ROS levels in *post mortem* measures of hippocampal MDA content.

ROS levels, as measured by MDA content, were positively correlated with other measures of the study. A positive Pearson correlation was revealed for total MDA content and uninhibited hippocampal AChE activity [r(1, 16) = 0.637, p=0.008]. Time in the target quadrant was negatively correlated with MDA content in the hippocampus [r(1,19) = -0.586, p=0.008]. These correlations demonstrate the relationship between increased oxidative stress (MDA levels) and both decreased reference memory capabilities and increased AChE activity, as it is relevant to AD models and clinical AD.

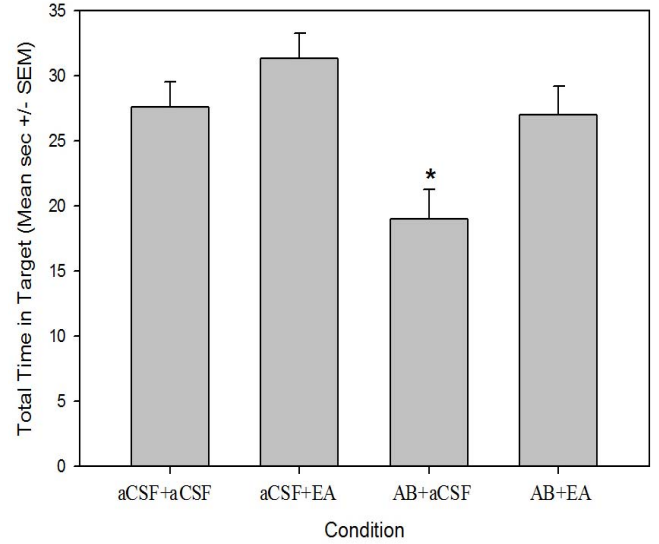


Figure 2. Spatial Reference Memory Test analysis of total time spent in target quadrant (mean sec \pm SEM). *A β +aCSF animals spent significantly less time in the target area compared to all other groups, indicating impaired reference memory capabilities (p<0.05). Variance indicators are SEM.

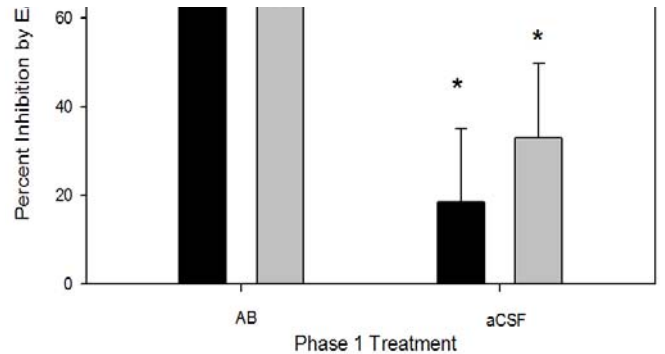
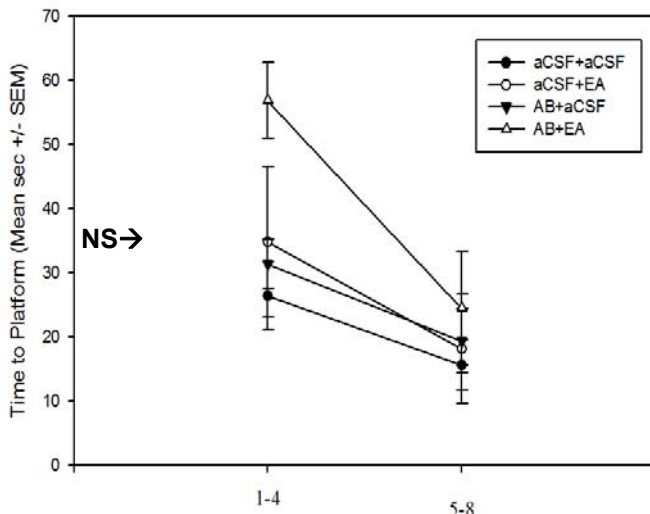


Figure 3. Mean percentage of AChE inhibition in hippocampal samples. Rats that received A β injections exhibited significantly more inhibition of AChE activity than did controls upon exposure of hippocampal tissue homogenates to EA. A two-way ANOVA showed a significant main effect of Treatment 1 (A β or aCSF). *Significantly less than both groups that received A β as phase 1 (p<0.05). Variance indicators are SEM.



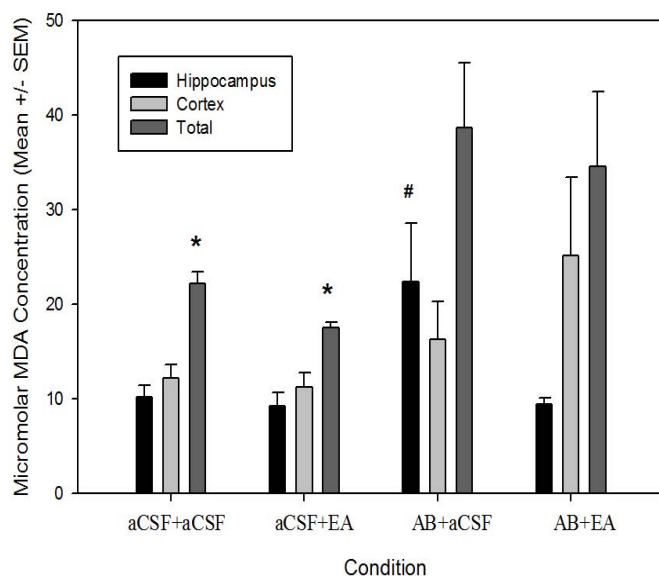


Figure 4. Results from TBARS assay. Micromolar Concentration of MDA (mean MDA concentration \pm SEM) showed area-dependent and additive changes due to experimental treatments. * = Total MDA content (hippocampal + cortical samples) was significantly less than both groups treated with A β in phase 1. # = Hippocampal MDA content was significantly greater than all other groups ($p < 0.05$). Variance indicators are SEM.

Discussion

This study not only demonstrated detrimental effects of a single A β mixed peptide infusion, but also some beneficial effects of multiple EA treatments in reducing these A β -induced deficits. In line with the hypotheses, A β -treatment (during phase 1) decreased working and reference memory capabilities while EA treatment (in phase 2) restored those capabilities (see Figures 1 and 2). Also consistent with the hypotheses, the measure of ROS activity (MDA content via the TBARS assay) in the hippocampus was significantly increased in animals treated with A β (phase 1) and only control aCSF solution in phase 2. However, when A β -treated animals were infused with EA in phase 2 of the study, they then showed MDA levels that were significantly lower than the A β +aCSF group and comparable to the aCSF (phase 1) animals (refer to Figure 4).

The AChE analyses proved to be ambiguous because, although EA treatment did decrease AChE activity *in vitro*, uninhibited AChE levels (measured directly after animal sacrifice, before addition of EA to homogenates, *in vitro*) were unaffected by the animal's prior EA treatment. Thus, *ad hoc* hypotheses regarding AChE were not supported. But some interesting observations and *post hoc* findings were elucidated that

are worthy of discussion. There was a significant main effect of A β phase 1 treatment on the percent inhibition of hippocampal AChE induced by the addition of EA (data illustrated in Figure 3). This may suggest that there is a real effect on the sensitivity of the AChE enzyme to EA exposure when significant neurotoxic A β exposure has occurred. This sort of interaction between AChE sensitivity to an inhibitor and A β treatment is not unlikely, given previous work that has demonstrated significant effects of A β on enzymatic and cellular function and its links to increased ROS in various parts of the cholinergic system (Melo et al., 2002; Darreh-Shori et al., 2006). Since initial levels of hippocampal AChE activity did not differ among any treatment groups, the observed hypersensitivity of AChE is most likely attributable to something other than a simple enzyme upregulation or subsequent increase of ROS by the A β treatment.

Correlations between behavioral and chemical measures (i.e., MDA content correlated with reference memory measures and hippocampal AChE activity) indicate that the acute A β infusions were related to some expected changes in the subsequent behavioral, ROS and AChE measures. Notably, the correlations show that increased oxidation in the hippocampus was positively associated with an increase in basal hippocampal AChE activity and possibly AChE sensitivity to the inhibitor, EA. Furthermore, correlations demonstrate that increased sensitivity of hippocampal AChE (reflected by the change in slope and percent inhibition) is related to reduced reference memory, as well as an increase in hippocampal MDA content that quantifies oxidative damage. Such relationships have been indicated by previous data, lending credibility to this animal model of AD (Melo et al., 2003, Stepanichev et al., 2003; 2004; 2005, Leutner et al., 2001) and allowing for some possible interpretations about hypersensitive AChE in AD. The first interpretation is that ROS induces an increase in AChE activity (Melo et al., 2003; Saez-Valero et al., 1999). Alternatively, high AChE activity could induce production and release of ROS. However, the results of this experiment suggest that the higher the MDA content is in neural tissue, the more sensitive the AChE enzyme in that tissue will be to the inhibitory effects of EA exposure. This could suggest an altered AChE protein structure in cases of AD, a structure that is possibly modulated by the higher presence of ROS.

There is evidence suggesting that there are indeed altered isoform ratios of AChE produced by the AD brain, which relates to altered glycosylation of AChE in the disease (Mimori et al., 1997; Saez-Valero et al., 1999). Mimori, Nakamura, and Yukawa (1995) showed a decreased sensitivity of AChE to various inhibitors (e.g., donepezil) in the AD brain, when compared to

samples from control subjects. Other research has also supported the idea of possible interference of A β with the binding of drugs to AChE, especially in and around senile plaque deposits (Darreh-Shori et al., 2006). Since altered sensitivity of AChE by the presence of A β has been demonstrated, to a degree, it is possible that data from this study reflected this alteration of binding in combination with the possible upregulation of AChE expression due to A β . Additionally, these results may indicate a different action of binding by EA to AChE, compared to other AChE-inhibiting drugs, such as donepezil. Donepezil and other AD drugs exhibit equal or lower inhibition of AChE in Alzheimer's disease samples versus control samples compared to what this present study has found for EA inhibition (Darreh-Shori et al., 2006; Saez-Valero et al., 1999). However a comparison of the percent inhibition by EA with percent inhibition by other AChE inhibitors would need to be made, using the same chemical method, before any conclusions could be drawn about the binding of EA to the various forms of AChE. Precise binding and kinetic studies, along with X-ray crystallography, also need to be performed before drawing final conclusions about drug mechanisms or verifying aforementioned hypotheses.

Spatial memory deficits are common with AD and easy to assess in rodent models of the disease (Stepanichev et al., 2004; 2005). Most rat studies detect working memory deficits before, or instead of, reference memory deficits (Stepanichev et al., 2004; 2005). Most rats in this study were able to learn the location of the platform during the working memory task and only the RM-ANOVA uncovered a non-significant decrease in the A β +aCSF, but a significant decrease in all other groups. Reference memory tests indicated an even stronger advantage of EA treatment. It is important to note that A β +aCSF animals had significantly decreased reference memory scores compared to aCSF (phase 1) control animals. This indicated that the injection was successful in generating measurable memory deficits that were crucial for the conclusions drawn from the remainder of the study.

Some investigators have argued that working memory is first to be affected by AD (Stepanichev et al., 2003; 2004; 2005). But, studies have shown that if attention to a task is maintained, early-stage AD patients do not show significant working memory deficits (Newman, Musgrave, & Lardelli, 2007). Thus, it is possible that reference memory capabilities, particularly those that are spatial in nature, are actually the first to noticeably depreciate following the onset of AD. A common symptom used to diagnose AD (although true diagnosis can only be done *post mortem*) is the propensity of patients to experience spatial disorientation, get lost in familiar places, or forget

common procedures they have learned years ago and practiced since (Newman, Musgrave, & Lardelli, 2007).

There could be a variety of methodological or theoretical reasons for the behavioral and chemical results of this study. The unique peptide infusate itself may be a reason for the differing observations between this and other studies. Currently there are no other studies that have employed this A β mixture (1:1 %w/v of A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎) *in vivo*. However, there are a variety of studies that show how these specific peptides act on biological and behavioral symptoms of AD in isolation. Sipos and his colleagues (2007) demonstrated that soluble A β ₍₁₋₄₀₎ and A β ₍₁₋₄₂₎ fragments can be infused into the rat brain and differentially form aggregates within 1 day to 5 weeks, but the fragments were not combined in the same infusate mixture. Rats infused with either fragment (A β ₍₁₋₄₀₎ or A β ₍₁₋₄₂₎) had impaired object recognition memory and delayed acquisition in spatial memory tasks (Sipos et al., 2007). Also, rats injected with 15-30nmol of either fragment demonstrated differential cognitive deficits in spatial learning and reference memory tasks (Stepanichev, Moiseeva, Lazareva, Onufriev, & Gulyaeva, 2003). Stepanichev et al. (2003) also demonstrated that a single intracerebroventricular injection of the A β ₍₂₅₋₃₅₎ fragment will produce oxidative damage, as shown by TBARS (thiobarbaturic acid reactive species) reactivity, within 30 days post-injection. These researchers observed cognitive deficits in a radial arm maze task within 60 days post-injection.

Snyder et al. (1994) suggested that there is some interference between the two different A β fragments and the observed aggregation varies compared to aggregation of the individual, isolated peptides, *in vitro*. It is suggested that the (1-40) fragment slows aggregation of (1-42) in a concentration-dependant manner. However, the rate of the (1-40) fragment may experience a seeding effect from (1-42) that actually enhances aggregation of the (1-40) fragments around fragments of (1-42) (Snyder et al., 1994). This interaction may have been beneficial in producing both detectable chemical and behavioral differences within the same general time frame. The slight heating of the peptide mixture induced partial aggregation and the injection of the partially aggregated A β ₍₁₋₄₂₎ probably provided a "seed" around which A β ₍₁₋₄₀₎ could further aggregate and this overall enhancement in aggregation potential may have made the protein structures more resistant to proteolysis (Snyder et al., 1994). Furthermore, incorporation of the A β ₍₁₋₄₂₎ core and A β ₍₁₋₄₀₎ surroundings along with the free or diffusely aggregated A β ₍₁₋₄₀₎ may present a better model of the disease compared to a model that injects only one of the peptides.

However, this specific model may have been disadvantageous given time frame for the study because

certain assumptions had to be made about the aggregation and aggregate clearing processes and behavioral correlates based on literature from just one of the two peptides. Such assumptions may not have allowed for proper manipulation of timed variables and this could have decreased the power of the paradigm and ultimately led to observed trends that failed to reach statistical significance. For example, it could be that ROS, AChE or aggregate levels were in the process of rising or falling, but had not yet reached the minimal or maximal levels that they may have if measurements had been taken weeks later or earlier (Melo et al., 2003; Shin et al., 1997; Sipos et al., 2007). If this study were to be repeated it would be worthwhile to try a variety of A β incubation times (a day, a week, two weeks, four weeks, etc.) using the same injection method. Also, despite methodological efforts to create a moderate to late stage model of AD, these current results point to a model of early AD. So, it would also be beneficial to inject treatment infusates into the entorhinal cortex, since that is actually the first brain region prone to A β aggregation. The hippocampus (CA1) incurs greater damage later in the disease (Shin et al., 1997; Sipos et al., 2007; Song et al., 1999).

A more thorough test of memory and attentional capabilities would also be useful to show the true potential of the A β infusion in producing AD-like symptoms and of EA, or its derivative, in reversing such deficits. Specifically, it would be interesting to use a set-shifting paradigm, since it is often used for rodent models of other neurological diseases that incur attentional deficits, such as Schizophrenia (Birrell & Brown, 2000). The set-shifting task would also add an aspect of divided and selective attention, which has emerged in recent years as a strong indicator of AD and other dementias in the clinical setting (Perry et al., 2000). Such an attentional task linked with specifically targeted working and reference memory tasks might allow for a more appropriate method of gauging AD-like progression in rodent models.

While this study demonstrated some significant benefits of EA in the *in vivo* rat model, the use of EA has some considerable disadvantages that make it an unattractive treatment for large-scale *in vivo* application. First of all, it is not easily dissolvable in many physiological, salt-based solvents used for purposes of *in vivo* administration. Along the same lines, the polarity of its chemical structure leads to poor passage through the blood-brain barrier. If, for example, EA were to be administered orally for the treatment of Alzheimer's in humans or animal models of the disease, such a small amount would actually reach the brain that the chance of central nervous system effects would be very low. The oral dosage of AChE inhibitors can only be so high before significant peripheral side effects appear like

stomach pain, diarrhea, blurred vision or muscle fasciculation (Punga et al., 2007).

Moreover, direct intracerebral injection of the EA is possible, but it involves invasive surgical procedures that carry certain risks and such injections may only deliver the drug to very localized parts of the brain. Clearly the mechanisms of EA action need further research both *in vitro* and *in vivo*, but new research should also focus on derivatives of EA that more easily pass through the blood-brain barrier. Specifically, acetylated derivatives of EA may have significant promise as advanced therapeutic agents. Acetylation is a common method of reformulating molecules so they are more permeable to the blood-brain barrier; as the chemicals pass the acetyl groups are lost, leaving the original molecule (Frautschy et al., 2001). Moreover, there are acetylated derivatives of EA found in natural sources. Some of these EA derivatives include 3,3',4-tri-O-methylelagic acid and ellagic acid-4-acetyl arabinoside (Kueete et al., 2007; Mullen et al., 2003). But, significant therapeutic benefits and increased permeability through the blood-brain barrier may be observed with a derivative as simple as acetyllelagic acid to tetra-acetyllelagic acid. Since this current study showed therapeutic potential for EA in cases of AD, such drug and methodological manipulations should be explored in future investigations.

REFERENCES

- Andersen, J.M., Myhre, O., Fonnum, F. (2003). Discussion of the role of the extracellular signal-regulated kinase-phospholipase A₂ pathway in production of reactive oxygen species in Alzheimer's disease. *Neurochemical Research*, 28(2), 319-326.
- Arnold, H.M., Burk, J.A., Hodgson, E.M., Sarter, M., Bruno, J.P. (2002). Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. *Neuroscience*, 114(2), 451-460.
- Bastianetto, S, Brouillette, J, Quirion, R. (2007). Neuroprotective effects of natural products: Interaction with intracellular kinases, amyloid peptides and a possible role for transthyretin. *Neurochemical Research*, 32, 1720-1725.
- Bimonte, H.A., Denenberg, V.H. (1999). Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology*, 24(2), 161-173.

- Birrell, J.M., Brown, V.J. (2000). Medial frontal cortex mediates perceptual attentional set-shifting in the rat. *Journal of Neuroscience*, 20, 4320-4324.
- Borges, G., Roowi, S., Rouanet, J, Duthie, G.G, Lean, M.E.J, Crozier, A. (2007). The bioavailability of raspberry anthocyanins and ellagitannins in rats. *Molecular Nutrition and Food Research*, 51, 714-725.
- Brookmeyer, R., Johnson, E., Ziegler-Graham, K., Arrighi, H.M. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimer's and Dementia*, 3, 186-191.
- Chacón, MA, Barriá, MI, Soto, C, Inestrosa, NC. (2003). β -sheet breaker peptide prevents A β -induced spatial memory impairments with partial reduction of amyloid-deposits. *Molecular Psychiatry*, 9, 953-961.
- Ellagic Acid: Meekers red raspberry. Millennium Health LLC. Accessed: 26 June 2008. <http://www.ellagicdirect.com/index.html>.
- Ellagic Acid*; MSDS No. 9923907; ScienceLab.com; Houston, TX, 28 October 2007. https://www.sciencelab.com/xMSDS-Ellagic_Acid-9923907.
- Engelhardt, B. Development of the blood-brain barrier. (2003) *Cell and Tissue Research*, 314(1), 119-129.
- Frautschy, S.A, Hu, W, Kim, P, Miller, S.A, Chu, T, Harris-White, M.E, Cole, G.M. (2001). Phenolic anti-inflammatory antioxidant reversal of A β -induced cognitive deficits and neuropathology. *Neurobiology of Aging*, 22, 993-1005.
- Gallic Acid*; MSDS; No. G0806; Mallinckrodt Baker, Inc.; Phillipsburg, NJ, 28 October 2007. <<http://www.jtbaker.com/msds/english/html/g0806.htm>>
- Garcia, Y.J, Rodríguez-Malaver, A.J, Peñaloza, N. (2005). Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. *Journal of Neuroscience*, 144, 127-135.
- Gassen, G.V, Annaert, W. (2003). Amyloid, presenilins, and Alzheimer's disease. *The Neuroscientist*, 9, 117-126.
- Giacobini, E. Cholinesterase inhibitors stabilize Alzheimer's disease. (2000). *Neurochemical Research*, 25, 1185-1190.
- Giacobini, E. Cholinesterases: New roles in brain function and in Alzheimer's disease. (2003). *Neurochemical Research*, 28, 515-522.
- Hardy, J. Amyloid, the presenilins and Alzheimer's disease. (1997). *Trends in Neuroscience*, 20, 154-159.
- Harris, M.E, Hensley, K, Butterfield, D.A, Leedle, R.A, Carney, J.M. (1995). Direct evidence of oxidative injury produced by the Alzheimer's β -amyloid peptide (1-40) in cultured hippocampal neurons. *Experimental Neurology*, 131, 193-202.
- Hassoun, E.A, Vodhanel, J, Abushaban, A. (2004). The modulatory effects of EA and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of rats after subchronic exposure. *Journal of Biochemical and Molecular Toxicology*, 18(4), 196-203.
- Hellstrom-Lindahl, E. (2000). Modulation of β -amyloid precursor protein processing and tau phosphorylation by acetylcholine receptors. *European Journal of Pharmacology*, 292 (1-3), 255-263.
- Hilliard, W.G, Dillistone, E.J, Oliver, W.T. (1968). A method for cerebroventricular cannulation of the rat. *Canadian Journal of Computational Medicine and Veterinary Science*, 32, 368-370.
- Hoz, L., Moser, E.I., Morris, R.G.M. (2005). Spatial learning with unilateral and bilateral hippocampal networks. *European Journal of Neuroscience*, 22, 745-755.
- Kane, M.D, Lipinski, W.J, Callahan, M.J, Bian, F, Durham, R.A, Schwarz, R.D, Roher, A.E, Walker, L.C. (2000). Evidence for seeding of β -amyloid by intracerebral infusion of Alzheimer brain extracts in β -amyloid precursor protein-transgenic mice. *The Journal of Neuroscience*, 20(10), 3603-3611.
- Kirk, R.E. (1982). Experimental design: procedures for the behavioral sciences (2nd ed.). Monterey (CA): Brooks-Cole Publishing.
- Kuete, V., Wabo, G.F., Ngameni, B., Mbaveng, A.T., Metuno, R., Etoa, F., Ngadjui, T., Beng, V.P., Meyer, J.J.M., Lall, N. (2007). Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). *Journal of ethnopharmacology*, 114, 54-60.

- Lassiter, T.L., Marshall, R.S., Jackson, L.C., Hunter, D.L., Vu, J.T., Padilla, S. (2003). Automated measurement of acetylcholinesterase activity in rat peripheral tissues. *Toxicology*, 186(3), 241-253.
- Lauderback, C.M., Harris-White, M.E., Wang, Y., Pedigo Jr., N.W., Carney, J.M., Butterfield, D.A. (1999). Amyloid β -peptide inhibits Na^+ -dependent glutamate uptake. *Life Sciences*, 65, 1977-1981.
- Leutner, S., Eckert, A., Muller, W.E. (2001). ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. *Journal of Neural Transmission*, 108(8-9), 955-967.
- Lui, J., Tan, M., Liang, C., Ying, K.B. (1996). Immobilized enzyme modulator microassay (IEMMA) for the detection of pesticide in fresh produce. *Analytica Chimica ACTA*, 329, 297-304.
- Markesbery, V.R. Oxidative stress hypothesis in Alzheimer's disease. (1997). *Free Radical Biology & Medicine*, 23(1), 134-147.
- Mates, J.M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. (2000). *Toxicology*, 153(1-3), 83-104.
- Mattson, M.P., Goodman, Y., Luo, H., Fu, W., Furukawa, K. (1997). Activation of NF- κ B protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of Mn-SOD and suppression of peroxynitrite production and protein tyrosine nitration. *Journal of Neuroscience Research*, 49, 681-697.
- Melo, J.B, Agostinho, P, Oliveira, C.R. (2003). Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neuroscience Research*, 45, 117-127.
- Mickley, G.A, Hoxha, Z, Biada, J, Kenmuir, C.L, Bacik, S. Acetaminophen self-administered in the drinking water increases the pain threshold of rats (*Rattus norvegicus*). *Journal of the American Association for Laboratory Animal Science*, 2006, 45(5): 48-54.
- Miguel-Hidalgo, JJ, Alvarez, XA, Cacabelos, R, Quack, G. Neuroprotection by memantine against neurodegeneration induced by β -amyloid (1-40). *Brain Research*, 2002, 958(1): 210-221.
- Mimori, Y., Nakamura, S., Yukawa, M. (1997). Abnormalities of acetylcholinesterase in Alzheimer's disease with special reference to effect of acetylcholinesterase inhibitor. *Research report*, 83(1-2), 25-30.
- Miranda, MI, Ferreira, G., Ramirex-Lug, L., Bermudez-Rattoni, F. (2003). Role of cholinergic system on the construction of memories: Taste memory encoding. *Neurobiology of Learning and Memory*, 80(3), 211-222.
- Mohs, RC, Kawas, C, Carrillo, MC. Optimal design of clinical trials for drugs designed to slow the course of Alzheimer's disease. *Alzheimer's & Dementia*, 2006, 2: 131-139.
- Morris, R. Developments of a water maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 1984, 11(1): 47-60.
- Mullen, W., Yokota, T., Lean, M.E.J., Crozier, A. (2003). Analysis of ellagitannins and conjugates of EA and quercetin in raspberry fruits by LC-MSⁿ. *Phytochemistry*, 64, 617-624.
- Muñoz-Ruiz, P, Rubio, L, Garía-Palomero, E, Dorronsoro, I, Monte-Millán, M, Valenzuela, R, Usán, P, Austria, C, Bartolini, M, Andrisano, V, Bidon-Chanal, A, Orozco, M, Luque, FJ, Medina, M, Martínez, A. Design, Synthesis, and biological Evaluation of Dual Binding Site Acetylcholinesterase Inhibitors: New Disease-Modifying Agents for Alzheimer's disease. *Journal of Medicinal Chemistry*, 2005, 48: 7223-7233.
- Nakai, J.S., Elwin, J., Chu, I., Marro, L. (2005). Effect of anaesthetics/terminal procedure on neurotransmitters from no-dosed and aroclor 1254-dosed rats. *Journal of Applied Toxicology*, 25, 224-233.
- Newman, M., Musgrave, FI., Lardelli, M. (2007). Alzheimer disease: Amyloidogenesis, the presenilins and animal models. *Biochimica et Biophysica ACTA*, 1772, 285-297.
- Niewoehner, B., Single, F.N., Hvalby, O., Jensen, V., Alten-Borglow, M., Seeburg, R.H., Rawlins, J.N.P., Sprengel, R., Bannerman, D.M. (2007). Impaired spatial working memory both spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. *European Journal of Neuroscience*, 25(3), 837-846.
- Ono, K., Yamada, M. (2006). Antioxidant compounds have potent anti-fibrillogenic and fibril-destabilizing effects for α -synuclein fibrils *in vitro*. *Journal of Neurochemistry*, 97, 105-115.

Parks, J.K, Smith, T.S, Trimmer, P.A, Bennett Jr., J.T, Parker Jr., W.D. (2001). Neurotoxic A β peptides increase oxidative stress *in vivo* through NMDA-receptor and nitric-oxide-synthase mechanisms, and inhibit complex IV activity and induce a mitochondrial permeability transition *in vitro*. *Journal of Neurochemistry*, 76(4),1050-1056.

Paxinos, G. & Watson, C. The rat brain in stereotaxic coordinates, 6th ed. Academic Press: Amsterdam, 2007.

Perry, R., Watson, P., Hodges, J. (2000). The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. *Neuropsychologia*, 38(3), 252-271.

Punga, AR., Sawada, M., Stalberg, EV. (2007). Electrophysiological signs and the prevalence of adverse effects of acetylcholinesterase inhibitors in patients with myasthenia gravis. *Muscle Nerve*, 37, 300-307.

Rajan, Rs., Illing, ME., Bence, NF., Kopito, RR. (2001). Specificity in intracellular protein aggregation and inclusion of body formation. *Cell Biology*, 98(23), 13060-13065.

Reichman, WE. (2003). Current pharmacologic options for patients with Alzheimer's disease. *Annals of General Hospital Psychiatry*, 2.

Roberts, A.T., Martin, C.K., Liu, Z., Amen, R.J., Woltering, E.A., Rood, J.C., Caruso, MK., Yu, Y., Xie, H., Greenway, FL. (2007). The safety and efficacy of a dietary herbal supplement and gallic acid for weight loss. *Journal of Medicinal Food*, 10(1), 184-188.

Rx List: The Internet Drug Index. WebMD, RxList Inc., 2007. Accessed:09Dec2007. <http://www.rxlist.com/scr ipt/main/hp.asp>.

Saez-Valero, J., Sberna, G, McLean, C.A., Small, D.H. (1999). Molecular isoform distribution and glycosylation of acetylcholinesterase are altered in brain and cerebrospinal fluid of patients with Alzheimer's disease. *Journal of Neurochemistry*, 72(4), 1600-1608.

Selkoe, D.J. (1996). Amyloid Beta-protein and the genetics of Alzheimer's disease. *The American Society for Biochemistry and Molecular Biology, Inc.*, 271(31), 18295-18298.

Shahidi, S., Fereshteh, M., Nasser, N. (2004). Effects of reversible inactivation of the supramammillary nucleus on spatial learning and memory in rats. *Brain Research*, 1026(2), 267-274.

Shin, R, Ogino, K, Kondo, A, Saido, T, Trojanowski, JQ, Kitamoto, T, Tateishi, J. (1997). Amyloid β -protein (A β) 1-40 but not A β (1-42) contributes to the experimental formation of Alzheimer's disease amyloid fibrils in rat brain. *The Journal of Neuroscience*, 17(21), 8187-8193.

Shin, RW, Ogino, K., Saido, TC., Kitamoto, T., Trojanowski, JQ, Tateishi, J. (2001). Assembly of soluble β -amyloid peptides into Alzheimer disease amyloid fibrils in rat brain. *Fifth international conference on Alzheimer's disease*, S64-253.

Shukitt-Hale, B, Cheng, V, Bielinski, DF, Joseph, JA. (2007). Walnuts can improve motor and cognitive function in aged rats. *Poster presentation at the Society for Neuroscience Conference*, 2007. 256.14/N14. www.sfn.org.

Siciarz, A., Weinberger, B., Witz, G., Hiatt, M., Hegyi, T. (2001). Urinary thiobarbituric acid-reacting substances as potential biomarkers of intrauterine hypoxia. *Archives of Pediatrics and Adolescent Medicine*, 155, 718-722.

Sipos, E, Kurunczi, A, Kasza, A, Horvath, J, Felszeghy, K, Laroche, S, Toldi, J, Parducz, A, Penke, B, Penke, Z. (2007). β -Amyloid pathology in the entorhinal cortex of rats induces memory deficits: Implications for Alzheimer's disease. *Neuroscience*, 147, 28-36.

Skinner, J.E. Neuroscience: A laboratory manual. Philadelphia, PA: W.B. Saunders Co. 1971.

Snyder, S.W., Lador, U.S., Wade, W.S, Wang, G.T, Barrett, L.W., Matayoshi, E.D., Huffaker, H.J., Krafft, G.A., Hozman, T.F. (1994). Amyloid-beta aggregation: selective inhibition of aggregation in mixtures of amyloid with different chain lengths. *Biophysical Journal*, 67, 1216-1228.

Song, J.H, Shin, S.H, Ross, G.M. (1999). Prooxidant effects of ascorbate in brain slices. *Journal of Neuroscience Research*, 58, 328-336.

Steckler, T. & Sahgal, A. (1995). The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. *Behavioural Brain Research*, 67, 165-199.

- Stepanichev, M.Y, Moiseeva, Y.V, Lazareva, N.A, Onufriev, M.V, Gulyaeva, N.V. (2003). Single intracerebroventricular administration of amyloid-beta (25-35) peptide induces impairment in short-term rather than long-term memory in rats. *Brain Research Bulletin*, 61, 197-205.
- Stepanichev, M.Y, Moiseeva, Y.V, Lazareva, N.A, Onufriev, M.V, Gulyaeva, N.V. (2005). Studies of the effects of fragment (25-35) of beta-amyloid peptide on the behavior of rats in a radial arm maze. *Neuroscience and Behavioral Physiology*, 35(5), 511-518.
- Stepanichev, M.Y.; Zdobnova, I.M.; Zarubenko, I.; Moiseeva, Yulia V.; Lazareva, Natalia A.; Onufriev, Mikhail V.; Gulyaeva, Natalia V. (2004). Amyloid-B(25-35)-induced memory impairments correlate with cell loss in rat hippocampus. *Physiology and Behavior*, 80(5): 647-655.
- Stéphan, A, Laroche, S, Davis, S. (2001). Generation of aggregated β -amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. *The Journal of Neuroscience*, 21(15), 5703-5714.
- Sun, M.-K., Alkon, D.L. (2004). Induced depressive behavior impairs learning and memory in rats. *Neuroscience*, 129 (1), 129-139.
- Trubetskaya, V.V, Stepanichev, M.Y, Onufriev, M.V, Lazareva, N.A, Markevich, V.A, Gulyaeva, N.V. (2003). Administration of aggregated beta-amyloid peptide (25-35) induces changes in long-term potentiation in the hippocampus in vivo. *Neuroscience and Behavioral Physiology*, 33(2), 95-
- Wang, Z-F., Li, H-L., Li, X-C., Zhang, Q., Tian, Q., Huaxi, X., Wang, J-Z. (2006). Effects of endogenous [bet]-amyloid overproduction on tau phosphorylation in cell culture. *Journal of Neurochemistry*, 98(4), 1167-1175.
- Weinstock, M. (1999). Selectivity of cholinesterase inhibition: 98.Clinical implications for the treatment of Alzheimer's disease. *CNS Drugs*, 12(4), 307-323.
- Wellman, P.J. (1994). Laboratory exercises in physiological psychology, 4th ed. Boston: Allyn and Bacon.
- Wilson, G.N, Marinis, J, Alexander, N, Matera, K.M. (2007). Dual inhibition of acetylcholinesterase by walnut extract. *Poster presentation at the Society for Neuroscience Conference*, 2007. 155.8/S24. www.sfn.org. Unpublished data.
- Yoshihisa, I., Moriyuki, I., Takagi, N., Saito, H., Ishige, K. (2003). Neurotoxicity induced by amyloid B-peptide and ibotenic acid in organotypic hippocampal cultures: protection by S-allyl-L-cysteine, a garlic compound.
- Zhang, L, Zhou, FM, Dani, JA. (2004). Cholinergic drugs for Alzheimer's disease enhance in vitro dopamine release. *Molecular Pharmacology*, 66(3), 538-544.
- Acknowledgements: The authors thank Henry Haenftling, Raymond Shively, Diana Barko, Dave Revta, Daniel Petersen, Jennifer Remus, Kyle Ketchesin, Ginger Portman, Douglas Placko, Orion Biesan for their contributions that motivated this project, their help with experimental procedures, and/or help with the preliminary presentations and writing of this manuscript.*
- Address correspondence to: Gina N. Wilson, 1288 Leeser Ave., Akron, OH 44314; Email: ginanwilson@gmail.com
- Received: 15 May 2009
Accepted: 14 Jun 2010
- Copyright © 2010 by G. Wilson, G.A. Mickley, K.M. Matera and Baldwin-Wallace College