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Myocardial Hypersensitivity to Ischemic Injury is not Reversed by Clonidine or Propranolol in a Predator-Based Rat Model of Posttraumatic Stress Disorder

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Abstract

Individuals with posttraumatic stress disorder (PTSD) are at increased risk for cardiovascular disease. We previously reported that a predator-based model of PTSD increases myocardial sensitivity to ischemic injury. Heightened sympathetic signaling has a well-established role in the formation of anxiety associated with PTSD and may also contribute to worsening of myocardial injury in the ischemic heart. Thus, we examined whether suppression of sympathetic tone protects the ischemic heart in rats subjected to this model of PTSD. Rats were treated with saline or clonidine throughout the 31-day stress paradigm. Behavior on the elevated plus maze (EPM) was assessed on Day 32, and hearts were subjected to an ischemic insult on day 33. Stressed rats exhibited increased anxiety on the EPM and significantly larger myocardial infarcts following ischemia. Clonidine reversed the anxiety-like behavior but had no impact on infarct size. In a subsequent experiment, rats were treated with propranolol in their drinking water throughout the stress paradigm. Propranolol had no effect on either anxiety or myocardial sensitivity to ischemic injury. These findings suggest that the myocardial hypersensitivity to ischemic injury observed in this model is not caused by increased sympathetic tone or chronic β -adrenergic receptor signaling.

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Ethical Statement

All animal experiments complied with the ARRIVE guidelines and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Conflict of interest

The authors have no conflicts of interest.

Keywords

posttraumatic stress disorder; ischemia; heart; sympathetic; adrenergic; anxiety

1 Introduction

Individuals who are exposed to life-threatening trauma such as wartime combat, assault, rape, or motor vehicle accidents are at risk of developing posttraumatic stress disorder (PTSD). People with PTSD experience extreme psychological distress by repeatedly reliving their trauma through intrusive flashback memories (Ehlers et al., 2004; Ehlers et al., 2002; Reynolds and Brewin, 1999). These memories are accompanied by other debilitating symptoms, including emotional numbing, avoidance of stimuli associated with the trauma, and a persistent state of increased arousal and hypervigilance (Nemeroff et al., 2006; Pitman et al., 2012; Zoladz and Diamond, 2013).

In addition to psychological manifestations, PTSD is also associated with cardiovascular abnormalities including increased heart rate and blood pressure, increased risk of cardiac arrhythmias, increased atherosclerosis, and increased risk of myocardial infarction (Ahmadi et al., 2011; Beristianos et al., 2016; Khazaie et al., 2013; Vaccarino et al., 2013). Despite evidence that PTSD increases the likelihood of experiencing a myocardial infarction, the impact of PTSD on the ischemic heart has not been well studied. Such investigations are difficult to conduct in human patients because PTSD is associated with increased rates of smoking, sedentary lifestyle, diabetes, depression, and other factors that influence cardiovascular function (Dedert et al., 2010; Zen et al., 2012) and potentially confound efforts to address the direct impact of posttraumatic stress on the heart.

Animal models provide a tool to examine the impact of posttraumatic stress on the myocardial response to ischemic injury. Predator-based animal models of PTSD have been the subject of multiple recent reviews (Deslauriers et al., 2018; Schoner et al., 2017; Zoladz and Diamond, 2016). Predator exposure is an appealing stressor to use in animal models of PTSD because of its high ecological validity and the instinctual fear response it elicits. Previous work demonstrated that rats exposed to a predator-based model of PTSD developed a number of physiological and behavioral abnormalities that are remarkably similar to those observed in people with PTSD, including a robust fear-conditioned memory of the traumatic experience, increased anxiety-related behavior, reduced baseline levels of corticosterone, and an exaggerated startle response (Zoladz et al., 2008). These animals also exhibited impaired memory for new information, greater cardiovascular and hormonal reactivity to an acute stressor, exaggerated physiological and behavioral responses to an α_2 -adrenergic receptor antagonist (yohimbine), reduced growth rate, reduced thymus weight, greater adrenal gland weight, enhanced negative feedback of the HPA axis, and increased DNA methylation in the hippocampus (Roth et al., 2011; Zoladz et al., 2008; Zoladz et al., 2012, 2013). Decreased brain serotonin levels, increased brain norepinephrine levels, increased production of reactive oxygen species, increased production of proinflammatory markers, and decreased production of anti-inflammatory markers have also been reported in rats exposed to this model (Wilson et al., 2014a; Wilson et al., 2014b; Wilson et al., 2014c;

Wilson et al., 2013). Importantly, many of these effects can be blocked by clinically used drugs including clonidine, amitriptyline, sertraline, and valproic acid (Wilson et al., 2014b; Wilson et al., 2014c; Zoladz et al., 2013). Rats subjected to the predator-based model of PTSD have been previously shown to exhibit increased sympathetic activity and to be hypersensitive to myocardial ischemic injury (Rorabaugh et al., 2015). Some of the behavioral and cardiovascular effects of this model can also be blocked by suppressing sympathetic tone with clonidine (Zoladz et al., 2013). Furthermore, prior studies have demonstrated that chronic β -adrenergic receptor stimulation worsens myocardial injury in the ischemic heart and that this can be blocked by propranolol (Hu et al., 2006; Rorabaugh et al., 2017a). Thus, the goal of the present study was to determine whether the myocardial hypersensitivity to ischemic injury that develops in animals exposed to this form of stress can be blocked by suppressing sympathetic tone or by blocking β -adrenergic receptors.

2 Methods

2.1 Animals

Male Sprague-Dawley rats were obtained from a breeding colony at Ohio Northern University. The colony was initially established using Sprague Dawley rats obtained from Charles River Laboratories (Wilmington, MA). The rats were housed on a 12-h light/dark cycle (lights on 0700–1900 hours) in standard polycarbonate cages with free access to food and water. This investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Ohio Northern University. Previous work has demonstrated that females do not develop myocardial hypersensitivity to ischemic injury following exposure to this predator-based model of PTSD (Rorabaugh et al., 2015). Thus, only males were included in the present study.

2.2 Predator Exposure and Psychosocial Stress

Rats were randomly assigned to “psychosocial stress” or “no stress” groups. On Day 1, rats in the psychosocial stress groups were immobilized in plastic DecapiCones (Braintree Scientific; Braintree, MA) and placed in a perforated wedge shaped Plexiglas enclosure (Braintree Scientific; Braintree, MA; 20×20×8 cm). This enclosure was then taken to a cat housing room and placed in a metal cage (61×53×51 cm) with an adult female cat for 1 h. The Plexiglas enclosure prevented any contact between the cat and rats, but the rats were still exposed to all non-tactile sensory stimuli associated with the cat. Canned cat food was smeared on top of the enclosure to direct the cat’s attention toward the rats. An hour later, the rats were returned to their home cages. Rats in the psychosocial stress group were given two acute stress sessions which were separated by 10 days. The first stress session took place during the light cycle (between 0800–1300 hours), and the second stress session occurred during the dark cycle (between 1900–2100 hours). The stress sessions took place during different times of the day to add an element of unpredictability as to when the rats might re-experience the predator exposure (Roth et al., 2011; Zoladz et al., 2008; Zoladz et al., 2012, 2013; Zoladz et al., 2015). Rats in the stressed group were also subjected to unstable housing conditions throughout the 31-day stress paradigm. The animals were pair

housed, and their cage mates were changed daily such that no rats in the psychosocial stress group had the same cage mate on two consecutive days during the 31-day stress period.

Animals in the “no stress” control group were also pair housed. These animals were not exposed to the cat and maintained the same cage mates throughout the duration of the study. Rats in this group were handled daily to control for potential handling effects on stressed animals.

2.3 Clonidine Treatments

Rats received daily intraperitoneal injections of clonidine (0.05 mg/kg) (obtained from Sigma Aldrich, St. Louis, MO) or saline starting 24 hours after the first cat exposure and continuing throughout the stress paradigm. The last injection was administered 30 minutes prior to behavioral testing on the elevated plus maze on day 32. Hearts were isolated on day 33. The injections were always administered in the morning (between 0900–1200 hours). The clonidine dose (0.05 mg/kg) was chosen based on previous findings that this dose ameliorates anxiety-like behavior on the elevated plus maze and also blocks increased heart rate and blood pressure in rats exposed to this stress paradigm (Zoladz et al., 2013).

2.4 Propranolol Treatments

Propranolol-treated rats were administered propranolol in their drinking water (0.5 g/l) starting on day 2 and continuing until day 33 when hearts were isolated and subjected to an ischemic insult. Water bottles were changed every other day. Control animals were given drinking water without propranolol. Prior work demonstrated that chronic treatment of rats with this propranolol dose decreases blood pressure (Asahi et al., 2001) and preserves cardiac function in a rat model of ischemia-induced cardiomyopathy (Warner et al., 1992). More recently, work in our lab demonstrated that chronic treatment with this dose of propranolol also protects the heart from ischemic injury in a transgenic mouse model that exhibits increased sympathetic tone (Rorabaugh et al., 2017a).

2.5 Elevated Plus Maze (EPM)

Anxiety-related behavior was measured on day 32 on the EPM as previously described (Rorabaugh et al., 2015; Zoladz et al., 2008). Rats were placed on the maze for 5 min, and their behavior was recorded by a video camera located above the maze. Behavior on the maze was evaluated offline by two separate investigators who were blind to the experimental conditions of the animals. Time spent in the open arms was used to assess anxiety. The number of closed arm entries was also measured to assess potential differences in overall locomotor activity.

2.6 Langendorff Isolated Heart Preparation and Measurement of Infarct Size

Rats were anesthetized with sodium pentobarbital (100 mg/kg) on day 33. Hearts were quickly removed and mounted on a Langendorff isolated heart apparatus. Contractile function of the left ventricle was measured as previously described (Rorabaugh et al., 2015). Hearts were equilibrated for 25 minutes prior to 20 minutes of global ischemia and 2 hours of reperfusion as previously described (Rorabaugh et al., 2017a; Rorabaugh et al., 2015; Rorabaugh et al., 2016; Rorabaugh et al., 2017b). Preischemic contractile function was

assessed immediately prior to the onset of ischemia, and postischemic recovery of contractile function was assessed following 1 hour of reperfusion. Hearts were perfused for an additional hour (2 hours total reperfusion) prior to staining with 1% triphenyltetrazolium chloride as previously described (Rorabaugh et al., 2015). Infarct size in stained hearts was measured using NIH Image J software as previously described (Rorabaugh et al., 2017a; Rorabaugh et al., 2015).

2.7 Statistical Analysis

Data are reported as the mean $\pm$ S.E.M. Infarct size, contractile function, and behavior on the EPM were analyzed by two-way analysis of variance (ANOVA) with stress (no stress vs. stress) and drug treatment (saline vs. clonidine or water vs. propranolol) as factors. Tukey's post hoc analysis was used to identify significant differences between specific groups. All analyses were performed using GraphPad Prism software (GraphPad Inc., San Diego, CA), and *P* values ≤ 0.05 were considered statistically significant.

3 Results

3.1 Clonidine inhibits anxiety-related behavior on the EPM

Previous work demonstrated that clonidine blocks anxiety-related behavior on the EPM and several other behavioral and physiological changes induced by this animal model of PTSD (Zoladz et al., 2013). The EPM was employed in the current study to verify both the efficacy of the stress regimen and the efficacy of clonidine prior to assessment of their myocardial effects. Two-way ANOVA indicated a significant interaction between stress and clonidine [$F = 6.9$ (1, 58), $p < 0.01$] as well as a significant overall effect of clonidine [$F = 10.1$ (1, 58), $p < 0.005$]. Consistent with previous work (Rorabaugh et al., 2015; Zoladz et al., 2008; Zoladz et al., 2013), salinetreated stressed rats spent significantly less time in the open arms of the EPM than salinetreated nonstressed rats (Fig. 1A). This effect was blocked by clonidine. Expression of the data as a ratio of the time spent in the open arms relative to total time [time in open arms / (time in open arms + time in closed arms)] resulted in the same outcome. Stress had no effect on locomotor activity assessed by the number of closed arm entries (Fig. 1B). Clonidine significantly decreased locomotor activity in both stressed and nonstressed rats [$F = 20.8$ (1, 57), $p < 0.0001$] (Fig. 1B). However, this effect was anticipated because of the sedative effect of clonidine. These data confirm the efficacy of both the stress regimen and clonidine treatment in rats that were subsequently subjected to myocardial ischemia.

3.2 Clonidine does not prevent stress-induced myocardial hypersensitivity to ischemic injury

Hearts isolated from stressed and nonstressed rats were subjected to 20 minutes ischemia and 2 hours reperfusion on a Langendorff isolated heart apparatus. Infarcts were significantly larger in hearts from stressed rats compared to hearts from nonstressed rats [$F = 12.5$ (1,28), $p = 0.001$] (Fig. 2). However, clonidine had no significant effect on infarct size (Fig. 2). Clonidine also had no impact on parameters of either preischemic or postischemic recovery of contractile function (Table 1).

3.3 Propranolol does not reverse anxiety-related behavior on the EPM

The impact of propranolol treatment on behavior on the EPM was assessed to determine whether anxiety-related behavior could be reversed by blockade of β -adrenergic receptors. Two-way ANOVA indicated a significant overall effect of stress [$F = (11.1 (1,75), p = 0.01)$] on time spent in the open arms of the maze. However, this stress-induced effect was not blocked by propranolol (Fig. 3A). Propranolol also had no effect on locomotor activity in the maze (Fig. 3B).

3.4 Propranolol does not prevent stress-induced myocardial hypersensitivity to ischemic injury

In light of evidence for increased sympathetic tone in rats subjected to the predator-based PTSD model (Zoladz et al., 2008), we examined the impact of chronic propranolol treatment on stress-induced myocardial hypersensitivity to ischemia. We found that propranolol treatment throughout the stress regimen did not block the increase in infarct size in stressed hearts (Fig. 4). Two-way ANOVA indicated a significant overall effect of stress [$F = 4.8 (1, 450, p < 0.05)$] on infarct size. However, there was no significant effect of propranolol and no interaction between propranolol and stress. Propranolol also had no impact on parameters of either preischemic or postischemic recovery of contractile function (Table 2).

4 Discussion

Excessive activity of the sympathetic nervous system is a hallmark characteristic of PTSD (Geraciotti et al., 2001; Kosten et al., 1987; Mellman et al., 1995; Yehuda et al., 1992). Thus, it is not surprising that PTSD is associated with an increased risk for cardiovascular abnormalities, including increased heart rate and blood pressure, increased risk of cardiac arrhythmias, and increased risk of myocardial infarction (Ahmadi et al., 2011; Beristianos et al., 2016; Khazaie et al., 2013; Vaccarino et al., 2013). We previously reported that exposure to the psychosocial stress model of PTSD causes rats to develop myocardial hypersensitivity to ischemic injury (Rorabaugh et al., 2015). The current study extends these findings by demonstrating that this adverse cardiac effect is not prevented by suppressing sympathetic tone or by blocking β -adrenergic receptors.

Clonidine, an α_2 -adrenergic receptor agonist, acts on presynaptic receptors in the central nervous system to decrease sympathetic outflow. In previous work, chronic administration of clonidine blocked many of the behavioral and physiological phenotypes (anxiety-related behavior, exaggerated startle response, tachycardia, and increased blood pressure) induced by our psychosocial stress model of PTSD (Zoladz et al., 2013). In the present study, we replicated the ability of clonidine to block the development of anxiety-like behaviors in this model. However, clonidine did not inhibit the development of myocardial hypersensitivity to ischemic injury. Other preclinical work has shown that a single administration of clonidine prevented stress-induced changes in behavior when it was administered immediately following stress exposure (Shinba et al., 2001) or normalized such changes when it was administered prior to behavioral testing (Olson et al., 2011). Clonidine has also been shown to effectively block the reconsolidation of an auditory fear memory (Gamache et al., 2012), implicating its potential utility in ameliorating the strength of traumatic memories in people

with PTSD. Nevertheless, clinical studies examining the impact of clonidine in PTSD patients have been limited, and we are unaware of any large scale placebo-controlled trials involving clonidine and PTSD patients. Of the studies that have been conducted, there is some evidence that clonidine suppresses nightmares and symptoms of hyperarousal in people with PTSD (Alao et al., 2012; Belkin and Schwartz, 2015; Boehnlein and Kinzie, 2007; Kinzie and Leung, 1989), but additional work is necessary to more fully evaluate clonidine's therapeutic efficacy in PTSD patients.

Most of the research examining the effects of propranolol on trauma-induced changes in brain and behavior has focused on its ability to mitigate traumatic memories, with the hope that if the traumatic memory is weakened, then fewer PTSD-like symptoms will ensue. For instance, preclinical studies have demonstrated that a single dose of propranolol administered immediately following an intense stressor (exposure to a predator / predator scent, electric shock) blocks subsequent expression of anxiety-related behavior (Adamec et al., 2007; Aykac et al., 2012; Burhans et al., 2018), possibly by impairing the consolidation of the stressful experience. Other work has shown that a single dose of propranolol administered after the reactivation of a fear memory impairs the reconsolidation of that memory (Debiec and Ledoux, 2004; Taherian et al., 2014). Such findings have not been unequivocal, though, as others have reported that propranolol treatment immediately following stress (exposure to predator scent) does not suppress the development of anxiety-related behavior (Cohen et al., 2011). There has also been evidence that propranolol does not effectively block the reconsolidation of fear memories (Muravieva and Alberini, 2010). The present study differs from previous preclinical work in that propranolol was administered via the drinking water starting 24 hours after the first cat exposure and continuing throughout the remainder of the 31-day psychosocial stress regimen. The inability of propranolol to block anxiety-related behavior in our study may have resulted from the fact that propranolol was not present immediately after the initial predator exposure when memory consolidation occurs (although it was present at the time of the second cat exposure, which may have initiated reconsolidation processes). Alternatively, it is possible that the use of a second predator exposure or the prolonged duration (31 days) of the psychosocial stress paradigm evokes anxiogenic signaling pathways that are independent of β -adrenergic receptors.

Clinical research examining the ability of propranolol to prevent or alleviate symptoms of PTSD has targeted the drug's ability to disrupt consolidation/reconsolidation of the traumatic memory. Some studies have reported that propranolol administration in the acute aftermath of trauma reduces the development of PTSD symptoms (Pitman et al., 2002; Vaiva et al., 2003). There is also work demonstrating that propranolol administration following the reactivation of a traumatic memory can ameliorate PTSD symptoms, possibly by weakening the intensity of the traumatic memory (Brunet et al., 2008; Brunet et al., 2014). However, research in this area has been highly inconsistent, as other investigators have reported minimal or no effects of propranolol on PTSD symptomology when administered shortly after the traumatic event or following reactivation of the traumatic memory (Hoge et al., 2012; Wood et al., 2015). Research suggests that, if anything, acute propranolol administration in the aftermath of trauma or following the reactivation of a traumatic memory might reduce subsequent physiological responses to trauma-related stimuli (Argolo

et al., 2015). However, this treatment approach may not effectively reduce the overall symptoms of PTSD.

Previous studies investigating the impact of β -adrenergic receptor blockade in the context of PTSD have almost exclusively focused on propranolol. One study reported that metoprolol reduced the likelihood of developing PTSD-like symptoms in women following cardiac surgery (Krauseneck et al., 2010). However, there is very little information available regarding the efficacy of other β blockers in human patients or in laboratory models of PTSD.

Chronic β_2 -adrenergic receptor signaling worsens myocardial ischemic injury (Chen et al., 2013; Hu et al., 2006; Hu et al., 2008). In light of evidence for increased sympathetic tone in rats subjected to the predator-based PTSD model (Zoladz et al., 2008), we anticipated that suppressing sympathetic tone with clonidine or blocking β -adrenergic receptors would prevent the development of myocardial hypersensitivity to ischemia. However, our data suggest that the hypersensitivity to ischemia observed in this stress model is not the result of increased sympathetic tone or chronic β_2 -adrenergic receptor signaling.

In summary, increased sympathetic tone is a hallmark of PTSD. Clonidine and propranolol have been reported to relieve some symptoms of PTSD in humans (Boehnlein and Kinzie, 2007; Detweiler et al., 2016; Donnelly, 2003). However, our data suggest that these drugs do not inhibit the detrimental impact of this form of stress on the ischemic heart. Further work is needed to identify the mechanism by which predator-induced stress potentiates myocardial ischemic injury. Elucidation of the mechanism may lead to the development of new therapeutic strategies to protect people with PTSD from ischemic heart disease.

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References

- Adamec R, Muir C, Grimes M, Pearcey K, 2007 Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. *Behav Brain Res* 179(2), 192–207. [PubMed: 17335916]
- Ahmadi N, Hajsadeghi F, Mirshkarlo HB, Budoff M, Yehuda R, Ebrahimi R, 2011 Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *The American journal of cardiology* 108(1), 29–33. [PubMed: 21530936]
- Alao A, Selvarajah J, Razi S, 2012 The use of clonidine in the treatment of nightmares among patients with co-morbid PTSD and traumatic brain injury. *International journal of psychiatry in medicine* 44(2), 165–169. [PubMed: 23413663]
- Argolo FC, Cavalcanti-Ribeiro P, Netto LR, Quarantini LC, 2015 Prevention of posttraumatic stress disorder with propranolol: A meta-analytic review. *Journal of psychosomatic research* 79(2), 89–93. [PubMed: 25972056]
- Asahi T, Shimabukuro M, Oshiro Y, Yoshida H, Takasu N, 2001 Cilazapril prevents cardiac hypertrophy and postischemic myocardial dysfunction in hyperthyroid rats. *Thyroid : official journal of the American Thyroid Association* 11(11), 1009–1015. [PubMed: 11762709]
- Aykac A, Aydin B, Cabadak H, Goren MZ, 2012 The change in muscarinic receptor subtypes in different brain regions of rats treated with fluoxetine or propranolol in a model of post-traumatic stress disorder. *Behav Brain Res* 232(1), 124–129. [PubMed: 22522023]

- Belkin MR, Schwartz TL, 2015 Alpha-2 receptor agonists for the treatment of posttraumatic stress disorder. *Drugs in context* 4, 212286. [PubMed: 26322115]
- Beristianos MH, Yaffe K, Cohen B, Byers AL, 2016 PTSD and Risk of Incident Cardiovascular Disease in Aging Veterans. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry* 24(3), 192–200. [PubMed: 25555625]
- Boehnlein JK, Kinzie JD, 2007 Pharmacologic reduction of CNS noradrenergic activity in PTSD: the case for clonidine and prazosin. *Journal of psychiatric practice* 13(2), 72–78. [PubMed: 17414682]
- Brunet A, Orr SP, Tremblay J, Robertson K, Nader K, Pitman RK, 2008 Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of psychiatric research* 42(6), 503–506. [PubMed: 17588604]
- Brunet A, Thomas E, Saumier D, Ashbaugh AR, Azzoug A, Pitman RK, Orr SP, Tremblay J, 2014 Trauma reactivation plus propranolol is associated with durably low physiological responding during subsequent script-driven traumatic imagery. *Canadian journal of psychiatry* 59(4), 228–232. [PubMed: 25007116]
- Burhans LB, Smith-Bell CA, Schreurs BG, 2018 Propranolol produces short-term facilitation of extinction in a rabbit model of post-traumatic stress disorder. *Neuropharmacology* 135, 386–398. [PubMed: 29578033]
- Chen M, Sato PY, Chuprun JK, Peroutka RJ, Otis NJ, Ibetti J, Pan S, Sheu SS, Gao E, Koch WJ, 2013 Prodeath signaling of G protein-coupled receptor kinase 2 in cardiac myocytes after ischemic stress occurs via extracellular signal-regulated kinase-dependent heat shock protein 90-mediated mitochondrial targeting. *Circulation research* 112(8), 1121–1134. [PubMed: 23467820]
- Cohen H, Kaplan Z, Koresh O, Matar MA, Geva AB, Zohar J, 2011 Early poststressor intervention with propranolol is ineffective in preventing posttraumatic stress responses in an animal model for PTSD. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* 21(3), 230–240. [PubMed: 21194896]
- Debiec J, Ledoux JE, 2004 Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience* 129(2), 267–272. [PubMed: 15501585]
- Dedert EA, Calhoun PS, Watkins LL, Sherwood A, Beckham JC, 2010 Posttraumatic stress disorder, cardiovascular, and metabolic disease: a review of the evidence. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine* 39(1), 61–78. [PubMed: 20174903]
- Deslauriers J, Toth M, Der-Avakian A, Risbrough VB, 2018 Current Status of Animal Models of Posttraumatic Stress Disorder: Behavioral and Biological Phenotypes, and Future Challenges in Improving Translation. *Biological psychiatry* 83(10), 895–907. [PubMed: 29338843]
- Detweiler MB, Pagadala B, Candelario J, Boyle JS, Detweiler JG, Lutgens BW, 2016 Treatment of Post-Traumatic Stress Disorder Nightmares at a Veterans Affairs Medical Center. *Journal of clinical medicine* 5(12).
- Donnelly CL, 2003 Pharmacologic treatment approaches for children and adolescents with posttraumatic stress disorder. *Child and adolescent psychiatric clinics of North America* 12(2), 251–269. [PubMed: 12725011]
- Ehlers A, Hackmann A, Michael T, 2004 Intrusive re-experiencing in post-traumatic stress disorder: phenomenology, theory, and therapy. *Memory (Hove, England)* 12(4), 403–415.
- Ehlers A, Hackmann A, Steil R, Clohessy S, Wenninger K, Winter H, 2002 The nature of intrusive memories after trauma: the warning signal hypothesis. *Behaviour research and therapy* 40(9), 995–1002. [PubMed: 12296496]
- Gamache K, Pitman RK, Nader K, 2012 Preclinical evaluation of reconsolidation blockade by clonidine as a potential novel treatment for posttraumatic stress disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 37(13), 2789–2796. [PubMed: 22871915]
- Geraciotti TD, Jr., Baker DG, Ekhtor NN, West SA, Hill KK, Bruce AB, Schmidt D, Rounds-Kugler B, Yehuda R, Keck PE, Jr., Kasckow JW, 2001 CSF norepinephrine concentrations in posttraumatic stress disorder. *The American journal of psychiatry* 158(8), 1227–1230. [PubMed: 11481155]

- Hoge EA, Worthington JJ, Nagurney JT, Chang Y, Kay EB, Feterowski CM, Katzman AR, Goetz JM, Rosasco ML, Lasko NB, Zusman RM, Pollack MH, Orr SP, Pitman RK, 2012 Effect of acute posttrauma propranolol on PTSD outcome and physiological responses during script-driven imagery. *CNS neuroscience & therapeutics* 18(1), 21–27. [PubMed: 22070357]
- Hu A, Jiao X, Gao E, Koch WJ, Sharifi-Azad S, Grunwald Z, Ma XL, Sun JZ, 2006 Chronic beta-adrenergic receptor stimulation induces cardiac apoptosis and aggravates myocardial ischemia/reperfusion injury by provoking inducible nitric-oxide synthase-mediated nitrative stress. *J Pharmacol Exp Ther* 318(2), 469–475. [PubMed: 16574780]
- Hu A, Jiao X, Gao E, Li Y, Sharifi-Azad S, Grunwald Z, Ma XL, Sun JZ, 2008 Tonic beta-adrenergic drive provokes proinflammatory and proapoptotic changes in aging mouse heart. *Rejuvenation Res* 11(1), 215–226. [PubMed: 18257661]
- Khazaie H, Saidi MR, Sepehry AA, Knight DC, Ahmadi M, Najafi F, Parvizi AA, Samadzadeh S, Tahmasian M, 2013 Abnormal ECG patterns in chronic postwar PTSD patients: a pilot study. *International journal of behavioral medicine* 20(1), 1–6. [PubMed: 21960258]
- Kinzie JD, Leung P, 1989 Clonidine in Cambodian patients with posttraumatic stress disorder. *The Journal of nervous and mental disease* 177(9), 546–550. [PubMed: 2769247]
- Kosten TR, Mason JW, Giller EL, Ostroff RB, Harkness L, 1987 Sustained urinary norepinephrine and epinephrine elevation in post-traumatic stress disorder. *Psychoneuroendocrinology* 12(1), 13–20. [PubMed: 3588809]
- Krauseneck T, Padberg F, Roozendaal B, Grathwohl M, Weis F, Hauer D, Kaufmann I, Schmoeckel M, Schelling G, 2010 A beta-adrenergic antagonist reduces traumatic memories and PTSD symptoms in female but not in male patients after cardiac surgery. *Psychological medicine* 40(5), 861–869. [PubMed: 19691871]
- Mellman TA, Kumar A, Kulick-Bell R, Kumar M, Nolan B, 1995 Nocturnal/daytime urine noradrenergic measures and sleep in combat-related PTSD. *Biological psychiatry* 38(3), 174–179. [PubMed: 7578660]
- Muravieva EV, Alberini CM, 2010 Limited efficacy of propranolol on the reconsolidation of fear memories. *Learning & memory* (Cold Spring Harbor, N.Y.) 17(6), 306–313.
- Nemeroff CB, Bremner JD, Foa EB, Mayberg HS, North CS, Stein MB, 2006 Posttraumatic stress disorder: a state-of-the-science review. *Journal of psychiatric research* 40(1), 1–21. [PubMed: 16242154]
- Olson VG, Rockett HR, Reh RK, Redila VA, Tran PM, Venkov HA, Defino MC, Hague C, Peskind ER, Szot P, Raskind MA, 2011 The role of norepinephrine in differential response to stress in an animal model of posttraumatic stress disorder. *Biological psychiatry* 70(5), 441–448. [PubMed: 21251647]
- Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, Milad MR, Liberzon I, 2012 Biological studies of post-traumatic stress disorder. *Nature reviews. Neuroscience* 13(11), 769–787. [PubMed: 23047775]
- Pitman RK, Sanders KM, Zusman RM, Healy AR, Cheema F, Lasko NB, Cahill L, Orr SP, 2002 Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biological psychiatry* 51(2), 189–192. [PubMed: 11822998]
- Reynolds M, Brewin CR, 1999 Intrusive memories in depression and posttraumatic stress disorder. *Behaviour research and therapy* 37(3), 201–215. [PubMed: 10087639]
- Rorabaugh BR, Chakravarti B, Mabe NW, Seeley SL, Bui AD, Yang J, Watts SW, Neubig RR, Fisher RA, 2017a Regulator of G Protein Signaling 6 Protects the Heart from Ischemic Injury. *J Pharmacol Exp Ther* 360(3), 409–416. [PubMed: 28035008]
- Rorabaugh BR, Krivenko A, Eisenmann ED, Bui AD, Seeley S, Fry ME, Lawson JD, Stoner LE, Johnson BL, Zoladz PR, 2015 Sex-dependent effects of chronic psychosocial stress on myocardial sensitivity to ischemic injury. *Stress (Amsterdam, Netherlands)* 18(6), 645–653.
- Rorabaugh BR, Seeley SL, Bui AD, Sprague L, D'Souza MS, 2016 Prenatal methamphetamine differentially alters myocardial sensitivity to ischemic injury in male and female adult hearts. *Am J Physiol Heart Circ Physiol* 310(4), H516–523. [PubMed: 26683901]

- Rorabaugh BR, Seeley SL, Stoops TS, D'Souza MS, 2017b Repeated exposure to methamphetamine induces sex-dependent hypersensitivity to ischemic injury in the adult rat heart. *PloS one* 12(6), e0179129. [PubMed: 28575091]
- Roth TL, Zoladz PR, Sweatt JD, Diamond DM, 2011 Epigenetic modification of hippocampal *Bdnf* DNA in adult rats in an animal model of post-traumatic stress disorder. *Journal of psychiatric research* 45(7), 919–926. [PubMed: 21306736]
- Schoner J, Heinz A, Endres M, Gertz K, Kronenberg G, 2017 Post-traumatic stress disorder and beyond: an overview of rodent stress models. *Journal of cellular and molecular medicine* 21(10), 2248–2256. [PubMed: 28374949]
- Shinba T, Shinozaki T, Mugishima G, 2001 Clonidine immediately after immobilization stress prevents long-lasting locomotion reduction in the rat. *Progress in neuro-psychopharmacology & biological psychiatry* 25(8), 1629–1640. [PubMed: 11642659]
- Taherian F, Vafaei AA, Vaezi GH, Eskandarian S, Kashef A, Rashidy-Pour A, 2014 Propranolol-induced Impairment of Contextual Fear Memory Reconsolidation in Rats: A similar Effect on Weak and Strong Recent and Remote Memories. *Basic and clinical neuroscience* 5(3), 231–239. [PubMed: 25337385]
- Vaccarino V, Goldberg J, Rooks C, Shah AJ, Veledar E, Faber TL, Votaw JR, Forsberg CW, Bremner JD, 2013 Post-traumatic stress disorder and incidence of coronary heart disease: a twin study. *J Am Coll Cardiol* 62(11), 970–978. [PubMed: 23810885]
- Vaiva G, Ducrocq F, Jezequel K, Averland B, Lestavel P, Brunet A, Marmar CR, 2003 Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biological psychiatry* 54(9), 947–949. [PubMed: 14573324]
- Warner AL, Bellah KL, Raya TE, Roeske WR, Goldman S, 1992 Effects of beta-adrenergic blockade on papillary muscle function and the beta-adrenergic receptor system in noninfarcted myocardium in compensated ischemic left ventricular dysfunction. *Circulation* 86(5), 1584–1595. [PubMed: 1330362]
- Wilson CB, Ebenezer PJ, McLaughlin LD, Francis J, 2014a Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex. *PloS one* 9(2), e89104. [PubMed: 24551226]
- Wilson CB, McLaughlin LD, Ebenezer PJ, Nair AR, Dange R, Harre JG, Shaak TL, Diamond DM, Francis J, 2014b Differential effects of sertraline in a predator exposure animal model of post-traumatic stress disorder. *Frontiers in behavioral neuroscience* 8, 256. [PubMed: 25126063]
- Wilson CB, McLaughlin LD, Ebenezer PJ, Nair AR, Francis J, 2014c Valproic acid effects in the hippocampus and prefrontal cortex in an animal model of posttraumatic stress disorder. *Behav Brain Res* 268, 72–80. [PubMed: 24675160]
- Wilson CB, McLaughlin LD, Nair A, Ebenezer PJ, Dange R, Francis J, 2013 Inflammation and oxidative stress are elevated in the brain, blood, and adrenal glands during the progression of post-traumatic stress disorder in a predator exposure animal model. *PloS one* 8(10), e76146. [PubMed: 24130763]
- Wood NE, Rosasco ML, Suris AM, Spring JD, Marin MF, Lasko NB, Goetz JM, Fischer AM, Orr SP, Pitman RK, 2015 Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: three negative psychophysiological studies. *Psychiatry research* 225(1–2), 31–39. [PubMed: 25441015]
- Yehuda R, Southwick S, Giller EL, Ma X, Mason JW, 1992 Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *The Journal of nervous and mental disease* 180(5), 321–325. [PubMed: 1583475]
- Zen AL, Whooley MA, Zhao S, Cohen BE, 2012 Post-traumatic stress disorder is associated with poor health behaviors: findings from the heart and soul study. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association* 31(2), 194–201.
- Zoladz PR, Conrad CD, Fleshner M, Diamond DM, 2008 Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. *Stress (Amsterdam, Netherlands)* 11(4), 259–281.

- Zoladz PR, Diamond DM, 2013 Current status on behavioral and biological markers of PTSD: a search for clarity in a conflicting literature. *Neuroscience and biobehavioral reviews* 37(5), 860–895. [PubMed: 23567521]
- Zoladz PR, Diamond DM, 2016 Predator-based psychosocial stress animal model of PTSD: Preclinical assessment of traumatic stress at cognitive, hormonal, pharmacological, cardiovascular and epigenetic levels of analysis. *Experimental neurology* 284(Pt B), 211–219. [PubMed: 27283115]
- Zoladz PR, Fleshner M, Diamond DM, 2012 Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. *Psychoneuroendocrinology* 37(9), 1531–1545. [PubMed: 22421563]
- Zoladz PR, Fleshner M, Diamond DM, 2013 Differential effectiveness of tianeptine, clonidine and amitriptyline in blocking traumatic memory expression, anxiety and hypertension in an animal model of PTSD. *Prog Neuropsychopharmacol Biol Psychiatry* 44, 1–16. [PubMed: 23318688]
- Zoladz PR, Park CR, Fleshner M, Diamond DM, 2015 Psychosocial predatorbased animal model of PTSD produces physiological and behavioral sequelae and a traumatic memory four months following stress onset. *Physiology & behavior* 147, 183–192. [PubMed: 25911267]

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Highlights

- The psychosocial stress model of PTSD causes myocardial hypersensitivity to ischemic injury.
- Clonidine blocks anxiety-like behavior but not myocardial hypersensitivity to ischemia.
- Propranolol does not block anxiety or hypersensitivity to ischemia in this animal model of PTSD.

Fig. 1.

Clonidine reverses anxiety-related behavior on the EPM. Stressed rats spent significantly [$F = 6.9 (1, 58), p < 0.01$] less time than nonstressed rats in the open arms of the EPM (A). Clonidine reversed this effect in stressed animals but had no effect on time in the open arms in nonstressed animals. Clonidine significantly decreased overall locomotor activity (number of closed arm entries) in both stressed and nonstressed rats (B). Data represent the mean $\pm$ S.E.M. The number of animals used in each group is indicated in each bar. ^a indicates a significant difference ($p < 0.05$) compared to vehicle-treated nonstressed animals. ^b indicates a significant difference ($p < 0.005$) compared to vehicle treated stressed animals. ^c indicates a significant difference ($p < 0.05$) compared to vehicle-treated animals.

Fig. 2.

Clonidine does not reverse stress-induced increase in myocardial infarct size. Hearts from stressed rats had significantly larger infarcts [$F = 12.5$ (1,28), $p = 0.001$] compared to hearts from nonstressed rats following a 20-minutes ischemic insult (A). Clonidine had no significant effect on infarct size in hearts from either stressed or nonstressed rats. Data represent the mean $\pm$ S.E.M, and the number of animals used in each group is indicated in each bar. Representative images of infarcts are shown in panel B. ^a indicates a significant effect of stress ($p = 0.01$).

Fig. 3.

Propranolol has no effect on anxiety-related behavior on the EPM. Stressed rats spent significantly [$F = (11.1 (1, 75), p = 0.01)$] less time than nonstressed rats in the open arms of the EPM (A). Propranolol had no effect on time spent in the open arms in either stressed or nonstressed rats. Propranolol had no effect on locomotor activity (number of closed arm entries) in either stressed or nonstressed rats (B). Data represent the mean $\pm$ S.E.M. The number of animals used in each group is indicated in each bar. ^a indicates a significant effect of stress ($p = 0.01$).

Fig. 4.

Propranolol does not reverse stress-induced increase in myocardial infarct size. Hearts from stressed rats had significantly larger infarcts [$F = 4.8$ (1, 450, $p < 0.05$)] compared to hearts from nonstressed rats following a 20 minute ischemic insult (A). Propranolol had no effect on infarct size in hearts from either stressed or nonstressed rats. Data represent the mean $\pm$ S.E.M, and the number of animals used in each group is indicated in each bar. Representative images of infarcts are shown in panel B. ^a indicates a significant effect of stress ($p < 0.05$).

Table 1.
Preischemic contractile function and postischemic recovery of contractile function in hearts from clonidine- and saline-treated rats.

	Preischemic						Postischemic Recovery					
	Devel oped Press ure (mm Hg)	He art Rat e (be ats / mi n)	End Dias tolic Pres sure (mm Hg)	+dP/d T (mmH g/sec)	-dP/dT (mmH g/sec)	Coron ary flow rate (ml/ min/g)	Devel oped Press ure (mm Hg)	He art Rat e (be ats / mi n)	End Dias tolic Pres sure (mm Hg)	+dP/ dT mmH g/sec	-dP/dT (mmH g/sec)	Coron ary flow rate (ml/ min/g)
Saline; Nonstr essed	147 ± 8	28 2 ± 8	4 ± 0.4	4465 ± 257	-3667 ± 227	16.2 ± 0.8	102 ± 11	28 0 ± 6	23 ± 5	3242 ± 361	-2295 ± 244	18.7 ± 1.3
Saline; Stress ed	168 ± 5	27 5 ± 9	2 ± 0.6	4491 ± 172	-4036 ± 156	14.1 ± 0.6	88 ± 13	26 5 ± 11	30 ± 9	2656 ± 401	-1969 ± 288	18.2 ± 1.5
Clonidi ne; Nonstr essed	138 ± 10	27 5 ± 9	3 ± 0.6	4221 ± 234	-3447 ± 249	14.7 ± 1.0	73 ± 16	27 8 ± 8	26 ± 6	2592 ± 393	-1947 ± 292	17.6 ± 1.2
Clonidi ne; Stress ed	137 ± 9 ^a	26 9 ± 7	4 ± 0.5	4290 ± 342	-3358 ± 266	14.1 ± 0.8	57 ± 14	27 4 ± 15	35 ± 8	2054 ± 316	-1508 ± 269	17.0 ± 0.5

Data represent the mean ± S.E.M of 7–9 hearts. Two-way ANOVA indicated a significant overall effect of clonidine [$F = 6.0$ (1, 30), $p < 0.05$] on preischemic developed pressure. There were no other significant effects of stress or clonidine on preischemic parameters of cardiac function. There were also no overall effects of stress or clonidine on parameters of postischemic recovery of contractile function following 1 hour reperfusion.

^a indicates a significant difference ($p < 0.05$) compared to preischemic developed pressure in hearts from saline-treated stressed rats.

Table 2.
Preischemic contractile function and postischemic recovery of contractile function in hearts from propranolol- and saline-treated rats.

	Preischemic						Postischemic Recovery					
	Devel oped Press ure (mmH g)	He art Rat e (be ats / mi n)	End Dias tolic Pres sure (mm Hg)	+dP/d T (mmH g/sec)	-dP/dT (mmH g/sec)	Coron ary flow rate (ml/m in/g)	Devel oped Press ure (mmH g)	He art Rat e (be ats / mi n)	End Dias tolic Pres sure (mm Hg)	+dP/d T mmH g/sec	-dP/dT (mmH g/sec)	Coron ary flow rate (ml/m in/g)
Vehicle ; Nonstr essed	136 ± 3	276 ± 5	3.8 ± 0.2	4455 ± 289	-3441 ± 152	16.9 ± 0.8	51 ± 9	232 ± 26	35 ± 4	1505 ± 302	-1056 ± 208	13.6 ± 0.4
Vehicle ; Stresse d	144 ± 8	282 ± 10	3.6 ± 0.2	4593 ± 314	-3366 ± 198	16.7 ± 1.0	43 ± 11	282 ± 10	38 ± 6	1297 ± 325	-896 ± 219	13.2 ± 0.9
Propra nolol; Nonstr essed	153 ± 7	292 ± 5	4.0 ± 0.4	4190 ± 247	-3299 ± 152	15.1± 0.7	70 ± 10	249 ± 22	30 ± 6	1802 ± 275	-1352 ± 200	12.7 ± 0.7
Propra nolol; Stresse d	150 ± 8	290 ± 4	4.0 ± 0.4	4096 ± 232	-3211 ± 149	17 ± 0.7	73 ± 7	279 ± 5	25 ± 5	1849 ± 199	-1440 ± 144	14.4 ± 0.6

Data represent the mean ± S.E.M of 11–14 hearts. Two-way ANOVA indicated no significant overall effects of propranolol or stress on parameters of preischemic contractile function or parameters of postischemic recovery of contractile function.