Mood, Memory and the Hippocampus: How Functioning of this Structure can be an Appropriate Depression Endophenotype

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Abstract

Previous research suggests anatomical differences in the hippocampuses of people with and without depression. In an effort to learn whether these differences pre-exit a mood disorder and are genetically derived, this experiment explored the hippocampus-dependent memory capabilities of first degree relatives (FDRs) of people with depression to see if poor hippocampal functioning would be present in unaffected, at-risk individuals. 16 participants provided information into personal and family history with depression, filled out BDI-II and Zung Self-Rating Depression Scale (ZSRDS) and were led through a series of computer generated memory tasks. It was predicted that FDRs would perform better than participants with depression but worse than healthy controls on hippocampus-dependent memory tasks. Results found near significant differences between groups on average total latencies in the Virtual Water Maze Task, and in average trials to completion in the Milner-Austin Maze ($df = 2$, $p=0.107$, $p= 0.097$ respectively). In these tasks FDRs typically scored between participants with depression and controls. No significant differences were found between groups in non-spatial memory related tasks (Kimura's recurring figures and Hebb's Digit and Block Span Tasks). The presence of slight spatial memory deficits in FDRs suggest that hippocampal functioning could be a useful endophenotype to detect risk for depression. Future genetic research should explore the role of genes controlling hippocampal development or neurogenesis in the generation of depression.

Mood Memory and the Hippocampus: How Functioning of this Structure can be an Appropriate Depression Endophenotype

There are anatomical differences in the hippocampuses of people with and without depression. Since the hippocampus plays a role in memory these structural differences might explain the variation in cognitive capabilities found between these two groups. Patients with depression have been found to be impaired across a range of cognitive domains, including attention-executive function, visuospatial learning and memory, and verbal memory (O'brien et al., 2004; Frodl et al., 2006). In focusing on one cognitive symptom of depression, this research is an effort to explore if hippocampal functioning is a potentially useful endophenotype. If so, tests of hippocampus-dependent memory could be used not only to diagnose high risk, but also to direct future genetic research on the biochemical underpinnings of mood disorders towards the development and functioning of the hippocampus.

Several MRI studies have directly demonstrated the disparity in hippocampal volumes between healthy controls and those with depression. Bremner et al. (2000) matched 16 patients with depression with 16 healthy controls to find the left hippocampus of the patients on average, and significantly, 19% smaller while the right hippocampus was on average, but insignificantly, 12% smaller. Such significance for the left hippocampus even remained after total brain size, age, education, and extent of alcohol exposure were included in the analysis. Additionally, other brain regions did not demonstrate such notable volumetric differences between groups. Similarly, Sheline et al., (1999) studied 24 women in remission from depression against matched healthy controls and found that subjects with a history of depression had smaller hippocampal

volumes bilaterally despite no differences in overall brain size. Still other studies have found decreases only in right hippocampal volume in patients with depression (O'brien et al., 2004). The discrepancies in the exact location of volume reductions might be due to differences in measurement processes but in general it is well accepted that patients with a long history of depression exhibit some type of volume reduction in this brain structure (Campbell et al., 2004). Although anatomical structures are often under the control of genes, these findings alone do not justify a "genetic cause" for depression because of recent research into the neurodegenerative effects of the disorder.

Although anatomical differences in the hippocampuses between these two groups are clear, it has still not been well established whether these differences are pre-existing the onset of the disorder. For example, volume reductions may be a result of the deleterious neurological effects of depression as duration of the disorder but not age of the participant is significantly correlated with the hippocampus size deficit (Sheline et al., 1999). Bremner et al. (2000), however, found no such correlation between number of depression episodes and left hippocampal volume. At least in bipolar disorder is has been demonstrated that both adults and children with the disease demonstrate near significant volume reductions in the hippocampus ($p<0.054$; Blumberg et al., 2003). Since the volume reductions were apparent even in the younger patients these researchers concluded that such structural abnormalities occurred early in the disorder if not even before illness onset and could act as a predictor for mood disorders. Interestingly, MacQueen et al., (2003) compared multiple episode depressives and never treated first episode depressives with healthy controls and found that while both patient groups exhibited lower hippocampal functioning than controls (as measured in recollection and

verbal memories), only the multiple episode depressives had a decreased hippocampal size. Thus first episode patients with depression might exhibit smaller structural changes not yet observable under an MRI but that still result in measureable cognitive deficits. Together these findings suggest that while hippocampal volume reductions could pre-date a depression episode they can also worsen during experience with the disorder.

Given that abnormalities in the volume of the hippocampus could pre-date a depression episode, it is possible that its structural formation is under genetic control and thus abnormalities might also exist in unaffected first degree relatives (FDRs). Gottesman and Gould (2003) describe a useful and legitimate endophenotype as one that is not only associated with depression but that is also clearly heritable, state-independent (measureable in the absence of a depression episode), and found within unaffected family members at a higher rate than in the general population. The search for altered hippocampus functioning in FDRs assumes that deficits in cognitive capabilities in this group would suggest underlying, heritable, volumetric alterations of this structure. Of course this is contingent on the viability of hippocampus-dependent memory tasks as indicative measures of hippocampus structure.

Studies into the functional significance of the hippocampal volume reduction highlight the potential use of memory tasks in quantifying hippocampal functioning. In elderly patients with depression, decreased hippocampal volume was associated with deficits in working, visual, and verbal memory (O'brien et al., 2004). Sheline et al. (1999) found that not only did patients with a history of depression present smaller bilateral hippocampal volumes than healthy controls but they also scored lower in verbal memory tests despite no significant difference found in IQ scores. Similarly, Frodl et al.

(2006) compared 34 patients with remitted or current depression to 34 healthy controls and found that lower hippocampal volume correlated with poor scores on the Wisconsin Card Sorting Test (WCST) which measures executive functions potentially mediated by hippocampus. Activation of the hippocampus has been observed during tasks requiring spatial memory and neuronal apoptosis in the CA1 region of the hippocampus has been associated with poorer performance in the Morris Water Maze in rats (Ludvig et al., 2004; Huang et al., 2007). In fact, the hippocampus might even contain neurons with "location-specific firing patterns" that are involved in the declarative formation of spatial memory (Ludvig et al., 2004). In general the hippocampus is associated with declarative or explicit memory formation and spatial memory abilities and is not necessarily thought to play a role in implicit learning (for review see Kim & Diamond, 2002). These findings suggest that comparative performances on explicit and spatial memory tasks could act as indirect measures of differences in hippocampal volume.

Research has already demonstrated that depression has an estimated heritability of 31-42% and FDRs are clearly at high risk for developing the disorder (Sullivan et al., 2000). FDRs do show slight cognitive similarities with their affected relatives as they are faster at recognizing fear faces than healthy controls, and present with an increased reaction time in recognizing positive personality characteristics as compared to negative characteristics (Le Masurier et al., 2007). Additionally a study by Christensen et al. (2006) revealed slight cognitive deficits as unaffected twins of people with depression performed much worse than unaffected twins of healthy controls in measures of selective and sustained attention, executive function, language processing, and working and declarative memory. Furthermore, at risk twins who were monozygotic (and thus more

closely genetically related to their affected twin) performed worse than individuals who were dizygotic with an affected sibling. Together these findings suggest that cognitive deficits do persist in unaffected FDRs and that genetic relatedness increases the degree of deficits observed.

To this author's knowledge little if any research has explored the potential existence of hippocampus-specific volume and function deficits in FDRs of people with depression. The MacQueen at al. (2003) study highlighted the use of cognitive functioning tasks to explore underlying hippocampus abilities even before structural alterations are apparent. In this study, therefore, it was hypothesized that if hippocampus structural alterations are initially genetically driven then slight hippocampus-dependent cognitive deficits should be apparent in FDRs. This study also sought to verify previous research that found cognitive deficits in the population of participants with depression under the hypothesis that such would persist to a greater degree than that found in FDRs because of the additional neurodegenerative effects of a depression episode. Participants provided information into their personal and family history with depression, filled out two current mood forms, and were then led through a series of computer generated memory tasks. It was predicted that FDRs would perform better than participants with depression but worse than healthy controls on hippocampus-dependent memory tasks. Since earlier volumetric studies did not find differences between healthy controls and people with depression in other brain regions it was further predicted that no difference would be found between the groups on measures of hippocampus-independent memory.

Materials and Methods

Participants

All participants were students at American University. Participants were recruited from an introductory psychology class and received 0.5 extra credit points towards their grade in that class for every half hour they participated. All participants were provided with informed consent and assured that they could end the experiment at any time while still remaining eligible for extra credit and without accruing any other negative consequences.

Apparatus

Paper Forms. A personal and family history form was used to collect information on the current diagnoses of the participant and his or her family members (see appendix). Additionally, paper forms of the Beck Depression Inventory (BDI-II) and the Zung Self-Rating Depression Scale (ZSRDS) were used to confirm reported diagnosis status and detect any undiagnosed symptoms. A final form consisted of four questions concerning participant's familiarity with and frequency of use of computer games and computers in general (see appendix).

Computerized Memory Tasks. Four computerized memory tasks, the Virtual Water Maze Task, Hebb's Digit and Block Supraspan Tasks, the Milner-Austin Stepping Stone Maze, and Kimura's Recurring Figures were administered to measure hippocampal functioning.

The Virtual Water Maze Task simulates the Morris Water Maze Task in which a rat swimming in a circular pool of water must learn to find a hidden platform. Similar to a first-person shooter computer game, the participants use the arrow keys on a computer keyboard to "swim" on the surface of a pool of water depicted on a computer monitor as

they try to locate an invisible platform. There is a brief training and practice session in which the participant has a chance to become familiar with the task and the controls. During these 6 practice trials, the platform is visible 5 times and invisible a final 6^{th} time and all trials take place in a room different from that during the actual task. After the practice session, the Learning 1 task involves a series of 6 Blocks of 4 Trials, with each Trial in a Block beginning at one of the 4 compass points (i.e., NESW) occurring randomly. During all 6 blocks the platform remains in the exact same location but is hidden unless the participant has reached the searching time limit at which point the platform becomes visible and the participant is asked to swim towards it. These 6 blocks are followed by a single Probe Trial, in which, unbeknownst to the participant, there is no platform. Next a single Block of 4 Trials follows in which the platform is visible. Finally, there is another series of 4 Blocks of 4 Trials called Learning 2. Here the details of the room remain the same but the participant has been informed that the hidden platform has moved and will remain in the same new location for the remainder of the trials. This concludes with a single Probe Trial, in which there is no platform, although, again, the participant is not aware of this. Each Trial can last a maximum of 90 seconds but if the platform is not located within 60 seconds after a Trial starts, it becomes visible. This task usually takes 25-45 minutes to complete.

Hebb's Digit and Block Supraspan Tasks are two memory span tasks. Hebb's Digits is a variation of the standard Forward Digit Span task that is part of the Wechsler Scales. In the first part of the task, the computer displays a series of random black numerical digits on a white background, one at a time, at a rate of one per second. Once the series is complete, as indicated by the word "Go" appearing on the screen, the

participant must recall and, using the keyboard, enter the digits in the same order as the computer presented them. These number sequences begin at a length of 3 digits, and increase by one digit after two trials at one length as long as the participant repeats at least one trial within a block of two of a particular length correctly. The computer is able to generate sequences up to 12 digits long, which should accommodate about 99% of the population.

Once the computer has determined a participant's Digit Span (the length of the longest sequence the participant can repeat correctly) the second part of the task, supraspan learning, occurs. In this phase, the computer generates random number sequences that are one digit longer than the participant's Digit Span and presents them to the participant in exactly the same manner as during the first part of the task. As before, the participant tries to repeat and enter the sequences into the keyboard in the correct order. There are 24 trials in this phase of the task. Unbeknownst to the participant one of the sequences of numbers is repeated every 3rd trial while all the others are random and never repeat.

The Block Span task in this set operates in exactly the same way as the Digit Span task. This time, however, instead of a series of digits, the computer generates a nonverbal spatial sequence by blinking individual squares displayed in an asymmetrical array on the computer screen. The participant must repeat the sequences by pressing keys on a special keyboard that has been altered to resemble the spatial array of squares on the screen. On this keyboard most of the keys including all letters, numbers, and command keys were covered in black tape. Keys that corresponded to the squares on the screen were covered in yellow tape. As before, the computer determines the participant's Block Span in the

first phase and then presents supraspan sequences in phase two. These two tasks take 15-25 minutes in total.

In the Milner-Austin Stepping Stone Maze participants face a ten-by-ten grid of squares displayed on the computer screen representing a pond covered by square stones, some of which can support a person's weight and others that, when stepped upon, sink. There is a single hidden path that runs from the bottom left to top right of the array that they must discover via trial and error by using the mouse button to click on each square one at a time in a sequence that is meant to begin at the indicated Start position and continue, step by step, moving up, down, left, or right only, until they reach the designated End square. If the participant "steps" on an incorrect square, the computer generates an error sound and the participant must retreat to the square most recently found to be part of the path and try again to discover the next square in the sequence that forms the path. Once begun, an attempt to cross the "pond" must proceed until the End position before another "crossing" can begin. Participant's continue trying to "cross the pond" until they can complete two consecutive error-free crossings. This task takes 10-25 minutes to complete.

In Kimura's Recurring Figures task, the computer display a series of line drawings of geometric and nonsense patterns. In the first phase, the participants merely watch as the computer shows a sequence of 20 patterns for 4 seconds each. The computer then shows participants a series of 100 drawings, one at a time for 4 seconds each, consisting of new patterns as well as some of the patterns from the first 20 presentations. Some of these patterns repeat and the participants must indicate which patterns they have

seen before (in either the first phase or earlier in the second phase) and which they are seeing for the very first time. This task takes about 15 minutes.

Procedure

Screening participants. Since the study sample was going to be small due to time constraints it was important to ensure that it included the required distribution of people from the three groups of interest (those with a depression diagnosis, those related to someone with a depression diagnosis, and healthy controls). Screening was achieved by having participants first contact a third party to express their interest and explain their personal and family history. Once histories with depression were removed from the text of the email, eligible participants were forwarded to the experimenter running the tests who then contacted the participants directly with possible times and set up a date for the study. In this manner the tester was able to remain blind to the condition of the participant.

Testing. Testing took place any time during the day from starting at 9am to starting at 9pm. Upon arrival at the testing site, the participant was welcomed, seated at a table, and handed an informed consent form. The investigator reviewed the main points of informed consent to ensure that the participant understood her or his rights, the tasks involved in the study, and to clarify any questions. The participant also received a copy of the consent form and the HSC contact list to keep. Following this participants were handed an inter-office envelope containing four paper forms (the personal and family history, the BDI-II, the ZSRDS, and the questionnaire on experience with computers and computer games) which they were instructed to complete and return to the envelope. All forms had the participant's identification number on them and remained unexamined

until the completion of all the tasks. Participants were asked not to put their names on any of the forms so that responses would remain anonymous and the tester left the room while participants filled them out. These forms took no more than 10-15 minutes to complete.

Once the forms were complete and returned to the envelope the investigator moved the participant to sit in front of a PC lap top and prepared the participant for the virtual water maze task by briefly explaining the history of the task, the purpose, the procedure and pointing out the arrow keys. At completion, the investigator moved the participant to a nearby Mac computer, explained the procedure for the Hebb Digit supraspan task and then ran it on the computer for the participant. After this the keyboards were switched out so that participants could then use the altered one for the block supraspan task. The investigator explained the similar procedure for the block supraspan task and ran the program after answering any questions. For both these tasks the program was stopped and restarted if the estimated digit or block spans were judged to be inaccurate by the tester. This judgment was made if participants performed with relative ease and accuracy in the second (supraspan) part of the tasks.

The investigator then explained the Milner-Austin stepping stone maze while changing the screen resolution of the Mac to maximize the size of the maze screen. The program was then opened and run.

Finally the tester explained Kimura's recurring figures and opened the program for the participant. During the second part of this task, the investigator assumed responsibility for clicking the participant's affirmative or negative responses to speed up the process. During this time the investigator tried to look away from the screen in order

to avoid pressing her own response to the image and any mistakes in responses were recorded and fixed in the system later before analyzing the data.

At the conclusion of testing the investigator asked the participant if he or she had any questions, and handed them a debriefing form to read over. During this time the investigator took the opportunity to check the answer to number 9 on the BDI-II inventory. Only once did a participant respond above a score of 0 for this question at which point the investigator sat with the participant and followed the appropriate debriefing procedures outlined by the HSC at American University. After this, participant contact information was documented if he or she was interested in hearing the results of the study, the extra credit card was filled out and participants were thanked for their co-operation and allowed to leave.

Data Analysis

Group assignments and scoring paper forms. Participants were assigned to the groups control, first degree relative (FDR) or depressed primarily based on their responses to the personal and family history form. Participants were identified as having been diagnosed with depression, as having a relative that was diagnosed with depression, as both, or as neither. If participants had only 2nd degree relatives with depression they were assigned to the control group and if participants had both a personal and family history with depression they were assigned to the depressed group. Responses on the BDI-II form had a point value ranging from 0 to 3 and scores were defined as the sum of these points. On this form, a total score of 0-13 is considered minimal or no depression range, 14-19 is mild, 20-28 is moderate, and 29-63 is severe. Responses to the Zung questionnaire had a point value ranging from 1 to 4 and scores were defined as the sum of these points. On

this form people with depression tend to score in the upper point range of 50-69 and the maximum score is 80. If a question had no response, the investigator assigned the median value. On the BDI-II form this was 1.5 points and on the Zung this was 2.5 points. If the BDI-II or Zung scores seemed unusually high for any response on the personal and family history form further analysis was taken to reconsider the group assignment. This was performed by combining the BDI-II and Zung scores of the participant in question with those of the controls and then with those of the FDRs and taking the mean scores and standard deviations. If the participant's scores approached or extended beyond two standard deviations from the means of these groups then he or she was assigned to the depressed group despite lacking a diagnosis of the disorder. On the computer experience form response to each question had a point value ranging from 0 to 3 with higher scores denoting more experience with computers and computer games. With four questions the scores were the sum of the points on the responses and the highest possible score was 12.

Virtual Water Maze Task. The total latency, as defined by the time in seconds it took the participant to reach the platform in each trial, was the measurement of interest in this task. For each trial block the average of four trials (in which the starting location of the participant in the pool ranged over the four compass points) was determined and this function was performed for both the Learning 1 and Learning 2 tasks. A repeated measures ANOVA was run to compare the average total latencies of all the participants across all the trials in the three groups and within and between subject effects were reported.

Milner-Austin Maze. In this task participants had to get through the maze two times consecutively without making any errors. Thus, the number of trials to completion was

used as a measure of learning and a one-way ANOVA was run to compare the number of trials to completion for all of the participants across the three groups.

Kimura's Recurring Figures. In this task points are gained by correctly recognizing a repeated image and points are lost by incorrectly "recognizing" a non-repeated image (by providing a false positive). Points were divided into the score for geometric figures, nonsense figures, total, and the difference between geometric and nonsense scores for any one participant. A one-way ANOVA was run comparing each of these scores by group.

Hebb's Digit and Block Supraspan Tasks. Since it was suspected that the computer did not accurately compute the digit and block spans of each of the participants it was necessary to devise a way to determine which of the participants were being accurately tested in this task. Assuming an accurate digit span has been determined, when presented with a supraspan sequence (the length of a digit span +1) the participant should theoretically remember all of the digits except the last one. Thus, the participant must guess the final digit and has a 1 in 9 chance of guessing correctly (the nine digits used to make the sequence range from 1 to 9). Out of the 24 supraspan sequences presented 16 are non-repeating meaning by chance alone participants are expected to get 1.78 sequences correct. In running a chi-square analysis with the expected values of 1.78 correct and (16-1.78)= 14.22 incorrect sequences it was determined that an observed value of 4 or fewer correct non-repeated sequences was not statistically significantly different from expected whereas 5 or more correct non-repeated sequences was significantly different (see tests below).

Observed	Expected	$(O-E)^2/E$
4	1.78	2.77
12	14.22	0.35
16	X^2=	**3.12**
	p=	0.0773

Observed	Expected	$(O-E)^2/E$
5	1.78	5.82
11	14.22	0.73
16	X^2=	**6.55**
	p=	*0.0105*

Consequently, if the difference between the number of times the repeated sequence was reported correctly and the number of times a non-repeated sequence was guessed correctly was greater than four then it was determined that the task was too easy for the participant, and that the computer made a poor estimate of the participant's digit or block span so his or her data was eliminated from the analysis. Since only a very small sample of participants met these requirements (had an accurately estimated digit or block span) statistical analyses were not run on these measurements and only a qualitative analysis of generated graphs was performed.

Results

Participant Information

In total the study ran 16 participants, 75% of which were female, and 94% of which (15 out of 16) ranged in age from 18 to 20 (the final participant was 61 years old). Based on responses on the personal and family history form, the Zung, and the BDI-II, 6 participants were assigned to the control group, 6 to the depression group, and 4 to the first degree relative group (FDR). Groups did not differ significantly by age (df=2, p=0.241) although they did differ somewhat in sex ratios as 4 out of 6 controls were female (67%), 2 out of 4 FDRs were female (50%) and 6 out of 6 participants with depression were female (100%). There were no significant differences in main scores on

the Virtual Water Maze or Milner-Austin Maze between sexes, however, so these differences were not strongly considered (Learning 1, p=0.113; Milner-Austin maze, p=0.696).

Fourteen participants were assigned to their groups solely based on responses to the personal and family history form. One participant reported a history using antidepressants and three relatives (including one first degree) with bipolar disorder although had never been personally diagnosed with depression. Further analysis of BDI-II and Zung scores demonstrated this participant had high scores for both. When included in analysis of controls, the BDI-II score was more than two standard deviations above the mean and the Zung score was just under two standard deviations away from the mean. When included in analysis of first degree relatives, both the BDI-II score and the Zung score were still greatly more than one standard deviation away from the mean. Thus this participant was categorized along with people with depression. A second participant reported having no personal history with depression but a mother with the disorder. Again, analysis of BDI-II and Zung scores with controls showed this participant more than two standard deviations above the mean, and in analysis with FDRs, scores were just under two standard deviations above the means. Consequently this participant was also categorized along with people with depression. Based on the 11 participants who filled out a questionnaire concerning computer familiarity, there was no significant difference across groups in their experience with computers and computer games (df = 2, p=0.962).

Virtual Water Maze

Learning 1. For the first learning set of 6 trial blocks there was a significant difference of total latency within-subjects across the trial blocks (df = 5, p= 0.004). Figure 1

graphically represents the average total latencies of the groups by trial block and supports these statistics by showing a general decreasing trend in latency for all three groups.

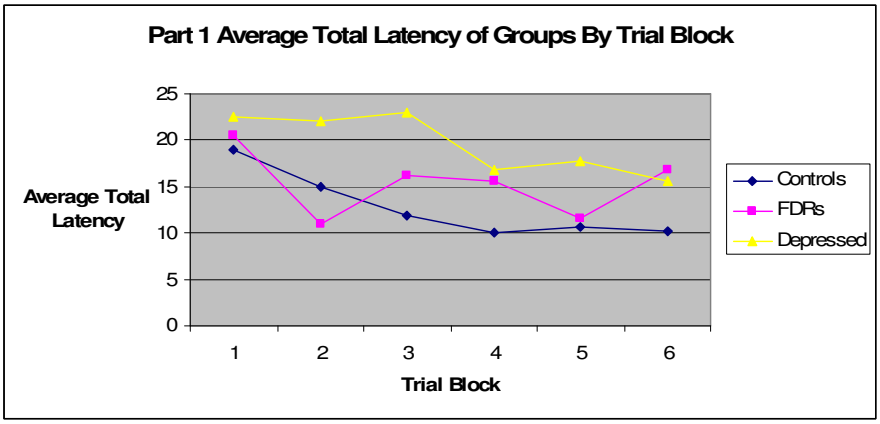

Figure 1: Average Total Latency per Trial Block for Each Group in Learning 1

The repeated measures ANOVA found no significant difference in average total latency between groups (df = 2, p=0.107).

Learning 2. In the second learning set of four trial blocks another repeated measures ANOVA found no significant difference in average total latencies within-subjects across trials (df= 3, p= 0.052) and again there was no significant difference in average total latency found between groups (df = 2, p=0.145). Figure 2 shows the learning curves of the three groups for the second learning set.

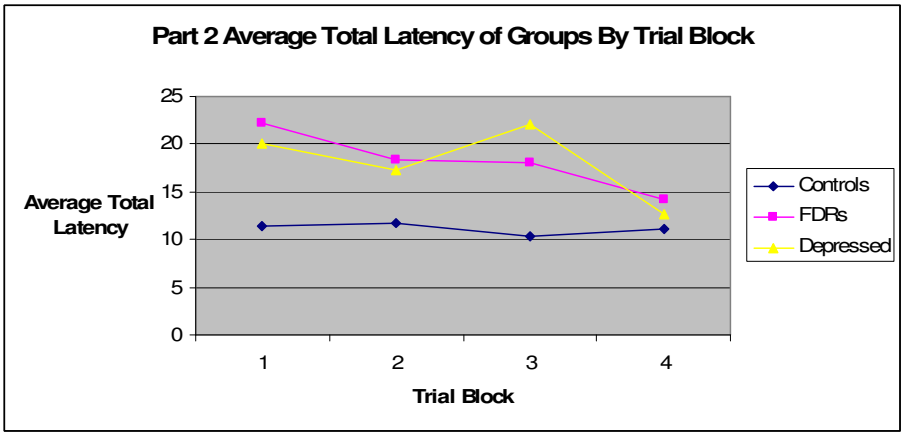

Figure 2: Average Total Latency per Trial Block for Each Group in Learning 2

Milner-Austin Maze

All participants completed the Milner-Austin Maze task. Figures 3 and 4 represent the average errors and completion latency respectively for each of the three study groups by trial. The large error bars represent the standard deviations of the average scores for these measures.

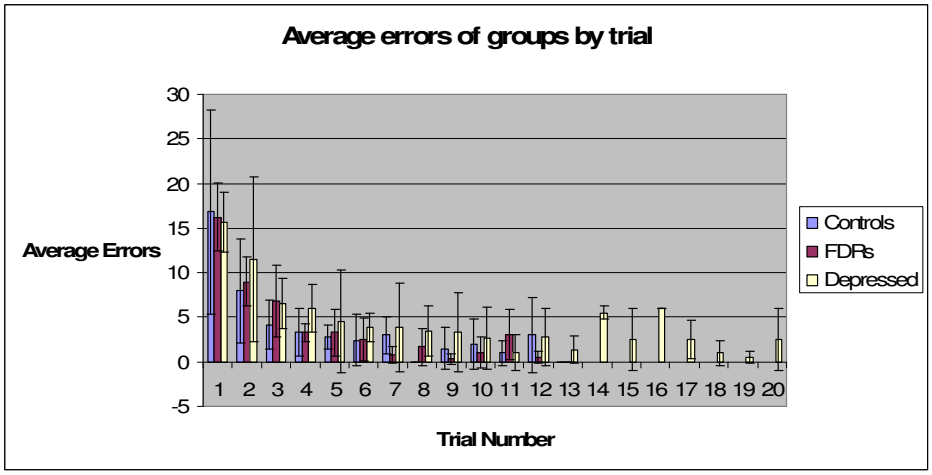

Figure 3: Average Errors per Trial for Each Group

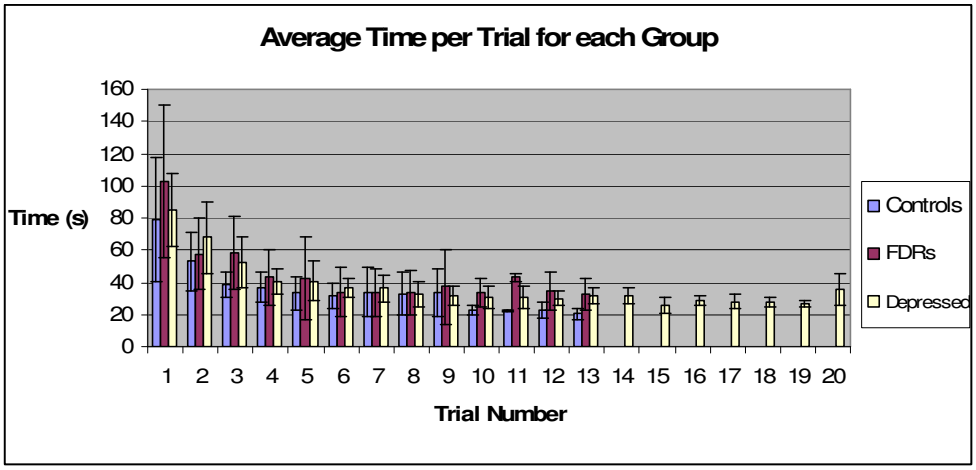

Figure 4: Average Time per Trial for Each Group

The one way ANOVA run to compare the average number of trials required for completion of the Milner-Austin Maze found no significant difference between the groups (df = 2, p= 0.097). Figure 5 graphically represents average trials to completion for

each of the three study groups along with standard deviations. Participants in the depressed group took, on average, the most amount of trials to complete the task, and the controls, on average, took the least amount of trials.

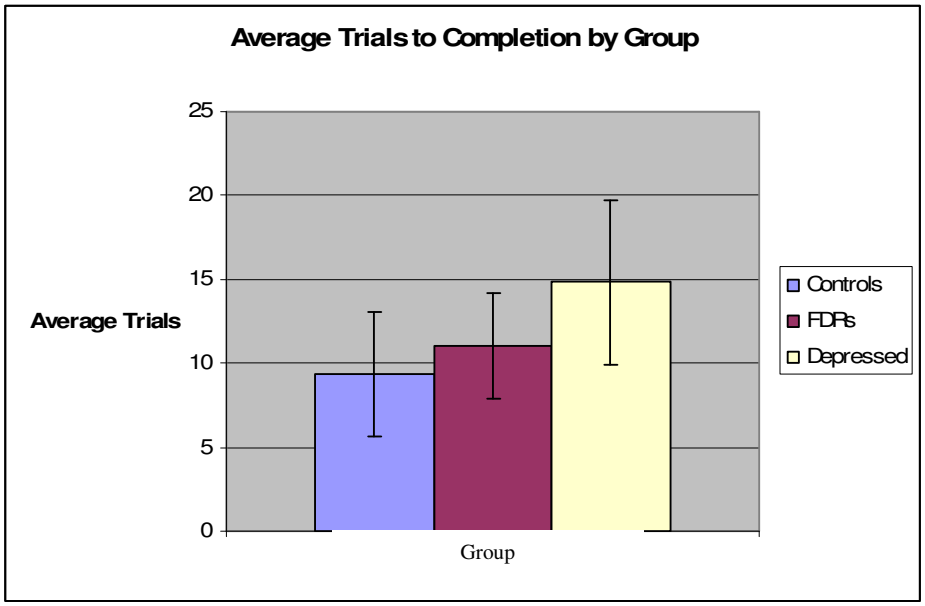

Figure 5: Average Trials to Completion of Milner-Austin Maze by Group

Kimura Recurring Figures

The one-way ANOVA run for four measures in the recurring figures test showed no significant differences in any of these scores across the groups (df = 2, p= 0.587, p=0.426, p= 0.367, & p= 0.506 for geometric scores, nonsense scores, total, and difference between geometric and nonsense respectively). All participants scored worse in remembering nonsense patterns in comparison to geometric shapes. Figure 6 reflects the similarities across groups in these tasks.

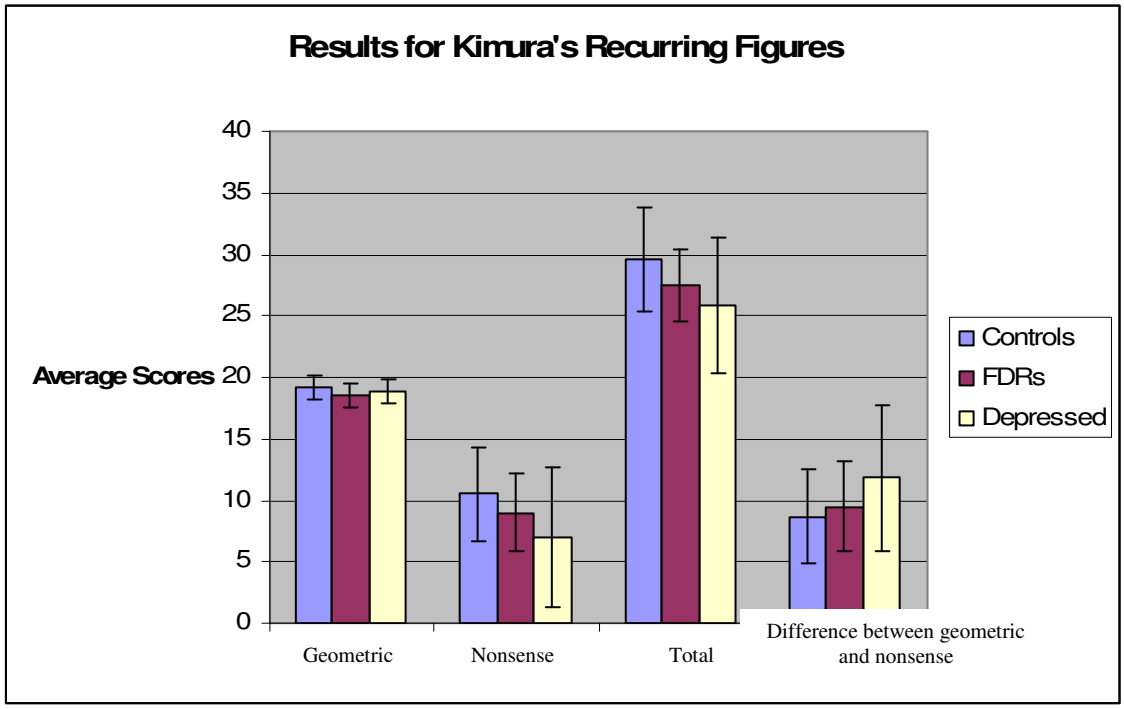

Figure 6: Average Scores and Standard Deviations for Kimura's Recurring Figures Task by Group

Supraspan learning

The two supraspan learning tasks (both the digit and block span) were judged to poorly demonstrate the learning abilities of the participants because the digit and block spans were most often inaccurately estimated by the computer. Since a chi square analysis determined a difference of 5 or more between random sequences right and repeated sequences correct, all participants with this difference or more between scores were eliminated from this analysis. As a result, 4 controls, no FDRs and 4 patients with depression had an accurately measured digit span, and 2 controls 3 FDRs and 3 patients with depression had an accurately measured block span. Such a small sample size rendered statistical analyses useless but Figure 7 compares the average digit and block spans of the three groups and suggests little if no difference between the groups.

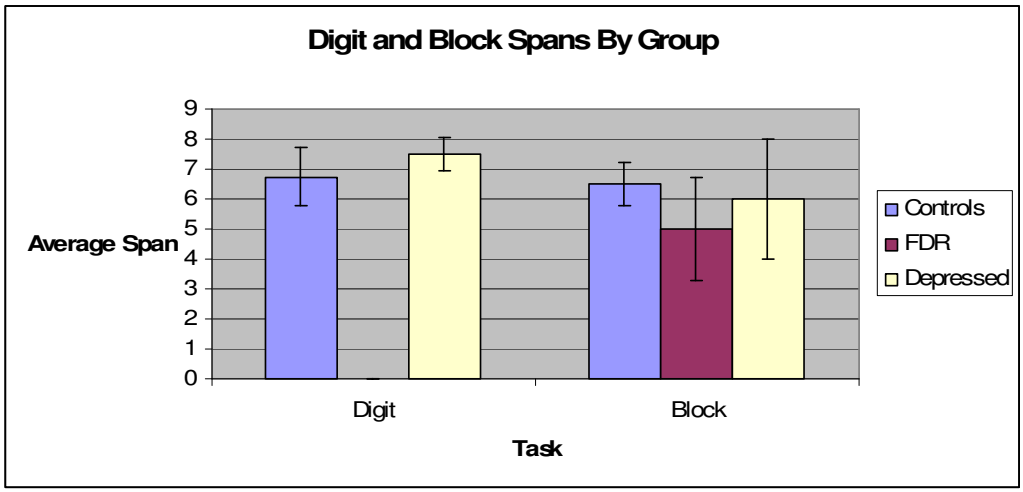

Figure 7: Average Digit and Block Spans for Participants in Groups Where Span Estimates were Considered Legitimate.

When the percent of correct repeated sequences out of total correct sequences was compared across groups (Figure 8) again few notable differences were found due to large standard deviations.

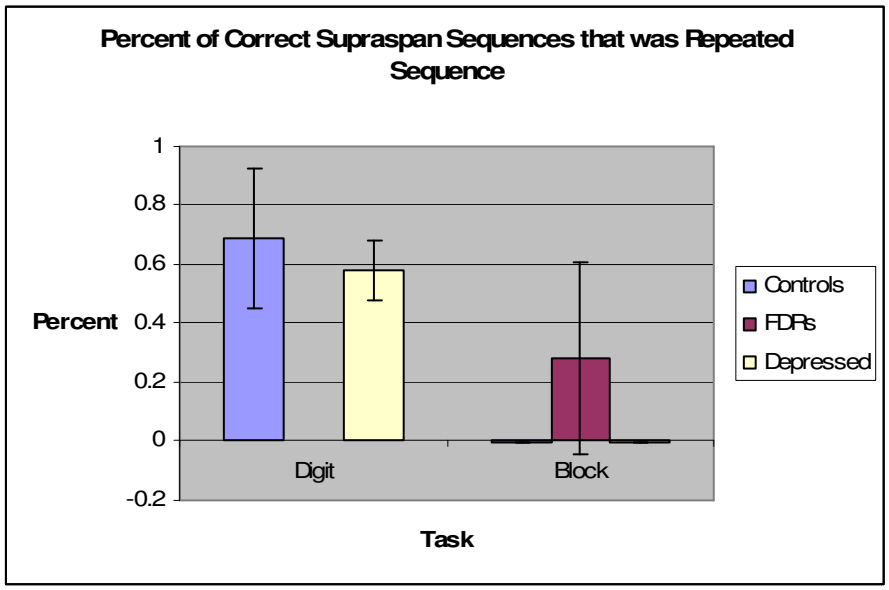

Figure 8: Percent of Correct Supraspan Sequences that was the Repeated Sequence by Group and Task.

Discussion

The results of this study partially support previous research which has found spatial memory deficits in people with depression and suggest that such deficits might exist in a less severe form in first degree relatives. Differences in learning between the three study groups approached significance for both the Virtual Water maze and the Milner-Austin maze. In learning 1, there was a significant difference of total latency within-subjects across the trial blocks suggesting that all participants were learning how to find the platform quickly ($df = 5$, $p= 0.004$). Figures 1 and 2 show the learning of the three groups in the Virtual Water maze task as measured by a decreasing average total latency over trial block. In Figure 1 although the average total latency decreased for both the control and depressed group, the participants with depression still took longer, on average, than the controls at all stages of the task suggesting that though they may learn, the quality of their learning is still lower. Additionally, while the average latencies of the FDR group seem to oscillate, at 4 of the 6 trial blocks FDR scores fell between those of controls and participants with depression. The one way ANOVA did not find a significant difference in average latency scores between the groups but the p-value was 0.107 which definitely approaches significance and suggests that a larger study sample might strengthen these differences. In learning 2 within-subject differences in average total latency were very close to significant ($p=0.052$) and perhaps failed to reach it because the control group did not demonstrate learning. This probably occurred because their average total latencies at trial block 1 were already so low that there was no realistic room to decrease their latency time (Figure 2). In comparison, both the FDRs and the participants with depression showed a much higher latency in the first trial block of learning 2

(suggesting their difficulty in finding the new platform location) and both showed relatively similar learning curves throughout this learning task (Figure 2). Again, the one way ANOVA found no significant difference in average total latency scores between the groups and this time the p-value only barely approached significance at 0.145. In both learning tasks, however, the depressed group consistently demonstrated greater average total latencies than the control group across trials confirming previous findings of a difference in spatial memory capabilities between these groups. A lack of significance was probably due to the FDR group scores which often, although not always, fell in between the other two groups. In total a larger sample size might generate similar but more significant findings.

Results for the Milner-Austin maze mirrored results from the Virtual Water Maze task in which differences between the groups approached but did not reach significance in measures of trials required until completion of the task ($p= 0.097$; Figure 5). Figure 5 further demonstrates that the FDRs scored, on average, in between controls and participants with depression in this measure. Interestingly, the learning curves of the three groups appeared very similar across the trials as measured by average number of errors per trial and average time per trial (Figures 3 and 4). The difference between these groups is only noticed near the end of these curves, where once the controls and FDRs have learned the maze, participants in the depressed group continue to make a low average number of errors until finally completing it twice through consecutively without any mistakes. Reasons for this are not clear and may suggest that learning curve differences between these three groups may only be observable under more strenuous conditions, or when most of the correct squares have been memorized and the memory is already

heavily taxed. In general, however, these results also show that people with depression took more trials to learn the path than the other two groups and that FDRs took more trials to learn the path than controls.

Although the groups had almost significant differences on tasks involving spatial orientation and spatial memory, they did not seem to differ on other measures of memory, as in the Kimura's Recurring Figures task and the supraspan tasks. The three groups did not differ significantly in the number of geometric and nonsense shapes that they remembered and Figure 6 supports these statistics by demonstrating large standard deviations which overlap across the means graphed. Additionally Figures 7 and 8 suggest little difference between the three groups in digit and block spans and supraspan learning. This last finding is surprising considering that the block span does have a spatial memory component but considering the low number of participants for whom the supraspan task was performed accurately these results probably carry very little external validity.

The separation of group differences across spatial-related and non-spatial related tasks does not support previous research which found patients with depression to have memory deficits in both domains. This might mean that the Kimura's Recurring Figures and supraspan tasks did not actively engage the hippocampus in the manner that the two mazes did and rather depended on memory structures less differentiated between the three groups resulting in similar scores. Importantly, the hippocampus is not necessarily associated with implicit learning, as would occur in the supraspan task (Kim & Diamond, 2002). Thus insignificant differences between the groups in Figure 8 might be expected since all of the groups should implicitly learn the repeated sequence at an equal rate. Of course then one would expected differences in the initial digit and block span measures

(Figure 7) which involves explicit memory and recall, which was not observed. Porter et al. (2003) similarly failed to find memory differences between medication-free people with depression and controls on measures of verbal learning such as immediate word-span and long-term recall even though there were clear visuospatial learning and memory deficits in the former group. Taken together perhaps a lack of significant differences in the digit supraspan task reflects activation and use of non-hippocampus related memory. Again, considering the relatively small pool of participants for whom the supraspan tasks accurately estimated a digit span, these results will not be heavily attended to as support for any predictions.

As for the Kimura's Recurring Figures task, it is also possible that the effect size was much smaller than the effect sizes for the two maze tasks such that far more participants would be needed to even find a suggestion of a difference. Additionally, the timing of this task could have rendered these results less accurately reflective of any real differences. This was the final task of the protocol and insignificant differences may be due to a universal exhaustion and lack of effort by all the participants leading to relatively similar results across groups.

There are several limitations to this study that should be addressed in future research. Clearly the sample size was very small and although there was no significant difference in ages between the groups, one of four FDR participants was over 40 years above the mean of the entire sample. The inclusion of this participant, reflecting a different generation of computer experience and clearly vulnerable to the typical memory deficits associated with old age, might have affected the FDR results pushing them toward a higher latency on the Water Maze task and toward more average trials to

completion on the Milner-Austin maze task. Additionally, another of the four first degree relatives had been using Cymbalta, a medication for major depressive disorder, general anxiety disorder and fibromyalgia, which might have exerted negative affects on cognitive capabilities (Porter et al., 2003). This participant was categorized as an FDR due to responses on the personal and family history form and presenting with seemingly average BDI and Zung scores. In comparison, the controls reported no medication use except for one participant who used Ritalin. Thus while results suggest FDRs to have memory capabilities between controls and people with depression, this may have been more due to the inclusion of an older participant, and a participant using psychotropic drugs, rather than any underlying neuro-anatomical differences. While exclusion of these participants would have rendered the FDR group far too small for adequate analysis, future work should look to more effectively control age and medication differences.

The sex ratios were not equal across the groups and this may have contributed to any differences observed in spatial memory tasks. This is not a huge concern for the Milner-Austin maze where there was no significant difference in scores by sex but in Learning 1 of the Virtual Water Maze differences approached significance at $p=0.113$ and future studies should control for sex ratios.

This study did not use the DSM IV diagnostic criteria for verifying any claims to having been diagnosed with depression and clearly had no means of verifying the diagnosis of family members with depression. Even though the BDI-II and Zung forms were used to control for current mood and to highlight discrepancies in the personal and family history reports, a more extensive personal and family history would be useful in more accurately categorizing the participants. Finally, despite findings related to the

supraspan task, this study did not have numerous clear measures of hippocampus-independent memory to control for global deficits that could be experienced by both the depressed and FDR groups. Clearly these are vital controls that would be necessary to clarify that the hippocampus is indeed the specific originating source of any memory deficits noted.

Despite the limited size of this study, these preliminary results do suggest that FDRs score between healthy controls and people with depression on measures of spatial memory. Additionally this study supports previous findings that people with depression perform worse than healthy controls on measures of spatial memory. This data suggests that FDRs may be harboring slight structural abnormalities in this brain region. Therefore hippocampal functioning, as measured by spatial memory abilities, could be used as a cognitive endophenotype to determine risk for the development of depression.

Since the FDRs performed better than people with depression, these results further support studies on the neurodegenerative effects of depression on hippocampal structure. It is suspected that hippocampal structural alterations might actually have a causal implication in the development of depression symptoms thus explaining the high risk found in the FDR population. The mechanism by which this relationship operates could be related to altered hypothalamic-pituitary-adrenal axis (HPA) reactivity. Individuals who suffer from depression experience a heightened HPA axis response to stressors (for review see Arborelius et al. 1999). Hyperactivity in the HPA axis leads to an excess of stress hormones like cortisol in the plasma creating a condition called hypercortisolaemia. The neurons in the hippocampus have a high density of low affinity glucocorticoid receptors which make them susceptible to damage from over-exposure to

these molecules when under chronic stress exposure (for review see Kim & Diamond, 2002; and Sheline et al., 1999). Lupien et al. (1998) noted that elderly humans with a history of high circulating levels of cortisol showed reduced hippocampal volume that correlated with the degree of cortisol elevation over time. Furthermore, this hippocampal atrophy was correlated with a decreased ability to perform on hippocampus dependent memory tasks. Since the hippocampus is involved in the negative feedback system for the HPA it has been postulated that its minimized volume leads to a breakdown in negative feedback mechanisms leading to more circulating levels of cortisol and the development of depression symptoms (Young, 2006). Interestingly, Holsboer et al (1995) found that weird HPA functioning was actually detectable in the first degree relatives of people with depression. Thus structural alterations in the hippocampus may actually be tightly interrelated with faulty HPA axis functioning and could be not only a result of but also a causal factor in such.

Of interest for future research would be an exploration of correlations between all these factors. Questions to consider would be whether first degree relatives also show decreased hippocampal volume (via MRI) in correlation with hyper HPA activity or poor spatial memory capabilities. Together these endophenotype studies might illuminate the importance of genes regulating neurogenesis in the hippocampus, or HPA axis functioning as a target for genetic research in depression disorders. Since volumetric alterations are associated with depression duration, it may be interesting to learn through a longitudinal study if lower memory scores, which are correlated with a greater degree of hippocampal atrophy, predict a higher chance of developing depression within the FDR group.

The results of this pilot study suggest that underlying hippocampal dysfunction may already exist in at-risk populations at a higher rate than in the general population and that this dysfunction co-segregates with the disorder such that it may be a viable endophenotype in screening for risk and in future genetic research. Furthermore this paper supports earlier conjectures that the HPA-axis interaction might explain the seemingly bi-directional relationship between depression and hippocampal damage. Consequently future research should continue to explore the subclinical conditions experienced by first degree relatives as they appear a valuable source of information in uncovering the genetic underpinnings of depression.

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Informed Consent

INFORMED CONSENT
Mood Disorders, Memory, and the Hippocampus

Investigator: Gianna Petito
Faculty Sponsor: Dr. Bryan Fantie

Purpose and Procedure: In the current project, we are hoping to explore memory capabilities as it relates to mood and mood disorders. You will be asked to fill out a form describing your personal and family history of depression, and two brief scales measuring symptoms of depression. You will then be asked to participate in a series of computerized memory tasks. This experiment should take about 2 hours to complete. Participants also must be at least 18 years old.

Confidentiality: Any data you supply will be used for research purposes only. Only the researchers involved will have access to the information you provide. This form will be kept separate from your other data. Your name will never be associated with your responses and everything will be stored in a secure location. You also will receive a copy of this form for your own records. Data will be disclosed through publication in scientific journals and conference presentations. Individual data will be stripped of all identifying information prior to data analysis. Only group data and/or anonymous, single case examples will be reported.

Compensation: Participation in this study is entirely voluntary. Participants will have the option of either 0.5 extra credit points in participating courses for every half hour or the opportunity to enter a raffle for a 1/20 chance to win $50. It is still possible to enter the raffle for a cash prize or to receive class credit even if the protocol is not completed.

Potential Risks: There are no foreseeable risks for your participation in the study. However, please be aware that you may withdraw from participation at any time for any reason and that there will be no penalty for this withdrawal and you will receive credit for all the time you have contributed. Also if at any time you feel uncomfortable or would like a rest you may let the investigator know.

Potential Benefits: Participants should gain insight into the research process by partaking in this experiment. Additionally the participant is contributing to the field of mood disorder research. The experimenter will be happy to discuss this research with you after it is completed.

Problems: If you have questions or concerns at any point in the study you may ask the experimenter. If you would like to receive a summary of the results of this study when it is completed [by the end of Spring semester 2009] or you have any additional concerns they can be directed to Dr. Bryan Fantie, bfantie@american.edu, Gianna Petito, gp9956a@american.edu, or to the chair of the Psychology Department's Human Subjects Committee

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I, (Please print name clearly)_____, have read the above information about the conduct of this investigation and understand the basic procedure of this study. I understand the potential risks as well as my rights and privileges outlined above. I am aware that I may discontinue participation at any time for any reason. I also understand that I must be at least 18 years old to participate in this study. I hereby give my consent to participate.

_____

Participant's Signature Date

Personal and Family History Form

Your answers to this form will be anonymous and we ask that after completion you place this sheet in the provided envelope.

Participant #:
Sex: M F
Age:

Have you ever been diagnosed with depression? If so, when?

Have you ever been diagnosed with any other psychiatric condition (e.g. bipolar disorder, anxiety disorder, schizophrenia, attention deficit disorder etc.)? If so, what and when?

Are you currently on any medications? If so, what, and for how long?

Have you ever undergone electro-convulsive therapy (ECT)? If so, when?

Have you ever suffered from any head injuries? If so, when?

How often do you drink alcohol?

How many drinks do you usually have?

Was there a time in the past when this quantity or frequency was different? If yes please explain

Do you or did you ever use drugs recreationally? If so, how often?

What kinds did/do you use?

Has anyone in your family ever been diagnosed with depression? If so, who and when (please list all that apply)?

Has anyone in your family ever been diagnosed with any other psychiatric condition (e.g. bipolar disorder, anxiety disorder, schizophrenia, attention deficit disorder etc.)? If so, who, what and when?

BDI-II Form (front)

Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____

Page 14 patient inits: _____

Baseline

BDI-II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

Subtotal Page 1

Continued on Back

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BDI-II (back)

Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____

Baseline

Page 15 patient inits: _____

11. Agitation

0 I am no more restless or wound up than usual.

1 I feel more restless or wound up than usual.

2 I am so restless or agitated that it's hard to stay still.

3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.

1 I am less interested in other people or things than before.

2 I have lost most of my interest in other people or things.

3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile and useful as I used to.

2 I feel more worthless as compared to other people.

3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.

1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.

2b I sleep a lot less than usual.

3a I sleep most of the day.

3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.

1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.

2b My appetite is much greater than usual.

3a I have no appetite at all.

3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

_____ Subtotal Page 2

_____ Subtotal Page 1

_____ Total Score

NR15645

Zung (Not available in digital)
Computer Experience Form (Not available in digital)

Debriefing Form

Debriefing Form **Spring 2009**

Mood Disorders, Memory, and the Hippocampus

We hope that this study will contribute to the growing research concerning the brain and genetic mechanisms involved in affective disorders. The tasks that you performed can be tied to specific brain regions and the pattern of your performance will give us clues into how these regions are involved in mood and memory.

We hope that you have gained some insight from participating in this study regarding the psychological assessment process.

Please feel welcome to contact either of us with any questions and/or concerns. There is also the Counseling Center on campus reachable at (202) 885-3500, if this experiment has caused you any distress and if you would like assistance from a trained therapist.

If you would like to receive a summary of the results of this study when it is completed [by the end of Spring semester 2009], please email Gianna Petito at the address given below.

Thank you for your participation in this research.

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