





## ORIGINAL RESEARCH ARTICLE

# Altered GABA<sub>A</sub> receptor function in women with endometriosis: a possible pain-related mechanism

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## Abstract

**Introduction:** The mechanism underlying endometriosis-related pain remains poorly understood. Previous studies have indicated that  $\gamma$ -aminobutyric acid (GABA) type A (GABA<sub>A</sub>) receptors and GABAergic substances (eg endogenous neurosteroids) play important mechanistic roles in various pain conditions. Our primary objective was to compare GABA<sub>A</sub> receptor function between women with endometriosis and healthy controls by performing a challenge test with diazepam, a GABA<sub>A</sub> receptor agonist, using the saccadic eye velocity as the main outcome. The secondary objective was to investigate the relation between GABA<sub>A</sub> receptor function and serum levels of allopregnanolone, an endogenous positive modulator of the GABA<sub>A</sub> receptor, in the participating women.

**Material and methods:** 15 women with pelvic pain and laparoscopically confirmed endometriosis and 10 healthy, symptom-free, control women, aged 18–40 years, underwent the diazepam challenge test during the follicular phase of the menstrual cycle. Basal serum allopregnanolone levels were measured prior to diazepam injection.

**Results:** Compared with healthy controls, women with pelvic pain and confirmed endometriosis had a significantly smaller change in saccadic eye velocity after GABA<sub>A</sub> receptor stimulation with diazepam, indicating lower sensitivity to diazepam. The saccadic eye velocity response was not correlated with the serum allopregnanolone levels.

**Conclusions:** Women with painful endometriosis show altered GABA<sub>A</sub> receptor function, depicted as a muted response to an exogenous GABA<sub>A</sub> receptor agonist.

## KEYWORDS

allopregnanolone, central sensitisation, endometriosis, GABA, pain

**Abbreviations:** GABA,  $\gamma$ -aminobutyric acid; GABA<sub>A</sub> receptor,  $\gamma$ -aminobutyric acid type A receptor; SEV, saccadic eye velocity;  $\Delta$ SEV, change of peak saccadic eye velocity from baseline.

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## 1 | INTRODUCTION

Endometriosis typically presents with various pain symptoms, ranging from dysmenorrhea (painful menstruation) and dyspareunia (painful intercourse) to more persistent, chronic pelvic pain. However, the extent of the endometriosis lesions is poorly correlated with the pain symptoms experienced by the patient, which is a puzzling finding.<sup>1</sup>

In recent years it has been hypothesized that endometriosis-associated pain might, beyond a nociceptive component, also involve a neuropathic component.<sup>2</sup> A central pain component may explain the poor correlation with the macroscopic stage of endometriosis and why conventional surgical and hormonal treatments sometimes fail.<sup>3</sup> Because women with endometriosis often have a lower pain threshold than healthy controls, it seems likely that central sensitization and alterations in the central nervous system might be involved in the pathogenesis of endometriosis-associated pain.<sup>4</sup>

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and appears to play a central role in pain regulation.<sup>5</sup> Inhibitory synapses are predominantly populated with GABA<sub>A</sub> receptors. The action of GABA<sub>A</sub> receptors can be potentiated by exogenous substances including benzodiazepines, as well as neurosteroids.<sup>6,7</sup> Neurosteroids are endogenous steroids produced in the central or peripheral nervous system, independently from the activity of endocrine glands.<sup>8</sup> They can modulate nervous system activity by acting through various membrane receptors. One neurosteroid, allopregnanolone, is secreted during stress and mirrors the fluctuations of serum progesterone levels during the menstrual cycle and pregnancy, and it plays important roles in various disorders in women, including premenstrual dysphoric disorder.<sup>9</sup> The binding of GABA agonists to GABA receptors facilitates the inhibitory actions of GABA and elicits hypnotic, anxiolytic and anticonvulsant effects.<sup>10</sup> The use of GABAergic substances induces tolerance to many of their therapeutic actions, as shown by a decrease in the GABA<sub>A</sub> receptor-mediated effect of the substance.<sup>11</sup> Chronic stress also inhibits GABA<sub>A</sub> receptor function.<sup>12</sup> Acute and chronic pain could be perceived as a type of stress and may thereby alter GABA<sub>A</sub> receptor function. Recent animal studies have provided increasing evidence that neurosteroids, and their action on the GABA<sub>A</sub> receptor, play a central role in the pathogenesis of neuropathic pain.<sup>13</sup> However, the role of GABA<sub>A</sub> receptor function in the development of endometriosis-related pain has not been studied to date.

A saccade is the rapid movement of the eye when moving from one point of fixation to another. The maximal velocity of a saccade varies between individuals but is stable within the same individual and is not voluntarily controlled.<sup>14</sup> Measurement of the saccadic eye velocity (SEV) can be used to study the effects of GABAergic substances in humans because the maximal SEV is affected by substances acting on the GABA<sub>A</sub> receptor and is reduced in a dose-dependent manner.<sup>15</sup> This method has frequently been used to study GABA<sub>A</sub> receptor function, especially the sensitivity to GABA<sub>A</sub> receptor modulators.<sup>16,17</sup>

### Key message

Women with endometriosis show a significantly decreased GABA<sub>A</sub> receptor-mediated inhibition compared to healthy controls, indicating changes in the central nervous system that could explain why treatment targeted at the nociceptive component of the pain sometimes fails in these women.

The primary objective of this study was to evaluate GABA<sub>A</sub> receptor function by performing a diazepam challenge test in patients with painful endometriosis and healthy controls. The secondary objective was to investigate whether altered GABA<sub>A</sub> receptor sensitivity is correlated to endogenous allopregnanolone levels in these patients.

## 2 | MATERIAL AND METHODS

Endometriosis patients were recruited from the gynecologic outpatient clinic at Sundsvall County Hospital, Sweden. All patients had pelvic pain and endometriosis according to European Society of Human Reproduction and Embryology criteria<sup>18</sup> and confirmed by laparoscopy. Women in the control group were healthy volunteers, without any symptoms indicative of endometriosis, recruited via an advertisement.

Women aged 18–40 years with regular menstrual cycles, suffering from severe dysmenorrhea and varying degrees of pelvic pain and a laparoscopically confirmed diagnosis of endometriosis were eligible for the endometriosis group. We excluded patients if they had recently used benzodiazepines or any other psychoactive drug or any hormonal contraceptive or other hormonal therapy in the last month. Women with signs of a gynecologic disorder (apart from endometriosis in the patient group), ongoing or past psychiatric illness, premenstrual dysphoric disorder, ongoing or past somatic diseases (including liver, kidney, heart, lung or neurological diseases), alcohol or drug abuse, and ongoing pregnancy were also excluded.

The screening phase commenced after the patients had provided consent to participate. The medical examination included a structured mental health interview (M.I.N.I.: International Neuropsychiatric Interview), a gynecologic examination, including a vaginal ultrasound to rule out other pathological conditions, a pregnancy test and blood sampling.

All study participants were examined in the follicular phase (cycle days 5–12) of the menstrual cycle, when sex hormone levels are generally low and allopregnanolone levels are more closely related to adrenal activity, rather than in the luteal phase, when allopregnanolone is produced mainly by the ovary.<sup>19</sup> Blood samples were taken to measure allopregnanolone levels. To avoid interference due to diurnal variations of allopregnanolone levels, all participants were tested during the same part of the day, before noon.

GABA<sub>A</sub> receptor function was evaluated during a diazepam challenge test, as described in an earlier study by our group on diazepam and SEV.<sup>16</sup> In brief, saccadic eye measurements were measured before and after the administration of a commonly used GABA<sub>A</sub> receptor agonist, diazepam (Stesolid®, Novum-Oripharm AB, Stockholm, Sweden; 5 mg/mL) at a dose of 0.1 mg/kg. SEV was measured at baseline (before diazepam injection) and then at 5, 30, 60 and 120 min after injection.

We used a video nystagmographic device (Ulmer VNG; Synapsys Micromega, Marseille, France), which uses a sophisticated algorithm to measure ocular movement accurately with high reliability. The theoretical accuracy of the measurements of eye movements is 0.25°. The system consists of software and hardware consisting of a computer, screen, color printer, projector and a VISIO mask (open mask) to record rapid eye movements using a camera permitting binocular analysis. The camera has an unlimited horizontal viewing angle and a vertical angle of view of +90°–20°, with sample frequencies of 50, 100 or 200 Hz. Eye movements are recorded by infrared video oculography. During the examination, the patient is seated in a stable chair with the head fixed in a headrest. For saccade measurement, we used high-frequency (200 Hz) infrared video oculography. The patient wears the VISIO mask on which the camera is fixed 10 cm from the nose and oriented to record both eyes simultaneously. The saccades are induced by light-emitting diodes (LEDs) presented on a ramp placed 1.5 m in front of the patient. The LEDs elicit 40° horizontal saccades ( $\pm 20^\circ$ ). The software uses “contrast image detection” and calculates the horizontal position of the centre of each pupil. The calculated parameters include the latency and maximal SEV. Delta SEV ( $\Delta$ SEV) was calculated as the difference in the maximal SEV at each timepoint relative to the baseline SEV.  $\Delta$ SEV was used because SEV varies markedly between individuals but shows high within-subject stability, both within testing sessions and between testing days.<sup>20</sup>

During the study, 5-mL blood samples were collected at baseline and the serum was separated by centrifugation and stored at  $-80^\circ\text{C}$  for later analysis of allopregnanolone levels. In the control group, the allopregnanolone levels were measured using a radioimmunoassay at our laboratory at Umeå University. The method is described in detail elsewhere.<sup>21</sup> In brief, after separation from cross-reacting steroids by celite chromatography, allopregnanolone was measured by radioimmunoassay using a polyclonal rabbit antiserum raised against 3 $\alpha$ -hydroxy-20-oxo-5 $\alpha$ -pregnan-11-yl-carboxymethyl ether, coupled to bovine serum albumin. The sensitivity of the assay was 0.025 ng/mL. The intra-assay coefficient of variation was 6.5% and the interassay coefficient of variation was 8.5%.

Although the sample preparation and extraction processes were the same in both study groups, the method used to quantify allopregnanolone differed between the groups because the laboratory at Umeå University had ceased activities when the blood samples for the patient group were ready for analysis. Therefore we received help from another laboratory in Oulo-Finland. In the patient group, we used the method described by Pekka Keski-Rahkonen et al. to quantify allopregnanolone.<sup>22</sup> In brief, the serum level was quantified by ultra-high performance liquid chromatography (UPLC/MS/MS

Waters Acquity) using a C18 column on a Waters XEVO-TQ-S triple quadrupole mass spectrometer at Admescope Oy laboratory (Oulu, Finland). The detection limit for allopregnanolone was 0.002 ng/mL (0.063 nmol/L).

## 2.1 | Statistical analyses

All statistical analyses were performed using SPSS version 28 (SPSS; IBM Corp., Armonk, NY, USA). Baseline characteristics were compared between the two groups using the Mann–Whitney *U* test. Outcome variables, maximal SEV, SEV latency and  $\Delta$ SEV were compared between the groups using two-way repeated-measures analysis of variance (ANOVA). Correlations among variables within each group were analyzed using Spearman's rank correlation test. A linear regression analysis was used to reveal causal relation between allopregnanolone (independent variable) and the latency, SEV and  $\Delta$ SEV (dependent variables). Values of  $p < 0.05$  were deemed statistically significant. For determination of the sample size, in our previous studies, we found that the standard deviation of SEV within individuals was  $\pm 7^\circ/\text{s}$ .<sup>16</sup> The smallest change in SEV between baseline and after injection of a GABAergic substance that we want to be able to detect is 15°/s. Therefore, at an  $\alpha$  of 0.05 and a power of 0.90, the calculated study size was seven participants per group.

## 2.2 | Ethics statement

All study participants received written and oral information about the study and provided signed consent before entering the study. The study was approved by the regional ethics review board in Umeå, Sweden (DNR 2012/63-31) on June 5, 2012.

## 3 | RESULTS

A total of 17 patients with endometriosis (endometriosis group) and 10 healthy controls (control group) were enrolled in the study. However, two patients in the endometriosis group were excluded because they missed the diazepam challenge test. Therefore, data for 15 patients with endometriosis and 10 healthy controls were analyzed (Table 1).

Regarding baseline characteristics, there were no differences in the age or body mass index between the two groups. All participants in the control group were non-smokers. In the patient group there were two smokers. We know that nicotine can affect GABA signaling and blood neurosteroid levels.<sup>23</sup> Comparison of smoking vs non-smoking patients did not, however, reveal any significant difference in serum allopregnanolone levels or SEV test results.

The endometriosis group had significantly longer latency and lower SEV at baseline compared with the control group. The serum allopregnanolone levels were also significantly lower in women in the endometriosis group (Table 1).

**TABLE 1** Background characteristics and baseline measurements of serum allopregnanolone and saccadic eye velocity parameters before diazepam injection in patients with endometriosis ( $n = 15$ ) and healthy controls ( $n = 10$ ).

|                                  | Endometriosis group      | Control group            | $p$    |
|----------------------------------|--------------------------|--------------------------|--------|
|                                  | Median (IQR)             | Median (IQR)             |        |
| Age (years)                      | 27 (6)                   | 25 (11)                  | 0.350  |
| BMI ( $\text{kg}/\text{m}^2$ )   | 24 (7)                   | 24 (4)                   | 0.147  |
| Serum allopregnanolone (nmol/L)  | 0.18 (0.24) <sup>a</sup> | 0.53 (0.16) <sup>b</sup> | <0.001 |
| Latency (ms)                     | 157 (59)                 | 221 (64)                 | 0.023  |
| Peak SEV ( $^{\circ}/\text{s}$ ) | 296 (84)                 | 426 (58)                 | 0.010  |

Note: Between-group differences were determined using the Mann-Whitney  $U$  test at a significance level of  $p < 0.05$ .

Abbreviations: BMI, body mass index; IQR, interquartile range; SEV, saccadic eye velocity.

<sup>a</sup>Analyzed by mass spectrometry.

<sup>b</sup>Analyzed by radioimmunoassay.

### 3.1 | Diazepam challenge test

#### 3.1.1 | Latency

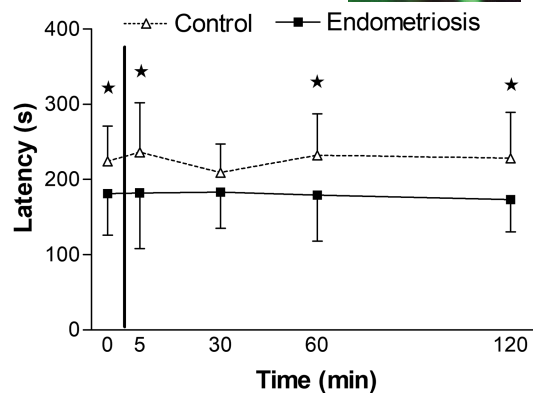
The latency of the onset of eye movements after a change in target did not change during the challenge test within each group ( $p = 0.602$ ). However, there was a significant overall difference between the groups ( $p = 0.019$ ), with a shorter latency in the endometriosis group (Figure 1).

#### 3.1.2 | Maximal SEV

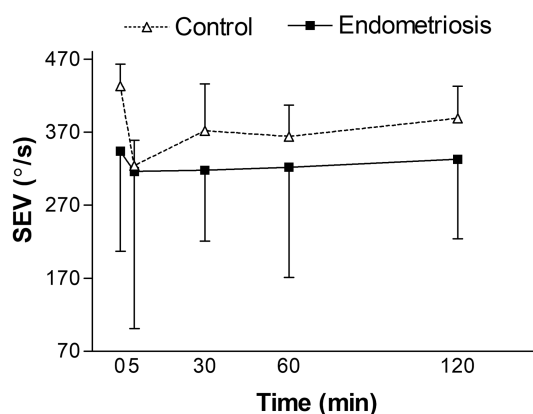
After diazepam injection, there were significant decreases in the SEV after 5 min in both groups. Within each group, the SEV was significantly different between the measurement timepoints ( $p = 0.002$ ). The overall differences between the two groups were not significant ( $p = 0.249$ ; Figure 2).

#### 3.1.3 | $\Delta$ SEV

The effect of diazepam was generally greatest immediately after injection in both groups. Its effect was more pronounced and disappeared more quickly in the control group. Statistical analyses over the whole study period (up to 120 min after diazepam injection) with repeated-measures ANOVA revealed a significant difference in  $\Delta$ SEV within the groups ( $p = 0.008$ ). For all timepoints combined, the  $\Delta$ SEV after diazepam injection was significantly smaller in the endometriosis group than in the control group ( $p = 0.005$ ; Figure 3).



**FIGURE 1** Saccadic eye velocity latency measured before and after a single injection of diazepam (0.1 mg/kg) in women with endometriosis ( $n = 15$ ) and healthy controls ( $n = 10$ ). There was a significant overall difference between groups ( $p = 0.019$ ). The vertical line shows the timing of diazepam injection. \* Significant difference between groups for the measured time point.



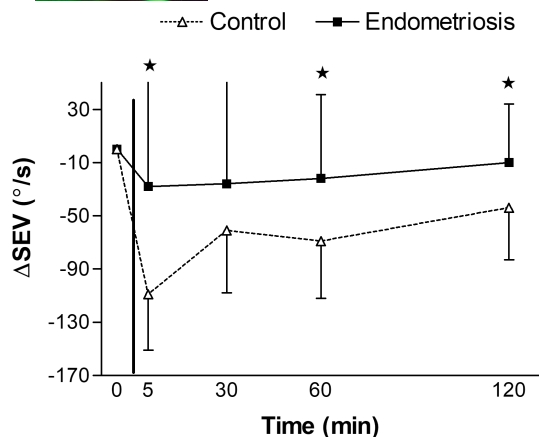
**FIGURE 2** Peak saccadic eye velocity (SEV) measured before and after a single injection of diazepam (0.1 mg/kg) in women with endometriosis ( $n = 15$ ) and healthy controls ( $n = 10$ ). The overall difference between groups was not significant ( $p = 0.249$ ). The vertical line shows the timing of diazepam injection.

### 3.2 | Correlations among study parameters

Because allopregnanolone is a mediator of the GABA<sub>A</sub> receptor, a correlation between allopregnanolone levels and SEV can be expected. However, the regression analysis did not detect an association or causal relation, in any of the groups, between the basal serum allopregnanolone levels (independent factor) and saccadic eye measurements (ie latency, SEV or  $\Delta$ SEV; dependent factors). Furthermore, in none of the groups, were age, body mass index or serum allopregnanolone levels correlated with the baseline measurements of latency or SEV or  $\Delta$ SEV values during the diazepam challenge test.

## 4 | DISCUSSION

The main finding of this study was that women with endometriosis and associated pain have a significantly reduced latency and  $\Delta$ SEV



**FIGURE 3** Change of peak saccadic eye velocity from baseline ( $\Delta$ SEV) after a single injection of diazepam (0.1 mg/kg) in women with endometriosis ( $n = 15$ ) and healthy controls ( $n = 10$ ). There was a significant overall difference between groups ( $p = 0.005$ ). The vertical line shows the timing of diazepam injection. \* Significant difference between groups for the measured time point.

after a diazepam challenge test, indicating an impaired inhibitory function of the GABA<sub>A</sub> receptors compared with a group of healthy women. Women with endometriosis also had significantly lower serum allopregnanolone levels, although this finding is uncertain because the analysis methods differed between the two groups. This is an obvious limitation to this study but is due to practical and resource-related problems. However, methodologic comparisons for other steroids between radioimmunoassay, after extraction and separation from cross-reactive steroids, and GC-MS have shown similar sensitivity at low serum levels and a high correlation.<sup>24</sup>

Oculographic measurement of SEV provides an objective and sensitive method of assessing GABA<sub>A</sub> receptor sensitivity. Alterations in SEV were previously demonstrated in various pain conditions, including patients with fibromyalgia who showed decreased SEV.<sup>25</sup> We found similar results in women with endometriosis and pain, who had significantly lower SEV and latency values compared with healthy controls. This could be explained by alterations in the GABAergic system and, more precisely the GABA<sub>A</sub> receptor, because SEV is directly affected by GABAergic substances.<sup>15</sup> Although the effect of diazepam was significantly smaller in the endometriosis group, as expected, the SEV decreased rapidly after diazepam injection in both groups. There is evidence to suggest that acute tolerance to the various pharmacologic effects of diazepam may develop after a single exposure to this drug.<sup>26</sup> Because the baseline SEV differed between the two groups, this difference cannot be explained solely by effects specific to diazepam, but rather as a chronic alteration of the GABA<sub>A</sub> receptor that is already present in women with endometriosis and associated pain. The present findings may thus be explained by altered GABA<sub>A</sub> receptor function, although we can only speculate on the underlying mechanism. Endometriosis typically presents with various pain symptoms, and there is a complex relation between stress and chronic pain. It is already known that stress, neurosteroids and GABA<sub>A</sub> receptor

function are intimately related.<sup>27</sup> Various stressful conditions, including pain, can acutely induce marked increases in the plasma and brain concentrations of neuroactive steroids, including allopregnanolone and allotetrahydrodeoxycorticosterone, and subsequently downregulate GABAergic transmission.<sup>28</sup> Neurosteroids are the most potent endogenous modulators of GABA<sub>A</sub> receptors, and their production is altered in many clinical conditions, including acute and chronic pain.<sup>29</sup> Increased allopregnanolone levels are associated with increasing GABA<sub>A</sub> receptor tolerance to GABAergic substances over time.<sup>30</sup> In contrast to these previous findings, we did not detect increased serum allopregnanolone levels or any correlation between GABA<sub>A</sub> receptor function and serum allopregnanolone levels in women with painful endometriosis.

Although cross-tolerance does not always occur to drugs that act at sites separated from the target site of the administered drug, chronic benzodiazepine treatment appears to decrease allosteric coupling of neuroactive steroid and benzodiazepine sites.<sup>31</sup> Furthermore, short-term benzodiazepine administration changes GABA<sub>A</sub> receptor function, and these changes impact modulatory sites differently, but it remains unclear whether acute diazepam administration causes cross-tolerance to the effect of neuroactive steroids or vice versa.<sup>32</sup> In this study, the lack of a correlation between peripheral allopregnanolone levels and GABA<sub>A</sub> receptor function could be explained by the fact that the GABA<sub>A</sub> receptors were stimulated with diazepam, not allopregnanolone, during the challenge test. Another explanation for the absence of correlation could be the small sample size in the groups.

Effects of acute and chronic stress also differ; acute stress is associated with elevated allopregnanolone levels,<sup>33</sup> whereas chronic stress has been shown to decrease GABA<sub>A</sub> receptor function and allopregnanolone levels.<sup>12</sup> Chronic pain has been associated with decreased serum allopregnanolone levels, and a previous study on war veterans in the USA showed that allopregnanolone levels were inversely correlated with pain severity in patients with chronic back pain.<sup>34</sup> Although women in the endometriosis group had significantly lower serum allopregnanolone levels compared with the healthy controls, conclusions from this comparison should be made very cautiously because the analysis methods differed between the two groups. Even though both methods are valid and well proven, this represents an apparent limitation to the study. Another limitation to the study is the small sample size. Even though we met the power needed, the small study size may still have an impact on the results. The fact that the control group did not undergo diagnostic laparoscopy to rule out asymptomatic endometriosis could be considered a limitation. As we find it unlikely that the endometriosis lesions themselves, but rather the pain associated with endometriosis, would be the cause of GABA<sub>A</sub>-receptor alterations, we found it sufficient to use controls without laparoscopy as long as the patients did not suffer from any pain symptoms associated with endometriosis.

Age and body mass index may also affect SEV, GABA<sub>A</sub> receptor function and serum allopregnanolone level.<sup>30,35</sup> However, the two groups did not differ with regard to these background characteristics.

Women with endometriosis are often hypersensitive to pain, indicating a possible change in the central nervous system leading to pain sensitization,<sup>4</sup> in which stimuli that normally do not evoke pain can cause an exaggerated perception of pain. Some studies have indicated that downregulation of GABA<sub>A</sub> receptors due to painful conditions is probably responsible for chronic pain later in life.<sup>36</sup> If the initial pain experienced by patients with endometriosis affects GABA<sub>A</sub> receptor function over time, the reduction in GABA<sub>A</sub> receptor function may lead to worse pain symptoms and central sensitization, which could explain the development of neuropathic pain in women with endometriosis. Increasing evidence suggests that GABA may play a role in neuropathic pain, and the loss of GABAergic inhibition within the spinal pain circuits is believed to be involved in the development of central sensitization.<sup>37</sup> Functional magnetic resonance imaging studies have revealed altered GABA levels in various regions of the brain in patients with different pain conditions.<sup>38</sup> A recent study on post-stroke pain in an animal model revealed that thalamic GABA<sub>A</sub> receptor expression and allopregnanolone levels decreased as allodynia developed after the insult. When allopregnanolone levels and GABA<sub>A</sub> receptor expression increased after intrathalamic injection of epoxyeicosatrienoic acid (an arachidonic acid metabolite involved in regulation of cerebral blood flow), the signs of allodynia were decreased. Interestingly, when allopregnanolone synthesis was inhibited or the GABA<sub>A</sub> receptor was blocked, allodynia persisted unchanged.<sup>39</sup>

## 5 | CONCLUSION

Women with endometriosis and associated pain show signs of impaired GABA<sub>A</sub> receptor function. This finding not only supports the concept of a central component and a neuropathic characteristic of the pain experienced by women with endometriosis, it also provides insight into the possible underlying mechanisms. To increase our understanding of the pain mechanisms in patients with endometriosis, further studies are needed to investigate how the levels of GABAergic substances and GABA<sub>A</sub> receptor function in the central nervous system are correlated with pain symptoms.

### AUTHOR CONTRIBUTIONS

AS, MB, TB and ST contributed substantially to the conception and planning of the study. AS, ST and AM collected the data. The data analysis was performed by AS and ST. AS, MB, TB and ST contributed to the interpretation of the data. AS wrote the first draft of the paper. The paper was revised by MB, TB, AM and ST together with AS. All five authors approved the final submitted version of the article.

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### CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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### REFERENCES

- Ye M, Guo H, Han J, et al. Relationship between endometriosis stage, characteristics of endometriotic lesions and severity of dysmenorrhoea. *Zhonghua Yi Xue Za Zhi*. 2015;95:685-688.
- Morotti M, Vincent K, Becker CM. Mechanisms of pain in endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2017;209:8-13.
- Sandström A, Bixo M, Johansson M, Bäckström T, Turkmen S. Effect of hysterectomy on pain in women with endometriosis: a population-based registry study. *BJOG*. 2020;127:1628-1635.
- Grundström H, Gerdle B, Alehagen S, Berterö C, Arendt-Nielsen L, Kjølhed P. Reduced pain thresholds and signs of sensitization in women with persistent pelvic pain and suspected endometriosis. *Acta Obstet Gynecol Scand*. 2019;98:327-336.
- Henderson LA, Peck CC, Petersen ET, et al. Chronic pain: lost inhibition? *J Neurosci*. 2013;33:7574-7582.
- Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA(a) receptors. *Neuropharmacology*. 2002;43:651-661.
- Pesold C, Caruncho HJ, Impagnatiello F, et al. Tolerance to diazepam and changes in GABA(a) receptor subunit expression in rat neocortical areas. *Neuroscience*. 1997;79:477-487.
- Baulieu EE, Robel P. Neurosteroids: a new brain function? *J Steroid Biochem Mol Biol*. 1990;37:395-403.
- Martinez PE, Rubinow DR, Nieman LK, et al. 5 $\alpha$ -reductase inhibition prevents the luteal phase increase in plasma allopregnanolone levels and mitigates symptoms in women with premenstrual dysphoric disorder. *Neuropsychopharmacology*. 2016;41:1093-1102.
- Lader M. Clinical pharmacology of benzodiazepines. *Annu Rev Med*. 1987;38:19-28.
- Cowley DS, Roy-Byrne PP, Radant A, et al. Benzodiazepine sensitivity in panic disorder: effects of chronic alprazolam treatment. *Neuropsychopharmacology*. 1995;12:147-157.
- Serra M, Pisu MG, Littera M, et al. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(a) receptor function in rat brain. *J Neurochem*. 2000;75:732-740.
- Meyer L, Taleb O, Patte-Mensah C, Mensah-Nyagan AG. Neurosteroids and neuropathic pain management: basic evidence and therapeutic perspectives. *Front Neuroendocrinol*. 2019;55:100795.
- Gentles W, Thomas EL. Commentary. Effect of benzodiazepines upon saccadic eye movements in man. *Clin Pharmacol Ther*. 1971;12:563-574.
- Hommer DW, Matsuo V, Wolkowitz O, et al. Benzodiazepine sensitivity in normal human subjects. *Arch Gen Psychiatry*. 1986;43:542-551.
- Sundstrom I, Nyberg S, Backstrom T. Patients with premenstrual syndrome have reduced sensitivity to midazolam compared to control subjects. *Neuropsychopharmacology*. 1997;17:370-381.
- Möller AT, Bäckström T, Nyberg S, Söndergaard HP, Helström L. Women with PTSD have a changed sensitivity to GABA-A receptor active substances. *Psychopharmacology (Berl)*. 2016;233:2025-2033.
- Becker CM, Bokor A, Heikinheimo O, et al. ESHRE guideline: endometriosis. *Hum Reprod Open*. 2022;2022:hoac009.
- Tiihonen Möller ABT, Söndergaard HP, Kushnir MM, Bergquist J, Helström L. Diurnal variations of endogenous steroids in the follicular phase of the menstrual cycle. *Neurochem Neuropharm*. 2016;2:1-6.

20. Wilson SJ, Glue P, Ball D, Nutt DJ. Saccadic eye movement parameters in normal subjects. *Electroencephalogr Clin Neurophysiol*. 1993;86:69-74.
21. Timby E, Balgard M, Nyberg S, et al. Pharmacokinetic and behavioral effects of allopregnanolone in healthy women. *Psychopharmacology (Berl)*. 2006;186:414-424.
22. Keski-Rahkonen P, Huhtinen K, Poutanen M, Auriola S. Fast and sensitive liquid chromatography-mass spectrometry assay for seven androgenic and progestagenic steroids in human serum. *J Steroid Biochem Mol Biol*. 2011;127:396-404.
23. Stokes PR, Benecke A, Myers J, et al. History of cigarette smoking is associated with higher limbic GABAA receptor availability. *Neuroimage*. 2013;69:70-77.
24. Stanczyk FZ, Lee JS, Santen RJ. Standardization of steroid hormone assays: why, how, and when? *Cancer Epidemiol Biomarkers Prev*. 2007;16:1713-1719.
25. Rosenhall U, Johansson G, Orndahl G. Eye motility dysfunction in chronic primary fibromyalgia with dysesthesia. *Scand J Rehabil Med*. 1987;19:139-145.
26. Ellinwood EH Jr, Linnoila M, Easler ME, Molter DW. Profile of acute tolerance to three sedative anxiolytics. *Psychopharmacology (Berl)*. 1983;79:137-141.
27. Biggio G, Concas A, Corda MG, Giorgi O, Sanna E, Serra M. GABAergic and dopaminergic transmission in the rat cerebral cortex: effect of stress, anxiolytic and anxiogenic drugs. *Pharmacol Ther*. 1990;48:121-142.
28. Barbaccia ML, Roscetti G, Trabucchi M, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. *Neuroendocrinology*. 1996;63:166-172.
29. Rustichelli C, Bellei E, Bergamini S, et al. Serum levels of allopregnanolone, progesterone and testosterone in menstrually-related and postmenopausal migraine: a cross-sectional study. *Cephalalgia*. 2020;40:1355-1362.
30. Hedström H, Bäckström T, Bixo M, et al. Women with polycystic ovary syndrome have elevated serum concentrations of and altered GABA(a) receptor sensitivity to allopregnanolone. *Clin Endocrinol (Oxf)*. 2015;83:643-650.
31. Friedman LK, Gibbs TT, Farb DH. Gamma-aminobutyric acidA receptor regulation: heterologous uncoupling of modulatory site interactions induced by chronic steroid, barbiturate, benzodiazepine, or GABA treatment in culture. *Brain Res*. 1996;707:100-109.
32. Gerak LR, McMahon LR, France CP. Acute cross tolerance to midazolam, and not pentobarbital and pregnanolone, after a single dose of chlordiazepoxide in monkeys discriminating midazolam. *Behav Pharmacol*. 2008;19:796-804.
33. Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type a receptor-active steroids in the rat brain. *Proc Natl Acad Sci U S A*. 1991;88:4553-4557.
34. Kilts JD, Tupler LA, Keefe FJ, et al. Neurosteroids and self-reported pain in veterans who served in the U.S. military after September 11, 2001. *Pain Med*. 2010;11:1469-1476.
35. Takahashi J, Hirano Y, Miura K, et al. Eye movement abnormalities in major depressive disorder. *Front Psych*. 2021;12:673443.
36. Janssen SP, Truin M, Van Kleef M, Joosten EA. Differential GABAergic disinhibition during the development of painful peripheral neuropathy. *Neuroscience*. 2011;184:183-194.
37. Zeilhofer HU, Wildner H, Yévenes GE. Fast synaptic inhibition in spinal sensory processing and pain control. *Physiol Rev*. 2012;92:193-235.
38. Peek AL, Leaver AM, Foster S, et al. Increased GABA+ in people with migraine, headache, and pain conditions—a potential marker of pain. *J Pain*. 2021;22:1631-1645.
39. Chen X, Li Z, Zhang B, et al. Alleviation of mechanical allodynia by 14,15-Epoxyeicosatrienoic acid in a central poststroke pain model: possible role of allopregnanolone and  $\delta$ -subunit-containing gamma-aminobutyric acid a receptors. *J Pain*. 2019;20:577-591.

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