ADRA2B deletion variant selectively predicts stress-induced enhancement of long-term memory in females

Phillip R. Zoladz a,*, Andrea E. Kalchik a, Mackenzie M. Hoffman a, Rachael L. Aufdenkampe a, Sarah M. Lyle a, David M. Peters a, Callie M. Brown a, Chelsea E. Cadle a, Amanda R. Scharf a, Alison M. Dailey a, Nicholas E. Wolters c, Jeffery N. Talbot b, Boyd R. Rorabaugh c

a Department of Psychology, Sociology, & Criminal Justice, Ohio Northern University, Ada, OH 45810, USA
b Research Center on Substance Abuse and Depression, Roseman University of Health Sciences, Henderson, NV 89014, USA
c Department of Pharmaceutical & Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University, Ada, OH 45810, USA

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Summary Clarifying the mechanisms that underlie stress-induced alterations of learning and memory may lend important insight into susceptibility factors governing the development of stress-related psychological disorders, such as post-traumatic stress disorder (PTSD). Previous work has shown that carriers of the ADRA2B Glu301-Glu303 deletion variant exhibit enhanced emotional memory, greater amygdala responses to emotional stimuli and greater intrusiveness of traumatic memories. We speculated that carriers of this deletion variant might also be more vulnerable to stress-induced enhancements of long-term memory, which would implicate the variant as a possible susceptibility factor for traumatic memory formation. One hundred and twenty participants (72 males, 48 females) submerged their hand in ice cold (stress) or warm (no stress) water for 3 min. Immediately afterwards, they studied a list of 42 words varying in emotional valence and arousal and then completed an immediate free recall test. Twenty-four hours later, participants’ memory for the word list was examined via free recall and recognition assessments. Stressed participants exhibiting greater heart rate responses to the stressor had enhanced recall on the 24-h assessment. Importantly, this enhancement was independent of the emotional nature of the learned information.
In contrast to previous work, we did not observe a general enhancement of memory for emotional information in ADRA2B deletion carriers. However, stressed female ADRA2B deletion carriers, particularly those exhibiting greater heart rate responses to the stressor, did demonstrate greater recognition memory than all other groups. Collectively, these findings implicate autonomic mechanisms in the pre-learning stress-induced enhancement of long-term memory and suggest that the ADRA2B deletion variant may selectively predict stress effects on memory in females. Such findings lend important insight into the physiological mechanisms underlying stress effects on learning and their sex-dependent nature.

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1. Introduction

Stress-induced alterations of learning underlie the formation of traumatic memories and therefore have important implications for understanding the development of post-traumatic stress disorder (PTSD). People with PTSD experience tremendous psychological distress by repeatedly reliving their trauma through intrusive, flashback memories (Zoladz and Diamond, 2013). It is believed that PTSD develops, at least in part, because an individual forms a memory for the traumatic experience that is hyper-consolidated (Hill et al., 2013), which, as William James (James, 1890) suggested, “leave[s] a scar upon the cerebral tissues.” Thus, developing a better understanding of the mechanisms responsible for stress-induced enhancements of long-term memory may provide insight into susceptibility factors for traumatic memory formation and PTSD onset.

The effects of stress on learning and memory are complex and depend on several factors. Post-learning stress often facilitates consolidation and results in enhanced long-term memory, while pre-retrieval stress frequently impairs retention (Wolf, 2009). Pre-learning stress effects on long-term memory are the least understood, as they have resulted in enhancements, impairments, or no effects at all (Diamond et al., 2007). Still, pre-learning stress effects on long-term memory may be a basic research model that provides the most relevant information regarding traumatic memory formation, as it involves stress exposure around the time of learning which influences the encoding and consolidation processes. One factor that plays an important role in dictating the type of effect that pre-learning stress exerts on long-term memory is the timing of the stressor relative to learning. A general consensus has begun to emerge that if a stressor occurs relatively close in time to the learning experience, then long-term memory will be enhanced, an effect that is likely associated with noradrenergic and corticosteroid signaling mechanisms and amygdala-induced enhancement of hippocampal neuroplasticity (Diamond et al., 2007; Joels et al., 2011; Schwabe et al., 2012). Similarly, investigators have speculated that an interaction between noradrenergic and corticosteroid signaling could underlie hyper-consolidation of traumatic memories in PTSD patients (Nicholson et al., 2014). Previous work from our laboratory revealed that exposing participants to a brief (i.e., 3-min) stressor immediately before learning enhanced long-term memory, an effect that was positively associated with participants’ heart rate response to the stressor (Zoladz et al., 2011). Such a stress-induced enhancement of learning would theoretically be adaptive, as it would allow an organism to remember information pertaining to threatening situations, which would later facilitate survival. However, left unchecked, such stress-induced enhancements of learning could also foster the development of powerful emotional memories that ultimately contribute to PTSD development.

Not everyone who is exposed to trauma develops PTSD, suggesting that genetic variation and/or differential peritraumatic responses might increase one’s susceptibility for the disorder. Over the past several years, researchers have established a clear association between certain genetic polymorphisms, emotional memory, and the onset of PTSD (Amstadter et al., 2009; Skelton et al., 2012; Wilker et al., 2014). Thus, it is possible that a particular genotype might predispose individuals to form stronger, potentially intrusive, emotional memories in response to trauma. One commonly occurring genetic variation related to emotional memory and PTSD is the Glu(301)-Glu(303) deletion variant of the ADRA2B gene, which codes for the α2b-adrenergic receptor. Although the variant is associated with both agonistic and antagonistic effects in vitro (Small et al., 2001), researchers have speculated that it likely results in greater norepinephrine availability during emotional events (Rasch et al., 2009), a physiological condition that has been associated with greater learning and memory (McGaugh, 2004). In healthy individuals, the ADRA2B deletion variant has been associated with enhanced perception and memory of emotional stimuli (de Quervain et al., 2007; Todd et al., 2013), as well as greater amygdala activation during the encoding of emotionally arousing information (Rasch et al., 2009; Cousijn et al., 2010). The deletion variant has also been associated with greater intrusiveness of traumatic memories in survivors of the Rwandan Civil War (de Quervain et al., 2007). However, it is unknown whether this deletion variant interacts with stress to influence encoding/consolidation, which would more directly assess, at least in a basic research model, whether the genetic variant could influence traumatic memory formation.

The purpose of the present study was to use an established model of stress effects on learning (Zoladz et al., 2011, 2013, 2014a,b) to test the hypothesis that individuals expressing the ADRA2B deletion variant would be more sensitive to stress-induced enhancements of long-term memory. Based on previous work indicating a relationship between HR and stress-induced enhancements of long-term memory (Zoladz et al., 2011; Larra et al., 2014), we also tested the hypothesis that stressed participants exhibiting a greater HR response to the stressor would demonstrate greater stress-induced enhancements of long-term memory. Finally,
extensive work has revealed sex differences with regards to emotional memory formation (Felmingham et al., 2012b; Schwabe et al., 2013) and PTSD susceptibility (Tolin and Foa, 2006), which, in some instances, have been associated with female menstrual cycle activity (Andreano et al., 2008; Ertman et al., 2011; Felmingham et al., 2012a). Thus, we explored whether or not the sex of participants and menstrual cycle activity in females would influence the interaction between ADRA2B genotype and stress effects on learning.

2. Methods

2.1. Participants

One hundred and twenty healthy men and naturally cycling women (72 males, 48 females; mean age = 19.70 years) from Ohio Northern University volunteered to participate in the experiment. Individuals were excluded from participating if they met any of the following conditions: diagnosis of Raynaud’s disease or peripheral vascular disease; presence of skin diseases, such as severe psoriasis, eczema or scleroderma; history of syncope or vasovagal response to stress; history of severe head injury; current treatment with psychotropic medications, narcotics, beta-blockers, steroids or any other medication that was deemed to significantly affect central nervous or endocrine system function; mental or substance use disorder; regular nightshift work. Individuals who smoked were allowed to participate in the study; information regarding individuals’ smoking habits was collected prior to the experiments via a short demographic survey. There were only three participants who reported smoking on a regular basis, and inclusion of the data from these participants in the statistical analyses did not alter the results. Participants were asked to refrain from using recreational drugs (e.g., marijuana) for 3 days prior to the experimental sessions; to refrain from drinking alcohol or exercising extensively for 24 h prior to the experimental sessions; and, to refrain from eating or drinking anything but water for 2 h prior to the experimental sessions. All of the experimental methodology was approved by the Institutional Review Board at Ohio Northern University.

2.2. Experimental procedures

To control for diurnal variations in cortisol levels, all testing was carried out between 1200 and 1800 h.

2.2.1. Cold pressor test (CPT)

Participants were asked to submerge their non-dominant hand, up to and including the wrist, in a bath of water for 3 min. Participants who had been randomly assigned to the stress condition (N = 62; 33 males, 29 females) placed their hand in a bath of ice cold (0–2 °C) water, while participants who had been randomly assigned to the control condition (N = 58; 39 males, 19 females) placed their hand in a bath of warm (35–37 °C) water. The water was maintained at the appropriate temperature by a VWR 1160S circulating water bath. To maximize the stress response, participants in each experiment were encouraged to keep their hand in the water bath for the entire 3-min period. However, if a participant found the water bath too painful, he or she was allowed to remove his or her hand from the water and continue with the experiment. Only one participant from the stress condition removed his or her hand from the water prior to 3 min elapsing (mean water time = 179.06 s), and all participants from the no stress condition kept their hand in the water for the entire 3-min period. Inclusion of the data from the stressed participant who removed his or her hand from the water early had no significant effect on the observed results.

2.2.2. Subjective pain and stress ratings

All participants were asked to rate the painfulness and stressfulness of the water bath manipulation at 1-min intervals on 11-point scales ranging from 0 to 10, with 0 indicating a complete lack of pain or stress and 10 indicating unbearable pain or stress. If a participant removed his or her hand from the water before 3 min had elapsed, the remaining data points were automatically scored as 10 s for each measure.

2.2.3. Word presentation

Immediately following the water bath manipulation, participants were presented with a list of 42 words selected from the Affective Norms for English Words (Bradley and Lang, 1999). Based on standardized valence and arousal ratings, we chose 14 neutral, 14 positive, and 14 negative words (7 arousing and 7 non-arousing words within each category), which, across valence and arousal categories, were balanced for word length and frequency. Participants were instructed to read each word aloud and rate its emotional valence on a scale from −4 (very negative) to +4 (very positive) and its arousal level on a scale of 0 (not arousing) to 8 (very highly arousing) on a sheet of paper containing the list of words (Schwabe et al., 2008; Zoladz et al., 2011, 2013, 2014a). These manipulations were performed to promote encoding of the words, and they allowed us to analyze the final memory data based on participants’ own ratings of the words.

According to the Affective Norms for English Words (Bradley and Lang, 1999), the mean (±SEM) valence and arousal ratings for the words that made up the list were as follows: positive arousing words (e.g., flirt): valence = 7.56 ± 0.20, arousal = 6.62 ± 0.22; positive non-arousing words (e.g., bunny): valence = 7.33 ± 0.08, arousal = 3.64 ± 0.19; negative arousing words (e.g., bloody): valence = 2.26 ± 0.14, arousal = 6.68 ± 0.13; negative non-arousing words (e.g., blister): valence = 2.40 ± 0.19, arousal = 4.18 ± 0.04; neutral arousing words (e.g., noisy): valence = 4.88 ± 0.15, arousal = 6.26 ± 0.24; neutral non-arousing words (e.g., bland): valence = 4.84 ± 0.21, arousal = 3.58 ± 0.11.

2.2.4. Memory testing

Immediately following word list encoding, participants were given 5 min to write down as many words as they could remember. This immediate free recall test was performed to verify that there were no group differences regarding short-term memory performance and to avoid a potential floor effect during long-term memory assessment. Twenty-four hours later, participants returned to the laboratory and were given 5 min to write down as many words as they could remember from the list of words that they studied on the previous day (i.e., delayed free recall). Immediate
and free recall performances were expressed as a percent of total words recalled for each valence/arousal category (i.e., percent of positive arousing words=positive arousing words recalled/7 × 100; percent of total words recalled=total words recalled/42 × 100). Participants’ forgetting rate was also calculated for each valence/arousal category by expressing delayed free recall performance as a percentage of immediate free recall performance (i.e., forgetting rate=delayed free recall/immediate free recall × 100).

Following delayed free recall testing, participants sat quietly for 10 min, after which they were given a recognition test. Participants were presented with a list of words containing 42 “old” words (i.e., words that were presented on the previous day) and 42 “new” words (i.e., words that were not presented on the previous day) and were instructed to label each word as “old” or “new.” The “new” words were matched to the “old” words on emotional valance, word length and word frequency, according to the ratings obtained from the Affective Norms for English Words (Bradley and Lang, 1999). To assess participants’ ability to discriminate between “old” and “new” words, we calculated a sensitivity index ($d′=z[\bar{p}(hit)]−z[\bar{p}(false alarm)]$) for each valence and arousal category to be used for statistical analysis.

2.2.5. Genotyping (see Fig. 1)

Subjects who carried the wild type $α_{2B}$-adrenergic receptor allele or the Glu$^{101}$-Glu$^{103}$ deletion (heterozygous or homozygous) variant of this gene were identified by polymerase chain reaction (PCR) using DNA isolated from buccal cheek swabs. PCR was performed as previously described by de Quervain and colleagues (de Quervain et al., 2007) using ‘‘Go Taq’’ DNA polymerase (Promega, Madison, WI) and the following primers: 5′-AGAAGGAGGGTTTGTGGGG-3′ and 5′-ACCTATAGACCCACGCCCT-3′. This PCR reaction produced 200 and 209 base pair PCR products for the Glu$^{101}$-Glu$^{103}$ deletion and wild type alleles, respectively. PCR conditions were as follows: 95 °C 5 min; 35 cycles: 95 °C 30 s, 58 °C 30 s, 72 °C 30 s; 72 °C 7 min. PCR products were separated on a 4% Amresco (Solon, OH) Super Fine Resolution agarose gel. A PCR reaction that contained no DNA was used as a negative control.

2.2.6. Cardiovascular analysis

Heart rate (HR) and blood pressure (BP) measurements were taken 2 min before (baseline), halfway through and approximately 10 min after cessation of the water bath manipulation. Cardiovascular activity was measured with a vital signs monitor (Mark of Fitness WS-820 Automatic Wrist Blood Pressure Monitor) placed on the wrist of each participant’s dominant hand.

2.2.7. Cortisol analysis

Saliva samples were collected from participants 2 min before (baseline) and 25 min following exposure to the water bath manipulation to analyze salivary cortisol concentrations. We collected the second sample 25 min after stress onset because stress-induced increases in salivary cortisol typically peak approximately 20–30 min following stress. The samples were collected in a Salivette saliva collection device (Sarstedt, Inc., Newton, NC). Participants were asked to place a synthetic swab in their mouths and chew on it so that it would easily absorb their saliva. Following 1 min of chewing, the synthetic swab was collected and placed in the Salivette conical tube and kept at room temperature until the experimental session was completed. The samples were subsequently stored at −20 °C until assayed for cortisol.

Saliva samples were thawed and extracted by low-speed centrifugation. Salivary cortisol levels were determined by enzyme immunoassay (EIA; Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer’s protocol. The minimum detectable concentration of cortisol was approximately 8 pg/ml, and the average inter- and intra-assay percent coefficients of variation were less than 6.6% and 3%, respectively.

2.3. Statistical analyses

Mixed-model ANOVAs were used to analyze all data; the between-subjects factors were genotype, stress and sex, and the within-subjects factors were word valence and arousal (for memory analyses) or time (for physiological and pain/stress ratings analyses). The analyses of participants’ valence and arousal ratings and memory for the words (i.e., immediate free recall, delayed free recall, forgetting rate and recognition) were performed based on categorizing the words (i.e., distributing the words to positive arousing, positive non-arousing, negative arousing, etc. groups) according to participants’ valence and arousal ratings that were obtained during the study.

Based on previous work indicating a relationship between HR and stress-induced enhancements of long-term memory (Zoladz et al., 2011; Larra et al., 2014), we conducted further analyses on the memory data by dividing stressed participants into HR Responder and HR Non-Responder groups. We performed a median split on stressed participants’ heart rate differences before and after stress exposure, which resulted in a median increase of 4 bpm and is comparable to previous work performing similar statistical manipulations before analyzing stress effects on memory (Larra et al., 2014). Because median splits can reduce the power of one’s statistical analyses, we also ran ANCOVAs on memory performance with HR response as a covariate; this resulted in larger sample sizes per cell. In these analyses, genotype, stress (as opposed to HR response group) and sex were utilized as the between-subjects factors. Finally, we performed analyses on female participants only to determine whether menstrual cycle stage influenced any observed effects. In order to do so, we divided female participants into follicular (0–14 days since last period; $N = 28$) or luteal (≥15 days since their last period; $N = 20$) phases of the menstrual cycle (Nielsen et al., 2013).

Some participants were excluded from some data analyses because of an inability to obtain their genotype ($N = 17$) or physiological (e.g., HR, BP) data ($N = 1$); the final sample size breakdown for all groups can be found in Table 1. Alpha was set at 0.05 for all analyses, and Bonferroni-corrected post hoc tests were employed when the omnibus $F$ test indicated the presence of a significant effect. SPSS (version 20) was used to perform all statistical analyses.
3. Results

3.1. Physiological responses (see Fig. 2)

Stress, overall, had no effect on HR (no significant effect of condition: $F(1,91) = 0.83, p > 0.05$). After performing the median split on HR responses to stress, we verified that HR Responders exhibited a greater change in HR relative to HR Non-Responders and non-stressed participants, $F(2,113) = 13.46, p < 0.001$. Stressed participants exhibited greater systolic and diastolic BP than non-stressed participants during the water bath manipulation [significant effects of condition: $F(1,91) = 5.68$ (systolic), $F(1,91) = 8.57$ (diastolic); significant Condition × Time interactions: $F(2,182) = 20.04$ (systolic), $F(2,182) = 29.47$ (diastolic) $(p's < 0.05)$]. Male participants also exhibited greater systolic and diastolic BP than female participants [significant effects of sex: $F(1,91) = 25.09$ (systolic), $F(1,91) = 21.42$ (diastolic) $(p's < 0.05)$]. There was a trend suggesting that carriers of the ADRA2B deletion had greater diastolic BP than non-carriers (effect of genotype approaching significance: $F(1,91) = 3.43, p = 0.067$). Stressed participants exhibited greater salivary cortisol levels than non-stressed participants after the water bath manipulation [significant effect of condition: $F(1,93) = 14.84$; significant Condition × Time interaction: $F(1,93) = 34.54$ $(p's < 0.001)$]. There was also a significant Condition × Sex interaction, $F(1,93) = 4.37, p < 0.05$, indicating that the difference between salivary cortisol levels of stressed and non-stressed participants was greater in females than in males.

3.2. Subjective ratings of water bath manipulation

Stressed participants (pain: $M = 6.78$; stress: $M = 5.51$) reported greater pain and stress ratings than non-stressed participants (pain: $M = 0.05$, stress: $M = 0.28$) throughout the water bath manipulation [significant effects of condition: $F(1,95) = 708.39$ (pain), $F(1,95) = 179.13$ (stress) $(p's < 0.05)$]. Furthermore, stressed females ($M = 7.31$) reported greater pain ratings than stressed males ($M = 6.25$) [significant effect of sex: $F(1,95) = 4.76$; significant Condition × Sex interaction: $F(1,95) = 4.09$ $(p's < 0.05)$].

![Image](image_url)

**Fig. 1** Identification of subjects who were wild type, heterozygous or homozygous for the deletion of nucleotides encoding Glu301-Glu303 of the $\alpha_{2b}$-adrenergic receptor. Polymerase chain reaction (PCR) was performed as previously described by de Quervain et al. (2007), and PCR products were separated on a 4% agarose gel. Wild type and deletion alleles produced PCR products that were 209 and 200 base pairs in length, respectively. A control PCR reaction that contained no DNA was used as a negative control. Lanes showing 200 base pair and 300 base pair size markers are indicated by "M".

**Table 1** Final sample sizes for Stress × Sex × ADRA2B genotype analyses.

<table>
<thead>
<tr>
<th></th>
<th>Deletion(N = 60)</th>
<th>No deletion(N = 42)</th>
<th>Total(N = 102)</th>
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<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
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<td>High HR</td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Low HR</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>No Stress</td>
<td>20</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>24</td>
<td>61</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
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<td></td>
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<tr>
<td>High HR</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Low HR</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>No Stress</td>
<td>12</td>
<td>5</td>
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</tr>
<tr>
<td>Total</td>
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<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>42</td>
<td>102</td>
</tr>
</tbody>
</table>
3.3. Word list ratings

Positive words were given more positive ratings than neutral words, which were given more positive ratings than negative words (significant effect of valence: F(2, 190) = 897.88, p < 0.001), and arousing words were rated as more arousing than non-arousing words (significant effect of arousal: F(1, 95) = 304.37, p < 0.001).

3.4. Memory testing

3.4.1. Immediate free recall (see Fig. 3)

When analyzing stress effects overall, there were no significant differences between stressed and non-stressed participants (no significant effect of condition: F(1, 95) = 0.11, p > 0.05). Participants recalled more negative and neutral words than positive words and more arousing words than non-arousing words (significant effect of valence: F(2, 190) = 15.34, p < 0.001; significant effect of arousal: F(1, 95) = 20.49, p < 0.001). Females also recalled more words than males (significant effect of sex: F(1, 95) = 12.25, p < 0.001). When analyzing stress effects by employing HR Responder as a between-subjects factor or by using HR response as a covariate in an ANCOVA, all effects remained unchanged. When analyzing females only, carriers of the ADRA2B deletion variant exhibited greater recall of arousing words if they were tested during the follicular phase, while non-carriers exhibited greater recall of arousing words.
Genetic variant predicts stress effects on memory

3.4.2. Delayed free recall (see Figs. 3 and 4)

3.4.2.1. Raw performance. When analyzing stress effects overall, there were no significant differences between stressed and non-stressed participants (no significant effect of condition: $F(1,95) = 0.78, p > 0.05$). Participants is they were tested during the luteal phase (significant Genotype \texttimes Menstrual Stage \texttimes Arousal interaction: $F(1,33) = 7.99, p < 0.01$; see Fig. 3). Analyses on males only, in contrast, indicated that they recalled more arousing words than non-arousing words, independent of genotype.

Fig. 3 Influence of ADRA2B genotype on recall of arousing versus non-arousing words in males and females. Males displayed greater immediate (inset a) and delayed recall (insets c and e) of arousing words, relative to non-arousing words, independent of genotype. Female carriers of the ADRA2B deletion variant exhibited greater immediate (inset b) and delayed (inset d) recall of arousing words, relative to non-arousing words, only when they were in the follicular stage of the menstrual cycle. In contrast, non-carriers of the deletion variant exhibited greater immediate and delayed recall of arousing words, relative to non-arousing words, only when they were in the luteal phase of the menstrual cycle. This Genotype \texttimes Menstrual Stage \texttimes Arousal interaction was reduced to a statistical trend when delayed recall was expressed as a percentage of immediate recall (inset f). Data are presented as means \pm SEM. *Significant main effect of arousing words, $p < 0.01$; **$p < 0.01$ relative to non-arousing words.
recalled more negative and neutral words than positive words and more arousing words than non-arousing words, particularly when they were negative or neutral in valence [significant effect of valence: \( F(2,190) = 19.74, p < 0.001 \); significant effect of arousal: \( F(1,95) = 37.06 \); significant Valence × Arousal interaction: \( F(2,190) = 16.53 \) \( (p < 0.001) \)]. Females also recalled more words than males, which was dependent in part on the valence and arousal level of the words [significant effect of sex: \( F(1,95) = 15.72, p < 0.001 \); significant Sex × Valence × Arousal interaction: \( F(2,190) = 6.15, p < 0.01 \)]. When analyzing stress effects by employing HR Responder as a between-subjects factor, there was, in addition to the aforementioned effects, a significant effect of HR Responder, indicating that stressed HR Responders exhibited greater recall than stressed HR Non-Responders and non-stressed participants, \( F(2,90) = 3.39, p < 0.05 \). Similarly, an ANCOVA that employed HR response as a covariate revealed an effect of stress that was approaching significance, \( F(1,93) = 3.51, p = 0.064 \). When analyzing females only, the effect observed for immediate free recall was also observed for delayed free recall (significant Genotype × Menstrual Stage × Arousal interaction: \( F(1,33) = 8.65, p < 0.01 \)). Males, again, recalled more arousing words than non-arousing words, independent of genotype.

### 3.4.3. Delayed recognition (see Fig. 5)

When analyzing stress effects overall, stressed female carriers of the ADRA2B deletion variant exhibited greater recognition memory than all other groups (significant Condition × Genotype × Sex interaction: \( F(1,94) = 9.28, p < 0.01 \)). Participants recognized more positive and neutral words than negative words (significant effect of valence: \( F(2,188) = 11.61, p < 0.001 \)). Participants also recognized more arousing words than non-arousing words (significant effect of arousal: \( F(1,94) = 44.92, p < 0.001 \)). When analyzing stress effects by employing HR Responder as a
Fig. 5  Recognition performance 24 h following encoding. Stressed female carriers of the ADRA2B deletion variant exhibited greater recognition memory than all other groups (inset a), which appeared to be driven by those female carriers demonstrating greater increases in heart rate during stress exposure (inset b). Insets c and d illustrate the interactive influences of stress, sex, ADRA2B genotype and arousal level of words on recognition memory. ARO = arousing words; NON = non-arousing words. Data are presented as means ± SEM. **p < 0.05 relative to all other groups; ’ p < 0.05 relative to the no stress group.

between-subjects factor, the HR Responder × Genotype × Sex interaction was significant, indicating that stressed female carriers of the ADRA2B deletion variant who were HR Responders exhibited greater recognition memory than non-stressed female carriers of the ADRA2B deletion variant, F(2,89) = 4.27, p < 0.05. This interaction remained significant in an ANCOVA that included HR response as a covariate, F(1,92) = 9.96, p < 0.01. When analyzing females only, no additional effects were observed.

4. Discussion

We have shown that brief, pre-learning stress enhances long-term memory in participants exhibiting greater HR responses to the stressor. This finding is consistent with our previous work (Zoladz et al., 2011), in which we found that the pre-learning stress-induced enhancement of long-term memory was positively correlated with participants’ HR response to the stressor. Most importantly, however, we have shown that female carriers of the ADRA2B deletion variant are selectively susceptible to stress-induced enhancements of long-term memory. This effect was also positively associated with HR response to the stress. Overall, our findings implicate autonomic mechanisms in the pre-learning stress-induced enhancement of long-term memory and suggest that the ADRA2B deletion variant may increase sensitivity to stress-induced enhancements of long-term memory in females.

4.1. Stress effects on long-term memory

We did not find that immediate, pre-learning stress enhanced long-term memory consolidation in general; rather, the enhancement was selective to stressed participants exhibiting greater HR responses to the stress and was observed for delayed recall, not recognition memory. Importantly, the stressed participants differing in HR responses exhibited statistically equivalent cortisol responses to the stressor, implicating the involvement of autonomic and perhaps noradrenergic activity in the observed memory enhancement. This finding is consistent with our previous work in humans (Zoladz et al., 2011), as described above, and with human and rodent work from other laboratories. For instance, Schwabe et al. (2008) found that pre-learning stress enhanced long-term memory and that the enhancement was independent of cortisol response; thus, the authors attributed the stress-induced enhancement to autonomic mechanisms. Also, despite observing an enhancement for delayed free recall, the investigators reported no overall effects of pre-learning stress on
long-term recognition memory, similar to the present findings. Others have extended pre-learning stress effects on learning and memory to rodents. Diamond and colleagues reported that brief predator stress immediately prior to learning enhanced long-term water maze memory and that the effect was blocked by the administration of a β-adrenergic receptor antagonist (Diamond et al., 2007; Halonen et al., 2007). Finally, our findings are consistent with the temporal dynamics model of emotional memory processing, proposed by Diamond et al. (2007), which includes the prediction that brief stress around the time of learning facilitates long-term memory via amygdala-induced enhancement of hippocampal plasticity, an enhancement that depends, at least in part, on noradrenergic activity.

Contrary to previous work, we did not consistently observe superior memory for positive and negative words, relative to neutral words. In fact, the only consistent effect that was observed for emotional information was better memory for arousing, relative to non-arousing, words. Because we manipulated both the valence and arousal level of the learned information, our findings might indicate that the arousal level of the information is more influential, relative to its valence, on memory performance. Indeed, we observed contradictory findings regarding the emotional valence of the studied words. Participants recalled more negative and neutral words than positive words but recognized more positive and neutral words than negative words. In our previous work, we have observed inconsistent effects for the influence of word valence on recall performance, sometimes reporting greater recall for positive and negative words, relative to neutral words (Zoladz et al., 2011), and other times reporting greater recall for positive and/or neutral words, relative to negative words (Zoladz et al., 2013, 2014a). Our observations for recognition memory in the present study are consistent with previous work from our laboratory (Zoladz et al., 2011, 2013, 2014a). Nevertheless, the discrepancies between our results and those from previous studies might be explained by the fact that we assigned words to valence/arousal categories based on participants’ ratings of the words, rather than simply utilizing standardized ratings. It is also possible that words do not evoke robust positive and negative emotional responses, relative to neutral words. Finally, that we have consistently observed better memory for arousing, relative to non-arousing, words suggests that the arousal levels of the learned information may be more influential on memory than its valence.

We also did not find that the stress-induced enhancement of long-term memory was selective to emotional information. Some work in this area has reported that pre-learning stress facilitates memory for emotional information, while impairing memory for neutral information (Jelicic et al., 2004; Payne et al., 2006, 2007). However, such a finding is not unequivocal. We previously found no effect of pre-learning stress that was selective to neutral information (Zoladz et al., 2011, 2013), and others have reported that pre- or post-learning stress can even enhance memory for neutral information, while leaving emotional information relatively unaffected (Schwabe et al., 2008; Preuss and Wolf, 2009). Some research has shown that whether or not information is related to the stressor is important in determining if emotional memory is affected by the stress. For instance, Smeets et al. (2009) found that stress enhanced participants’ memory for words only if the words were highly arousing and directly related to the stressor that was employed in the study. Thus, we may not have observed effects of stress that were selective to emotional information because this information was not related to the stressor. This is consistent with some of our previous work on stress-memory interactions (Zoladz et al., 2013, 2014a).

4.2. Influence of ADRA2B deletion variant on stress—memory interactions

Previous work concerning the relationship between genetic variation and emotional memory has called attention to the possible relevance of the ADRA2B deletion variant for understanding traumatic memory formation (Skelton et al., 2012; Wilker et al., 2014). This deletion variant has been associated with enhanced perception and recall of emotional stimuli (de Quervain et al., 2007; Rasch et al., 2009; Todd et al., 2013), greater amygdala responses to emotional stimuli (Rasch et al., 2009; Cousijn et al., 2010) and greater intrusiveness of traumatic memories in Rwandan Civil War survivors (de Quervain et al., 2007). Up to this point, however, there has been no examination of how the ADRA2B deletion variant might influence stress-induced alterations of learning. That the stress-induced enhancements of learning reported here were associated with participants’ cardiovascular responses to the stressor is not surprising, as such a finding implicates noradrenergic influences on the enhancements. Indeed, extensive work has linked noradrenergic activity in the amygdala with enhanced emotional learning (McGaugh, 2004). Moreover, researchers have speculated that people who develop PTSD produce hyper-consolidated memories of the trauma as a result of an unrestrained sympathetic-adrenomedullary response to the trauma, which significantly enhances the neural mechanisms underlying learning (Zoladz and Diamond, 2013). It is noteworthy that the ADRA2B deletion variant was selectively associated with stress-induced enhancements of memory in females who exhibited increased HR following stress. This finding supports the possibility that the ADRA2B deletion variant results in greater norepinephrine availability during emotional events, which then strengthens one’s memory for such events, at least in females.

Despite our findings regarding the ADRA2B deletion variant, it should be emphasized that we did not replicate all of the previous work conducted on this genetic alteration. Previous studies have shown that carriers of the ADRA2B deletion variant exhibit greater recall of positive and/or negative pictures, relative to non-carriers of the variant (de Quervain et al., 2007; Rasch et al., 2009). However, in the present study, the ADRA2B deletion variant predicted stress effects on female recognition memory for emotional and non-emotional words combined. Thus, in contrast to previous work, our effects were observed for recognition, not recall, and were not selective to emotional information. In fact, the ADRA2B variant was predictive of greater emotional memory (i.e., better memory for arousing, relative to non-arousing, words) only in female carriers who were tested during the follicular phase of the menstrual cycle (see Fig. 3; described more below). It is possible that we did not replicate previous work because we utilized a
4.3. Sex differences

That some stress effects on memory were observed for females, but not males, is consistent with previous work in this area. For instance, females appear to be more sensitive to the influences of emotion on memory, and they recall emotional information better than males, an effect that has been associated with greater noradrenergic activity in females (Felmingham et al., 2012b; Schwabe et al., 2013). There is also work indicating that females are more susceptible to stress-induced enhancements of long-term memory, relative to males (Felmingham et al., 2012b). Related to menstrual cycle activity, we found that female carriers of the ADRA2B deletion variant had better memory for arousing words, relative to non-arousing words, only when they were tested during the follicular phase of the menstrual cycle, while female non-carriers exhibited the same effect only when they were tested during the luteal phase. Most of the research concerning menstrual cycle influences on emotional memory has revealed that any observed effects are greater during the luteal phase, a phase when progesterone levels peak. Indeed, researchers have observed correlations between stress-induced cortisol and memory only when females are in the luteal phase of the menstrual cycle (Andreano et al., 2008), and females also exhibit stronger responses of the amygdala-hippocampus neural network when they are in the luteal phase (Andreano and Cahill, 2010). Furthermore, females exhibiting high levels of progesterone demonstrate better memory for emotional information, greater stress-induced elevations of salivary cortisol and stronger stress-enhanced levels of memory than women with low levels of progesterone (Ertman et al., 2011; Felmingham et al., 2012a). Thus, if anything, we would have expected emotional memory to be greater for females in the luteal phase. It is not clear why this was evident for non-carriers of the ADRA2B deletion variant only, nor why female carriers of the variant exhibited greater emotional memory during the follicular, but not luteal, phase. It is possible that the physiological consequences of the ADRA2B deletion variant interact with female hormone fluctuations to exert differential effects on emotional learning. Future work should utilize measures of noradrenergic activity and menstrual cycle hormones to test this possibility.

4.4. Limitations

By including several factors in our analyses and employing a median split for HR response to stress, some of our effects were limited by small sample sizes (e.g., N=5–6) in individual cells (see Table 1). For instance, the contribution of HR response to the Genotype × Stress × Sex interaction observed for recognition memory was based on 6 female deletion carriers identified as HR Responders. On the other hand, most of our findings implicating HR response in stress effects on memory were supported by follow-up ANCOVAs that included HR response solely as a covariate. Though our findings suggest a role for autonomic mechanisms in the observed effects, additional work is necessary to substantiate this claim. Similarly, since so many factors have been introduced into the present analyses, it is worth noting that other interactions between certain variables could have influenced the present findings. For instance, menstrual cycle activity could influence physiological responses to stress, such as HR, BP and/or cortisol responses, which could impact stress–memory interactions. Future studies should explore the possibility that such interactions modify the types of effects stress exerts on long-term memory.

4.5. Conclusions

The present findings corroborate our previous work on pre-learning stress-induced enhancements of long-term memory and are the first to show that the ADRA2B deletion variant can increase healthy participants’ susceptibility to stress-induced enhancements of learning. The results provide important insight into one possible susceptibility factor for traumatic memory formation and, perhaps, the development of PTSD. Because the observed association between the ADRA2B deletion variant and enhanced long-term memory was observed in females, but not males, additional work is required to delineate what physiological mechanisms might underlie such sex differences, as they may tell us a great deal about why females are more vulnerable to stress-related disorders like PTSD (Tolin and Foa, 2006). As an example, future studies should examine how noradrenergic activity interacts with cortisol and menstrual cycle hormone levels to influence the relationship between the ADRA2B deletion variant and stress effects on learning.

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