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Neurosteroids and Self-Reported Pain in Veterans Who Served in the U.S. Military After September 11, 2001

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Abstract

Objective—Nearly half of Operation Enduring Freedom / Operation Iraqi Freedom (OEF/OIF) veterans experience continued pain post-deployment. Several investigations report analgesic effects of allopregnanolone and other neurosteroids in animal models, but few data are currently available focusing on neurosteroids in clinical populations. Allopregnanolone positively modulates $GABA_A$ receptors and demonstrates pronounced analgesic and anxiolytic effects in rodents, yet studies examining the relationship between pain and allopregnanolone in humans are limited. We thus hypothesized that endogenous allopregnanolone and other neurosteroid levels may be negatively correlated with self-reported pain symptoms in humans.

Design—We determined serum neurosteroid levels by gas chromatography / mass spectrometry (allopregnanolone, pregnenolone) or radioimmunoassay (dehydroepiandrosterone [DHEA], progesterone, DHEA sulfate [DHEAS]) in 90 male veterans who served in the U.S. military after September 11, 2001. Self-reported pain symptoms were assessed in four areas (low back pain, chest pain, muscle soreness, headache). Stepwise linear regression analyses were conducted to investigate the relationship between pain assessments and neurosteroids, with the inclusion of smoking, alcohol use, age, and history of traumatic brain injury as covariates.

Setting—Durham VA Medical Center.

Results—Allopregnanolone levels were inversely associated with low back pain (p=0.044) and chest pain (p=0.013), and DHEA levels were inversely associated with muscle soreness (p=0.024). DHEAS levels were positively associated with chest pain (p=0.001). Additionally, there was a positive association between traumatic brain injury and muscle soreness (p=0.002).

Conclusions—Neurosteroids may be relevant to the pathophysiology of self-reported pain symptoms in this veteran cohort, and could represent future pharmacological targets for pain disorders.

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Keywords

neuroactive steroid; allopregnanolone; pregnenolone; DHEA; nociception; pain; neurosteroid

1. Introduction

More than 1.64 million military personnel have served during Operation Enduring Freedom / Operation Iraqi Freedom (OEF/OIF) to date (1). OEF/OIF deployments can involve high levels of combat stress, and many soldiers serve multiple tours of duty (2–4,1); greater than one fourth of these returning veterans receive mental health or psychosocial diagnoses (5,1). An even greater proportion (nearly half) of these returning veterans report persistent pain (6), and chronic widespread pain is a frequent occurrence in otherwise healthy OEF/OIF veterans (7). As a higher percentage of wounded military personnel fortunately now survive than ever before (2,3), reduction and alleviation of pain in this cohort is an acute need. The amelioration of persistent pain symptoms via supplementation or modulation of endogenous compounds with analgesic actions may represent a promising treatment strategy, and neurosteroids represent potential candidates for this indication.

The neurosteroid allopregnanolone, a metabolite of progesterone, positively modulates inhibitory γ-aminobutyric acid type A ($GABA_A$) receptors (8,9). Other neurosteroids, including the sulfated metabolites of pregnenolone and dehydroepiandrosterone (DHEA), exhibit the opposite profile of $GABA_A$ receptor action (10–14). Extensive data demonstrate that various neurosteroids with positive $GABA_A$ receptor-modulating activities exert anxiolytic (15–18), anticonvulsant (19–23), and anti-aggression effects (24,25), but additional evidence indicates roles for neurosteroids in analgesia as well (26–31). Given that $GABA_A$ receptors are integral to nociceptive processing (32–34), neurosteroids may play a significant role in the pathophysiology of pain.

The predominance of studies demonstrating analgesic neurosteroid effects reports the importance of allopregnanolone in preclinical pain models. Allopregnanolone increases response latencies to thermal stimuli in both rats (26) and invertebrates (30), increases response latencies to tailflick in rats (28), and protects against noxious mechanical visceral stimuli in rats (27). Allopregnanolone also may be responsible for the analgesic effects of its precursor progesterone, as multiple reports indicate that allopregnanolone is the active metabolite responsible for the anesthetic actions of progesterone (35,29). It is presently unclear whether other neurosteroids modulate analgesic responses in rodents, as examination of dehydroepiandrosterone sulfate (DHEAS) reveals lack of analgesic effect (28), and studies examining pregnenolone sulfate differ, demonstrating both attenuation of capsaicin-induced nociception (36,37) and failure to increase tailflick response latency in rats (28).

Compared to the number of studies investigating neurosteroids and pain in animal models, few studies in humans have been performed. Additionally, study findings to date have not been in complete agreement. For example, while endogenous DHEAS levels are no different between men with chronic osteoarthritis pain and pain-free controls (38), endogenous DHEAS levels inversely correlate with musculoskeletal pain in women during menopause transition (39). Further, supplementation with DHEA does not improve pain in postmenopausal women with fibromyalgia (40), but it does improve joint pain in aging men with partial androgen deficiency (41). The single investigation examining the relationship between allopregnanolone and pain in humans suggests that elevated endogenous allopregnanolone levels are associated with decreased pain tolerance in non-Hispanic white individuals but not in African Americans (42). Thus, additional clinical studies are needed to clarify the role of neurosteroids in human nociception.

This study therefore examined possible neurosteroid associations with self-reported pain measures as assessed by four questions of the Symptom Checklist-90-R [SCL-90-R; (43)] instrument in males who served in the military after September 11, 2001. The neurosteroids measured were allopregnanolone, pregnenolone, progesterone, DHEA, and DHEAS, and the four SCL-90-R items evaluated low back pain, chest pain, muscle soreness, and headache. Based on the prior animal literature and multiple reports in humans that synthetic steroid anesthetics structurally similar to allopregnanolone produce analgesic effects (44–47), we hypothesized that endogenous serum allopregnanolone levels would be negatively associated with self-reported pain.

2. Methods

2.1 Study Population

All participants provided written informed consent, and the protocol was approved by the local institutional review board. The first 90 male subjects recruited into the VA Mid-Atlantic MIRECC Registry having a blood draw between 10:30 AM and 2:30 PM were included in this investigation. These 90 male subjects were enrolled between June 2005 and April 2006 at the Durham VA site. All participants in this Registry served in the U.S. military after September 11, 2001. Of the 82 veterans in this cohort with no missing data, 40 had served in the Army, 7 in the Navy, 12 in the Marines, 4 in the Air Force, 12 in the National Guard, 4 in the Reserves, 1 in both the Army and Marines, 1 in both the Air Force and the Marines, and 1 in both the National Guard and the Reserves. Of the 65 veterans who had served in the Army, Navy, Marines, or Air Force, approximately half (n=33) had also served in the National Guard and/or Reserves. At least 79 of the 82 veterans in this cohort served in a warzone (information not available for 3 veterans), with 36 of those 79 being combat veterans, 33 serving in combat support roles, and 10 providing warzone service support. Two veterans were receiving opioids (oxycodone/acetaminophen) for pain at the time of the evaluation, 5 were receiving non-steroidal anti-inflammatory agents, 1 was taking acetaminophen, and 1 was taking aspirin.

2.2 Self-reported Pain Measures and SCL-90-R

Subjects completed the SCL-90-R, which asked them to rate severity of symptoms over the past 7 days and included four items assessing pain: low back pain, chest pain, muscle soreness, and headache (43,48). Each pain condition was rated with five possible response options: none at all (level 0), a little (level 1), moderate pain (level 2), quite a bit (level 3), and extreme (level 4).

2.3 Neurosteroid Analyses

Pregnenolone and allopregnanolone levels in serum were determined by a highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) method in the negative ion chemical ionization mode, as previously described (49,50). One mL of serum was extracted three times in ethyl acetate prior to high-performance liquid chromatography (HPLC) purification utilizing tetrahydrofuran, ethanol, and hexane in the mobile phase. A subset of samples (30%) was injected in duplicate. The mean coefficients of variation for pregnenolone and allopregnanolone in this subset were 1.9% and 6.8%, respectively. The limit of detection with this method was 2 picograms for both pregnenolone and allopregnanolone. Other serum steroid levels were determined utilizing commercially available radioimmunoassay (RIA) kits according to manufacturer directions (progesterone and DHEAS: Diagnostic System Laboratories, Webster, TX). DHEA serum levels were determined utilizing a kit from ICN Pharmaceuticals (Costa Mesa, CA).

2.4 Stepwise Linear Regression Analysis

In this exploratory investigation, four stepwise linear regression analyses utilizing SAS software (SAS Version 9.1, Cary, NC) were performed examining possible relationships between neurosteroid levels (predictor variables) and pain ratings (response variables) (i.e. ratings of low back pain, chest pain, muscle soreness, and headache reported on the SCL-90-R). Several factors that potentially affect both neurosteroid levels and psychiatric symptoms (age, smoking status, alcohol use, and history of traumatic brain injury (TBI; defined as a past TBI with loss of consciousness by self-report; modified from Ivins et al and Kay et al (51,52)) were included as covariates in the regression analyses. SAS default mode $p<0.15$ was used for terms entering or leaving the stepwise linear regression models in this pilot study.

3. Results

3.1 Study Population

Table 1 summarizes the demographic and clinical data for this study sample. Of 90 male veterans who served since September 11, 2008 and who were enrolled at the Durham VA site, 82 subjects had no missing data. Subject mean age was 37.3 ± 10.3 SD years. Forty-eight percent were African American, 46% Caucasian, 4% Hispanic, and 2% Native American. Fifteen percent (12/82) of the sample reported a history of TBI with loss of consciousness (LOC). Of these 12 veterans, 9 sustained one TBI with LOC and 3 veterans sustained two TBIs, both with LOC. Of the 15 total TBIs with LOC, estimated duration of LOC by self-report was 1–20 minutes for 11 TBIs, 21–59 minutes for 1 TBI, 60 minutes or greater for 2 TBIs, and an unknown period of LOC for 1 TBI. Seventy-six percent were non-smokers (either never smoked or had quit). The average alcohol use score on the Alcohol Use Disorders Identification Test [AUDIT; (53)] was 6.2 ± 6.6 (SD).

3.2 Allopregnanolone

In the stepwise linear regression model, there was a significant, inverse relationship between allopregnanolone levels in serum and ratings of back pain (Table 2; $p=0.044$). Allopregnanolone levels in serum are higher in subjects reporting a 0 or 1 for low back pain severity (no/little pain) compared to those reporting a 2, 3, or 4 for low back pain severity (moderate to extreme pain) on the SCL-90-R (mean levels 102.5 ± 58.9 SD pg/ml vs. 81.1 ± 34.3 SD pg/ml, respectively; n=42 for no/little pain group and n=40 for moderate-to-extreme pain group). Allopregnanolone levels in serum also were significantly, inversely related to ratings of chest pain in this model (Table 2; $p=0.013$); allopregnanolone levels in serum are higher in subjects reporting a 0 or 1 for chest pain severity compared to those reporting a 2, 3, or 4 for chest pain severity on the SCL-90-R (mean levels 97.9 ± 52.0 SD pg/ml vs. 67.9 ± 25.7 SD pg/ml, respectively; n=66 for no/little pain group and n=16 for moderate-to-extreme pain group). Thus, participants who had lower levels of serum allopregnanolone reported higher levels of back pain and chest pain. Serum allopregnanolone levels were not significantly related to ratings of headache and muscle soreness (data not shown).

3.3 Other Neurosteroids

Stepwise linear regression analyses were also used to examine the relationship of pregnenolone, DHEA, DHEAS, and progesterone levels to pain ratings. These analyses revealed two additional, significant relationships between neurosteroids and pain ratings. First, DHEA levels in serum were inversely related to ratings of muscle soreness (Table 2; $p=0.024$). DHEA levels in serum are higher in subjects reporting a 0 or 1 for muscle soreness severity compared to those reporting a 2, 3, or 4 for muscle soreness severity on the SCL-90-R (mean levels 10.8 ± 6.2 SD ng/ml vs. 8.8 ± 4.6 SD ng/ml, respectively; n=54 for

no/little pain group and n=28 for moderate-to-severe pain group). Second, DHEAS levels were positively related to ratings of chest pain (Table 2; p=0.001). DHEAS levels are lower in subjects reporting a 0 or 1 for chest pain severity compared to those reporting a 2, 3, or 4 for chest pain severity on the SCL-90-R (mean levels 243.9 ± 145.5 SD μg/dl vs. 299.9 ± 188.6 SD μg/dl, respectively; n=66 for no/little pain group and n=16 for moderate to extreme pain group).

3.4 Demographic correlations

As noted above, we included several demographic and clinical variables as covariates in the stepwise regression analyses predicting pain ratings: age, smoking, alcohol use, and history of TBI. None of these covariates were found to be correlated with pain ratings, with the exception of a positive association between TBI and ratings of muscle soreness (Table 2; p=0.002).

4. Discussion

Consistent with our hypothesis, the present study demonstrates an inverse association between serum allopregnanolone and ratings of low back pain and chest pain in subjects who served in the U.S. military since September 11, 2001. These data support the possibility that there may be antinociceptive effects for this neurosteroid in humans. Future studies are needed to replicate these findings and to definitively evaluate the precise roles of allopregnanolone in pain disorders. Also, the finding that DHEA demonstrates an inverse association with a pain measure (muscle soreness) in this cohort raises the possibility of a more general antinociceptive feature for neurosteroids as a class.

To date, the existing literature contains few studies examining the relationship between neurosteroid levels and pain in clinical populations. When the field is narrowed further to only allopregnanolone, a neurosteroid with considerable evidence of analgesic properties in rodents (26,35,27–31), only one human study remains and this was conducted in a pain-free healthy volunteer cohort rather than a population that included subjects with pain symptoms (42). This previous study reported that individuals who showed higher endogenous allopregnanolone levels in response to cold pressor, thermal heat, or tourniquet ischemia pain tests were less likely to report an increase in pain tolerance. In addition, this study reported that baseline allopregnanolone levels correlated significantly with a decrease in pain tolerance during pain testing in one ethnic group in the study sample (i.e., non-Hispanic whites).

Several experimental differences from the present study, however, should be noted when comparing the findings of the Mechlin et al. and the current study. Perhaps most importantly, these studies may involve different pathophysiological mechanisms, as the present study examines correlations in baseline perceived pain levels, whereas the study by Mechlin et al. examines effects on pain threshold and tolerance via several acute stressor paradigms. Whereas the present study included a clinical population reporting pain complaints, the Mechlin et al. study focused on healthy volunteers free of chronic pain conditions. Further, while the present study included only males, Mechlin et al. included both males and females, thereby potentially influencing mean baseline allopregnanolone levels since this neurosteroid fluctuates across the menstrual cycle (54–56). This point may be especially relevant in light of the fact that the reported pain tolerances were gender specific in the previous study (42). Methodologically, it also should be noted that Mechlin et al. determined allopregnanolone levels via radioimmunoassay, a technique that may identify several GABAergic neurosteroids simultaneously based on an 84–169% cross-reactivity of the method with 3α-hydroxy-4-pregnen-20-one (55,57) and 6.6% cross-reactivity with pregnanolone (55), potentially also contributing to divergent results.

While the present study finds that higher endogenous allopregnanolone levels correlate with decreased reporting of some types of pain symptoms, the mechanisms responsible for potential analgesic effects of this neurosteroid remain to be determined. Some possible explanations are currently supported in the literature, including a role for allopregnanolone in anti-inflammatory actions. For example, in both mice and rats, allopregnanolone administration reduces levels of inflammatory cytokines (58,59). Longer term, the effects of allopregnanolone may result from protection against cell degeneration and/or cell death, as considerable evidence exists implicating allopregnanolone in several neuroprotective actions. For example, in a mouse model of the fatal, autosomal recessive neurodegenerative Niemann-Pick type C disease, there is a substantial reduction in the synthesis of allopregnanolone at birth (60), and neonatal administration of allopregnanolone delays neurological symptom onset and doubles lifespan (61,62). Further, neonatal administration of allopregnanolone increases neuronal myelination (62), a process previously demonstrated to involve $GABA_A$ receptors (63). Also, allopregnanolone inhibits apoptosis in rat models of TBI, decreasing caspase-3 activity and DNA fragmentation (64), decreasing glial fibrillary acid protein-positive astrocytes, and improving performance in spatial learning tasks (65). Multiple mechanisms may thus contribute to a possible analgesic and neuroprotective effects of this neurosteroid in humans.

Examination of additional neurosteroids yielded no association of either pregnenolone or progesterone with any of the pain measures, but findings did emerge for both DHEA and DHEAS. While we found that DHEA levels in serum were inversely associated with muscle soreness, levels of its sulfated form were positively associated with chest pain. Prior evidence has yielded inconsistent analgesic profiles for both DHEA and DHEAS (39,41,40,38) in humans. However, the differences in findings for each of these neurosteroids have been between inverse correlations with pain vs. no correlations at all. Thus, the positive correlation between DHEAS and chest pain in the present study is surprising both in that the response differs from that of DHEA, and in its incongruity with previous studies. Why these two neurosteroids demonstrate opposite serum profiles vis-a-vis pain ratings in this subject population is not yet clear. Further examination of various pain types and severities is needed to determine whether these findings are specific to muscle soreness or chest pain, as most previous studies reporting no correlation or a negative correlation for DHEAS have examined other types of pain (e.g., joint pain). Also, an examination of the same neurosteroids and pain measures in women could provide important information about possible gender-specific associations, as a negative correlation between DHEAS and musculoskeletal pain has been reported in a subject population of 57 women undergoing the menopausal transition (39).

Finally, while age, current smoking, and history of alcohol use are not associated with any of the pain measures in the present study, a history of TBI is associated with muscle soreness. This association is not a surprising one (66–69), especially given the high rates of polytrauma in this cohort of returning veterans (70,4,71). However, the lack of an association with other pain measures such as headache is unexpected. It is possible that a larger sample size or the use of a more comprehensive pain assessment may be required to capture these additional associations.

Limitations of this exploratory pilot study must be acknowledged. Our R^2 values were relatively low, with a maximum result of 0.17. We also conducted four separate analyses, thereby potentially increasing the likelihood of Type I error. Nevertheless, with the exception of TBI, only neurosteroid variables were significant in our analyses, supporting our hypothesis for a neurosteroid role in pain modulation. Further, the instrument utilized does not query the specific type of headache, potentially limiting assessments of relationships between neurosteroid levels or other findings and headache. Results will

clearly require replication in a larger cohort, but these initial findings are promising, as they are consistent with the preclinical literature and provide data for future hypothesis testing.

In summary, the present study demonstrates that increased levels of endogenous allopregnanolone are associated with decreased self-reported low back pain and chest pain in subjects who served in the U.S. military after September 11, 2001. In addition, increased levels of DHEA are associated with decreased muscle soreness pain, and higher DHEAS levels are associated with increased chest pain in this population. This analysis used exploratory techniques (stepwise linear regression) and results should be replicated in an independent sample. These findings, particularly if supported by future replication studies, suggest that modulation of allopregnanolone or other neurosteroid levels may be logical targets for therapeutic intervention in pain disorders.

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References

1. Tanielian, T.; Jaycox, LH.; Schell, TL.; Marshall, GN.; Burnam, MA.; Eibner, C.; Karney, BR.; Meredith, LS.; Ringel, JS.; Vaiana, ME. Invisible Wounds Study Team. Rand Report. Center for Military Health Policy Research; 2008. Invisible Wounds of War.
2. Gawande A. Casualties of war--military care for the wounded from Iraq and Afghanistan. The New England Journal of Medicine. 2004; 351(24):2471–2475. [PubMed: 15590948]
3. Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, Koffman RL. Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. The New England Journal of Medicine. 2004; 351(1):13–22. [PubMed: 15229303]
4. Hoge CW, McGurk D, Thomas JL, Cox AL, Engel CC, Castro CA. Mild traumatic brain injury in U.S. Soldiers returning from Iraq. The New England Journal of Medicine. 2008; 358(5):453–463. [PubMed: 18234750]
5. Seal KH, Bertenthal D, Miner CR, Sen S, Marmar C. Bringing the war back home: mental health disorders among 103,788 US veterans returning from Iraq and Afghanistan seen at Department of Veterans Affairs facilities. Archives of Internal Medicine. 2007; 167(5):476–482. [PubMed: 17353495]
6. Gironda RJ, Clark ME, Massengale JP, Walker RL. Pain among veterans of Operations Enduring Freedom and Iraqi Freedom. Pain Medicine. 2006; 7(4):339–343. [PubMed: 16898945]
7. Helmer DA, Chandler HK, Quigley KS, Blatt M, Teichman R, Lange G. Chronic widespread pain, mental health, and physical role function in OEF/OIF veterans. Pain Medicine. 2009; 10(7):1174–1182. [PubMed: 19818029]
8. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science. 1986; 232(4753):1004–1007. [PubMed: 2422758]
9. Morrow AL, Suzdak PD, Paul SM. Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. European Journal of Pharmacology. 1987; 142(3):483–485. [PubMed: 2828079]
10. Majewska MD, Schwartz RD. Pregnenolone-sulfate: an endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Brain Research. 1987; 404(1-2):355–360. [PubMed: 3032339]

11. Majewska MD, Demirgoren S, London ED. Binding of pregnenolone sulfate to rat brain membranes suggests multiple sites of steroid action at the GABAA receptor. European Journal of Pharmacology. 1990; 189(4-5):307–315. [PubMed: 2177701]
12. Majewska MD, Demirgoren S, Spivak CE, London ED. The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABAA receptor. Brain Research. 1990; 526(1):143–146. [PubMed: 1964106]
13. Imamura M, Prasad C. Modulation of GABA-gated chloride ion influx in the brain by dehydroepiandrosterone and its metabolites. Biochemical and Biophysical Research Communications. 1998; 243(3):771–775. [PubMed: 9501004]
14. Park-Chung M, Malayev A, Purdy RH, Gibbs TT, Farb DH. Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites. Brain Research. 1999; 830(1):72–87. [PubMed: 10350561]
15. Crawley JN, Glowa JR, Majewska MD, Paul SM. Anxiolytic activity of an endogenous adrenal steroid. Brain Research. 1986; 398(2):382–385. [PubMed: 2879610]
16. Wieland S, Lan NC, Mirasedeghi S, Gee KW. Anxiolytic activity of the progesterone metabolite 5 alpha-pregnan-3 alpha-o1–20-one. Brain Research. 1991; 565(2):263–268. [PubMed: 1688192]
17. Bitran D, Klibansky DA, Martin GA. The neurosteroid pregnanolone prevents the anxiogenic-like effect of inescapable shock in the rat. Psychopharmacology. 2000; 151(1):31–37. [PubMed: 10958114]
18. Jain NS, Hirani K, Chopde CT. Reversal of caffeine-induced anxiety by neurosteroid 3-alpha-hydroxy-5-alpha-pregnane-20-one in rats. Neuropharmacology. 2005; 48(5):627–638. [PubMed: 15814098]
19. Landgren S, Aasly J, Backstrom T, Dubrovsky B, Danielsson E. The effect of progesterone and its metabolites on the interictal epileptiform discharge in the cat's cerebral cortex. Acta Physiologica Scandinavica. 1987; 131(1):33–42. [PubMed: 3673611]
20. Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5 alpha-pregnan-3 alpha-ol-20-one. European Journal of Pharmacology. 1989; 166(2):325–329. [PubMed: 2792198]
21. Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. The Journal of Pharmacology and Experimental Therapeutics. 1994; 270(3):1223–1229. [PubMed: 7932175]
22. Devaud LL, Purdy RH, Morrow AL. The neurosteroid, 3 alpha-hydroxy-5 alpha-pregnan-20-one, protects against bicuculline-induced seizures during ethanol withdrawal in rats. Alcoholism, Clinical and Experimental Research. 1995; 19(2):350–355.
23. Kokate TG, Cohen AL, Karp E, Rogawski MA. Neuroactive steroids protect against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. Neuropharmacology. 1996; 35(8):1049–1056. [PubMed: 9121607]
24. Kavaliers M. Inhibitory influences of the adrenal steroid, 3 alpha, 5 alpha-tetrahydrodeoxycorticosterone [correction of tetrahydroxycorticosterone] on aggression and defeat-induced analgesia in mice. Psychopharmacology. 1988; 95(4):488–492. [PubMed: 3145520]
25. Pinna G, Dong E, Matsumoto K, Costa E, Guidotti A. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proceedings of the National Academy of Sciences USA. 2003; 100(4):2035–2040.
26. Kavaliers M, Wiebe JP. Analgesic effects of the progesterone metabolite, 3 alpha-hydroxy-5 alpha-pregnan-20-one, and possible modes of action in mice. Brain Research. 1987; 415(2):393–398. [PubMed: 3111642]
27. Winfree CJ, Coombs DW, DeLeo JA, Colburn RW. Analgesic effects of intrathecally-administered 3 alpha-hydroxy-5 alpha-pregnan-20-one in a rat mechanical visceral pain model. Life Sciences. 1992; 50(14):1007–1012. [PubMed: 1552820]
28. Frye CA, Duncan JE. Progesterone metabolites, effective at the GABAA receptor complex, attenuate pain sensitivity in rats. Brain Research. 1994; 643(1–2):194–203. [PubMed: 8032914]
29. Korneyev A, Costa E. Allopregnanolone (THP) mediates anesthetic effects of progesterone in rat brain. Hormones and Behavior. 1996; 30(1):37–43. [PubMed: 8724177]

30. Kavaliers M, Perrot-Sinal TS, Desjardins DC, Cross-Mellor SK, Wiebe JP. Antinociceptive effects of the neuroactive steroid, 3alpha-hydroxy-5alpha-pregnan-20-one and progesterone in the land snail, Cepaea nemoralis. Neuroscience. 2000; 95(3):807–812. [PubMed: 10670448]

31. Pathirathna S, Brimelow BC, Jagodic MM, Krishnan K, Jiang X, Zorumski CF, Mennerick S, Covey DF, Todorovic SM, Jevtovic-Todorovic V. New evidence that both T-type calcium channels and GABAA channels are responsible for the potent peripheral analgesic effects of 5alpha-reduced neuroactive steroids. Pain. 2005; 114(3):429–443. [PubMed: 15777868]

32. Yokoro CM, Pesquero SM, Turchetti-Maia RM, Francischi JN, Tatsuo MA. Acute phenobarbital administration induces hyperalgesia: pharmacological evidence for the involvement of supraspinal GABA-A receptors. Brazilian Journal of Medical and Biological Research. 2001; 34(3):397–405. [PubMed: 11262592]

33. Zou X, Lin Q, Willis WD. The effects of sympathectomy on capsaicin-evoked fos expression of spinal dorsal horn GABAergic neurons. Brain Research. 2002; 958(2):322–329. [PubMed: 12470868]

34. Enna SJ, McCarson KE. The role of GABA in the mediation and perception of pain. Advances in Pharmacology. 2006; 54:1–27. [PubMed: 17175808]

35. Mok WM, Krieger NR. Evidence that 5 alpha-pregnan-3 alpha-ol-20-one is the metabolite responsible for progesterone anesthesia. Brain Research. 1990; 533(1):42–45. [PubMed: 2085732]

36. Chen SC, Wu FS. Mechanism underlying inhibition of the capsaicin receptor-mediated current by pregnenolone sulfate in rat dorsal root ganglion neurons. Brain Research. 2004; 1027(1-2):196–200. [PubMed: 15494172]

37. Chen SC, Liu BC, Chen CW, Wu FS. Intradermal pregnenolone sulfate attenuates capsaicin-induced nociception in rats. Biochemical and Biophysical Research Communications. 2006; 349(2):626–633. [PubMed: 16945330]

38. Khoromi S, Muniyappa R, Nackers L, Gray N, Baldwin H, Wong KA, Matheny LA, Moquin B, Rainer A, Hill S, Remaley A, Johnson LL, Max MB, Blackman MR. Effects of chronic osteoarthritis pain on neuroendocrine function in men. The Journal of Clinical Endocrinology and Metabolism. 2006; 91(11):4313–4318. [PubMed: 16912126]

39. Finset A, Overlie I, Holte A. Musculo-skeletal pain, psychological distress, and hormones during the menopausal transition. Psychoneuroendocrinology. 2004; 29(1):49–64. [PubMed: 14575729]

40. Finckh A, Berner IC, Aubry-Rozier B, So AK. A randomized controlled trial of dehydroepiandrosterone in postmenopausal women with fibromyalgia. The Journal of Rheumatology. 2005; 32(7):1336–1340. [PubMed: 15996074]

41. Genazzani AR, Inglese S, Lombardi I, Pieri M, Bernardi F, Genazzani AD, Rovati L, Luisi M. Long-term low-dose dehydroepiandrosterone replacement therapy in aging males with partial androgen deficiency. Aging Male. 2004; 7(2):133–143. [PubMed: 15672938]

42. Mechlin B, Morrow AL, Maixner W, Girdler SS. The relationship of allopregnanolone immunoreactivity and HPA-axis measures to experimental pain sensitivity: Evidence for ethnic differences. Pain. 2007; 131(1–2):142–152. [PubMed: 17292548]

43. Derogatis, LR. Symptom Checklist-90-R, Administration, Scoring, and Procedures Manual. 3.

44. Gilron I, Coderre TJ. Preemptive analgesic effects of steroid anesthesia with alphaxalone in the rat formalin test. Evidence for differential GABA(A) Receptor modulation in persistent nociception. Anesthesiology. 1996; 84(3):572–579. [PubMed: 8659786]

45. Goodchild CS, Robinson A, Nadeson R. Antinociceptive properties of neurosteroids IV: pilot study demonstrating the analgesic effects of alphadolone administered orally to humans. British Journal of Anaesthesia. 2001; 86(4):528–534. [PubMed: 11573627]

46. Nadeson R, Goodchild CS. Antinociceptive properties of neurosteroids III: experiments with alphadolone given intravenously, intraperitoneally, and intragastrically. British Journal of Anaesthesia. 2001; 86(5):704–708. [PubMed: 11575348]

47. Winter L, Nadeson R, Tucker AP, Goodchild CS. Antinociceptive properties of neurosteroids: a comparison of alphadolone and alphaxalone in potentiation of opioid antinociception. Anesthesia and Analgesia. 2003; 97(3):798–805. [PubMed: 12933405]

48. Peebles JE, McWilliams LA, MacLennan R. A comparison of symptom checklist 90-revised profiles from patients with chronic pain from whiplash and patients with other musculoskeletal injuries. Spine. 2001; 26(7):766–770. [PubMed: 11295898]
49. Marx CE, Trost WT, Shampine L, Behm FM, Giordano LA, Massing MW, Rose JE. Neuroactive steroids, negative affect, and nicotine dependence severity in male smokers. Psychopharmacology. 2006; 186(3):462–472. [PubMed: 16402195]
50. Marx CE, Trost WT, Shampine LJ, Stevens RD, Hulette CM, Steffens DC, Ervin JF, Butterfield MI, Blazer DG, Massing MW, Lieberman JA. The neurosteroid allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease. Biological Psychiatry. 2006; 60(12):1287–1294. [PubMed: 16997284]
51. Kay T, Harrington DE, Adams R. Definition of mild traumatic brain injury. The Journal of Head Trauma Rehabilitation. 1993; 8(3):86–87.
52. Ivins BJ, Schwab KA, Warden D, Harvey LT, Hoilien MA, Powell CO, Johnson CS, Salazar AM. Traumatic brain injury in U.S. Army paratroopers: prevalence and character. The Journal of Trauma. 2003; 55(4):617–621. [PubMed: 14566111]
53. Reinert DF, Allen JP. The alcohol use disorders identification test: an update of research findings. Alcoholism, Clinical and Experimental Research. 2007; 31(2):185–199.
54. Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Nappi RE, Luisi S, Palumbo M, Purdy RH, Luisi M. Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. The Journal of Clinical Endocrinology and Metabolism. 1998; 83(6): 2099–2103. [PubMed: 9626145]
55. Girdler SS, Straneva PA, Light KC, Pedersen CA, Morrow AL. Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. Biological Psychiatry. 2001; 49(9): 788–797. [PubMed: 11331087]
56. Genazzani AD, Luisi M, Malavasi B, Strucchi C, Luisi S, Casarosa E, Bernardi F, Genazzani AR, Petraglia F. Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroid, during the menstrual cycle and in amenorrheic subjects. European Journal of Endocrinology. 2002; 146(3):347–356. [PubMed: 11888841]
57. Smith CD, Wekstein DR, Markesbery WR, Frye CA. 3alpha,5alpha-THP: a potential plasma neurosteroid biomarker in Alzheimer's disease and perhaps non-Alzheimer's dementia. Psychopharmacology. 2006; 186(3):481–485. [PubMed: 16231167]
58. Ghezzi P, Santo ED, Sacco S, Foddi C, Barbaccia ML, Mennini T. Neurosteroid levels are increased in vivo after LPS treatment and negatively regulate LPS-induced TNF production. European Cytokine Network. 2000; 11(3):464–469. [PubMed: 11022133]
59. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Experimental Neurology. 2004; 189(2):404–412. [PubMed: 15380490]
60. Mellon S, Gong W, Griffin LD. Niemann pick type C disease as a model for defects in neurosteroidogenesis. Endocrine Research. 2004; 30(4):727–735. [PubMed: 15666818]
61. Griffin LD, Gong W, Verot L, Mellon SH. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. Nature Medicine. 2004; 10(7):704–711.
62. Ahmad I, Lope-Piedrafita S, Bi X, Hicks C, Yao Y, Yu C, Chaitkin E, Howison CM, Weberg L, Trouard TP, Erickson RP. Allopregnanolone treatment, both as a single injection or repetitively, delays demyelination and enhances survival of Niemann-Pick C mice. Journal of Neuroscience Research. 2005; 82(6):811–821. [PubMed: 16273542]
63. Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. Journal of Neurochemistry. 2003; 86(4):848–859. [PubMed: 12887683]
64. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. Neuroscience. 2004; 123(2):349–359. [PubMed: 14698743]

65. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. Journal of Neurotrauma. 2005; 22(1):106–118. [PubMed: 15665606]

66. Uomoto JM, Esselman PC. Traumatic brain injury and chronic pain: differential types and rates by head injury severity. Archives of Physical Medicine and Rehabilitation. 1993; 74(1):61–64. [PubMed: 8420522]

67. Lahz S, Bryant RA. Incidence of chronic pain following traumatic brain injury. Archives of Physical Medicine and Rehabilitation. 1996; 77(9):889–891. [PubMed: 8822679]

68. Bryant RA, Marosszeky JE, Crooks J, Baguley IJ, Gurka JA. Interaction of posttraumatic stress disorder and chronic pain following traumatic brain injury. The Journal of Head Trauma Rehabilitation. 1999; 14(6):588–594. [PubMed: 10671704]

69. Walker WC, Seel RT, Curtiss G, Warden DL. Headache after moderate and severe traumatic brain injury: a longitudinal analysis. Archives of Physical Medicine and Rehabilitation. 2005; 86(9): 1793–1800. [PubMed: 16181945]

70. Batten SV, Pollack SJ. Integrative outpatient treatment for returning service members. Journal of Clinical Psychology. 2008; 64(8):928–939. [PubMed: 18561182]

71. U.S., Department, of, Veterans, Affairs. VHA Office of Public Health and Environmental Hazards Reports on Returning Combat Veterans. 2008.

Table 1

Demographic and Clinical Data.

	Mean	SD
Age (years)	37.3	10.3
Alcohol use (AUDIT score)	6.2	**6.6**
	%	
African American	48 % (n=39)	
Caucasian	46 % (n=38)	
Hispanic	4 % (n=3)	
Native American	2 % (n=2)	
Traumatic brain injury (with loss of consciousness)	15 % (n=12)	
Current smoker	26 % (n=21)	

Table 2

Stepwise Linear Regression Model Correlations.

Psychiatric Rating Scale	β Coeff	Model P-value	R^2	Individual P-value
Lower Back Pain (SCL-27)		0.0435	0.05	
Allopregnanolone	−0.0064			0.0435
Chest Pain (SCL-12)		<0.0028	0.16	
Allopregnanolone	−0.0054			0.0134
DHEAS	0.0024			0.0012
Muscle Soreness (SCL-42)		0.0021	0.17	
DHEA	−0.0630			0.0238
TBI	1.2284			0.0017
Headache (SCL-1)		0.0734	0.04	