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*Original Article*

# The sedation effect of electro-acupuncture on Bilateral Zusanli (ST 36) and Neiguan (PC 6) in general anesthesia may not be mediated by the benzodiazepines-GABA pathway

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**Abstract:** Objective: To explore the mechanisms of electro-acupuncture (EA) induced sedation during general anesthesia through investigating the role of the benzodiazepines-GABA signaling pathway. Method: 80 patients undergoing abdominal surgery were randomly divided into four groups (n=20): C group (propofol only group), F group (propofol + flumazenil group), EA group (propofol + electro-acupuncture group), and EA + F group (propofol + electro-acupuncture + flumazenil group). Before induction, acupuncture needles were inserted at the points of Zusanli (ST 36) and Neiguan (PC 6) bilaterally to elicit "DeQi". Target-controlled infusion (TCI) of propofol was used for the induction and maintenance of anesthesia. After 15 minutes, equilibrium of Narcotrend Index (NT index) was achieved; patients were then assigned to receive different interventions. In EA group and EA + F group, patients received EA for 30 minutes under general anesthesia, and flumazenil with the dosage of 0.1 mg/kg was added in the latter at the end of EA. In C group and F group, patients did not receive EA, and flumazenil with the dosage of 0.1 mg/kg was administered in F Group at the same time point as EA + F group. Heart rate (HR), mean arterial pressure (MAP) and NT index were recorded at different time points. Result: There was a significant decrease in NT index of EA group 15 minutes after EA stimulation compared with control C group ($P<0.05$). To our surprise, after administering flumazenil, there was no significant difference in NT index between EA group and EA + F group ($P>0.05$). The HR and MAP between the two groups also showed no significant difference ($P>0.05$). Conclusion: The drop of NT index which induced by EA didn't reverse after administration of flumazenil. The effect of EA in deepening anesthesia may not be mediated by the benzodiazepines-GABA signaling pathway.

**Keywords:** Acupuncture, flumazenil, narcotrend, general anesthesia, sedation

## Introduction

Acupuncture and related techniques can serve as important adjuvants in perioperative care [1]. Electro-acupuncture (EA) could effectively lower the minimal alveolar concentration of enflurane (1.67 to 1.15 Vol%), and reduce the inhalation dosage of enflurane (29.73%) for oesophagectomy [2]. Other studies on animals and patients also showed similar results [3, 4]. In our recent study, EA at bilateral Zusanli (ST36) and Neiguan (PC6) significantly increased the depth of general anesthesia, as measured by Narcotrend index [5]. However, the underlying mechanisms for EA's sedation effects are still unclear.

GABA receptors (GABA-Rs) are the major inhibitory receptors in the central nervous system (CNS). The typical $GABA_A$ receptors are positively modulated by benzodiazepines, which allosterically enhance the inhibitory actions of GABA by binding to the modulatory site. This plays an important role in sedation/hypnotic and anxiolytic effects of benzodiazepines [6]. A previous study showed that EA at H7 significantly attenuated morphine withdrawal syndrome via activation of the $GABA_A$ receptor and

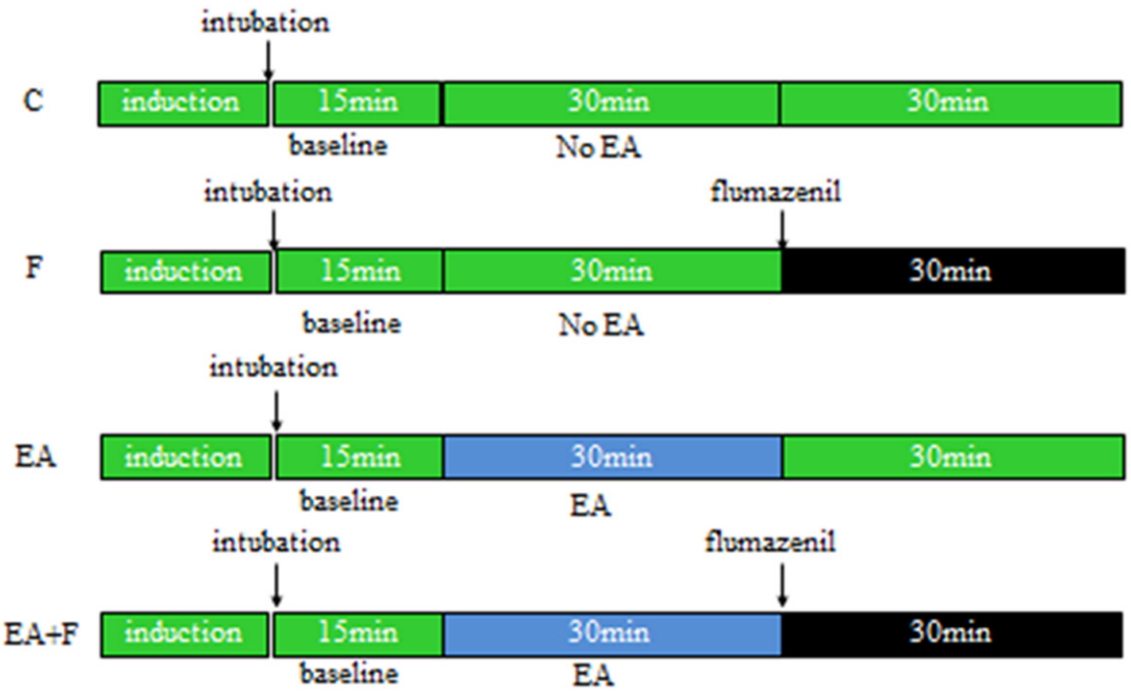


**Figure 1.** Study design. Patients were randomly assigned to four groups: C group, F group, EA group, EA + F group.

this effect was blocked by the GABA-R antagonist [7]. In a recent publication, EA on Zusanli (ST36) and Neiguan (PC6) of insomniac rats can considerably up-regulate the expression of hypothalamic GABA and $GABA_A$ receptor [8]. These data suggests EA may enhance sedation depth through the classic benzodiazepine-GABA signaling pathway.

Flumazenil is an imidazole benzodiazepine that promptly reverses the sedative effect of benzodiazepines through competitive inhibition on GABA receptors [9]. When administered alone, flumazenil's effect is limited. Midazolam is a benzodiazepine that can significantly lower the bispectral index (BIS), an index for sedation deepness. Flumazenil can be used in clinic to rescue patients that are in a deep sedation state under the influence of midazolam and help to bring the BIS index to normal [10].

In the present study, we hypothesize that EA's effect on deepening anesthesia is similar to benzodiazepine, which acts on the GABA system, and can also be blocked by flumazenil. By observing the change of sedation index before and after flumazenil administration, we intend to find the impact of flumazenil on EA assisted anesthesia and uncover clues on the mechanism of EA in general anesthesia.

## Methods

*Patient and method*

This study was approved by medical ethics committee of Sichuan cancer hospital, and all patients who agreed to participate signed informed consent. 80 abdominal surgical patients aged 30 to 60 years old, weighing 50-70 Kg, ASA I-II, who scheduled to receive general anesthesia were enrolled to this trial. Exclusion criteria were 1. Hypertension or severe heart and lung diseases, 2. Conditions that might affect the consciousness or NT index, such as seizures, psychosis, brain lesions, and treatment with sedative drugs, 3. Contraindications to EA, such as severe hemorrhagic disease, hyper-sensitive patient, and tumor or ulcer exist on the skin of the acupuncture points.

Using a computer-generated program, 80 patients were randomly assigned to four groups (n=20): C group (propofol only group), F group (propofol + flumazenil group), EA group (propofol + electro-acupuncture group), and EA + F group (propofol + electro-acupuncture + flumazenil group). The study protocol is schematically illustrated in **Figure 1**.

**Table 1.** Comparison of the general information of the patients in four groups ($\bar{x} \pm s$) (n=20 in each group)

| Groups | Sex ratio (Cases, M/F) | Age (years) | Height (m) | Weight (Kg) |
|---|---|---|---|---|
| EA | 8/12 | 48.5 ± 6.6 | 1.588 ± 0.083 | 61.167 ± 9.154 |
| C | 7/13 | 45.7 ± 7.4 | 1.573 ± 0.054 | 56.167 ± 7.056 |
| EA + F | 8/12 | 46.8 ± 6.7 | 1.592 ± 0.077 | 59.430 ± 7.415 |
| P + F | 8/12 | 47.2 ± 6.6 | 1.596 ± 0.063 | 58.480 ± 6.901 |

C group: propofol induced general anesthesia, without electro-acupuncture or flumazenil. F group: C + flumazenil. EA group: C + 30 min electro-acupuncture. EA + F group: C + 30min electro-acupuncture + flumazenil at the end of electro-acupuncture.

The allocation information (group C, F, EA or EA + F) was placed in 80 numbered, sealed, opaque envelopes. After intubation, a neutral anesthesia assistant would open an envelope, and assign the group number. The general anesthesia and EA of the patients was implemented by the same and well-trained anesthesiologist. The researchers who were responsible for the data collection and analysis were blinded to the participant group assignments.

The patients were brought to the induction room. Three disposable electrodes were placed on the forehead of each patient. These electrodes were attached to a Narcotrend monitor (Narcotrend-Compact, Schiller, Switzerland), for continuous recording the NT indices.

Before intubation, all patients received EA at the points of *Zusanli* (ST 36) and *Neiguan* (PC 6) bilaterally. If the needle reached the exact point, patients would have a special feeling, called "*De Qi*".

After establishment of the intravenous catheter, an anesthesiologist intubated for all patients. Intravenous administration of fentanyl in 3 ug/kg, rocuronium in 0.9 mg/kg, and target-controlled infusion (TCI) of propofol in 3 ug/ml of the target concentration of effect-compartment (Ce) were used achieve general anesthesia. Patients were then intubated. TCI is a software based primarily on the Marsh pharmacokinetic model. After we input the age, height, weight, gender and the necessary drug concentration, the software will calculate the proper infusion dose and speed to reach the target concentration at the effect site [11]. After intubation, the propofol Ce was adjusted to 1.5 ug/ml to maintain anesthetic state, and this act as a control in this study.

*Interventions*

After 15 minutes stabilization of NT index, patients were assigned to receive different interventions according to the groups' allocation. Patients who were subjected to 60 min of continuous propofol infusion, without EA or flumazenil were set as the control group. Patients with 30 min EA stimulation were allocated to EA group. Acupuncture needles were connected to HANS-200A electro-acupuncture device (Gensun Medical, Nanjing, China), with a stimulus intensity of 2 mA, diffuse/dense wave frequency of 2/15 Hz. Patients with or without EA stimulation, who received intravenously administered flumazenil (0.1 mg/kg) at the end of EA stimulation, were assigned to EA + F group and F group, respectively. As shown in **Figure 1**. The endpoint of the study was set at 30 minutes after EA stimulation, and then the patients carried on with their respective operations.

In each group, we observed the heart rate (HR), mean arterial pressure (MAP) and NT index values at a total of 18 time points: before induction, after induction, immediately after intubation, 5 min, 10 min, 15 min after intubation, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min after onset of EA stimulation and 5 min, 10 min, 15 min, 20 min, 25 min, 30 min after EA. We calculated the average value of NT index of 10 min and 15 min after induction as the equilibrium NT values. $NT_{equilibrium}=(NT_{10\ min} + NT_{15\ min})/2$. This value was used for data calculation and analysis.

*Data analysis*

The aim of the statistical analysis was to compare the HR, MAP, NT index values of the 18 time points among the four groups. All of the values are presented as mean ± SE. One-way ANOVA was applied for the comparisons between two groups at each time point. In each group, The NT indices were compared with $NT_{equilibrium}$ using t-test. The values after administering flumazenil between EA and EA + F group were analyzed using the General Linear Model (GLM) with repeated measures (SPSS 16.0). $P<0.05$ were considered statistically significant.

**Table 2.** The NT Indices at different time points in EA & C groups ($\bar{x}$ ± SE) (n=20 in each group)

| Time Points | EA Group | *p* (EA)[1] | C Group | *p* (C)[2] | *p* (EA vs C)[3] |
|---|---|---|---|---|---|
| Before induction | 98.60 ± 0.112 | | 98.70 ± 0.128 | | 0.56 |
| After induction | 63.55 ± 2.969 | | 66.90 ± 2.235 | | 0.37 |
| Intubation | 58.50 ± 3.927 | | 59.45 ± 2.354 | | 0.84 |
| Intubation 5 min | 69.00 ± 2.420 | | 69.50 ± 2.294 | | 0.88 |
| Intubation 10 min | 68.80 ± 2.032 | | 68.10 ± 1.783 | | 0.80 |
| Intubation 15 min | 64.80 ± 2.439 | | 66.90 ± 1.765 | | 0.49 |
| Equilibrium Value | 67.00 ± 2.209 | | 67.65 ± 1.572 | | |
| | | | | | 0.01*,[4] |
| Stimulation 5 min | 62.45 ± 2.343 | 0.17 | 65.55 ± 1.730 | 0.37 | 0.29 |
| Stimulation 10 min | 60.35 ± 2.580 | 0.06 | 63.50 ± 1.902 | 0.10 | 0.33 |
| Stimulation 15 min | 56.55 ± 2.389 | 0.00* | 63.65 ± 1.907 | 0.11 | 0.03* |
| Stimulation 20 min | 56.30 ± 2.111 | 0.00* | 64.10 ± 2.211 | 0.20 | 0.01* |
| Stimulation 25 min | 53.60 ± 2.104 | 0.00* | 65.00 ± 2.255 | 0.34 | 0.00* |
| Stimulation 30 min | 54.45 ± 2.348 | 0.00* | 64.65 ± 2.112 | 0.26 | 0.00* |
| Stop 5 min | 54.55 ± 2.178 | 0.00* | 64.95 ± 2.257 | 0.33 | 0.00* |
| Stop 10 min | 56.45 ± 2.154 | 0.00* | 65.10 ± 1.762 | 0.29 | 0.00* |
| Stop 15 min | 58.90 ± 2.864 | 0.03* | 65.90 ± 1.775 | 0.46 | 0.04* |
| Stop 20 min | 59.35 ± 2.355 | 0.02* | 67.05 ± 1.882 | 0.80 | 0.01* |
| Stop 25 min | 59.10 ± 2.694 | 0.02* | 67.00 ± 1.355 | 0.76 | 0.01* |
| Stop 30 min | 57.65 ± 2.554 | 0.00* | 66.40 ± 2.353 | 0.66 | 0.02* |

1. Comparing NT index values with the equilibrium value in EA group by t test. 2. Comparing NT index values with the equilibrium value in C group by t test. 3. Comparing NT index values at 18 time points between two groups by one-way ANOVA. 4. Comparing NT index values at time points after EA stimulation between two groups by GLM with repeated measures. *: *P*<0.05 represents statistical significance.

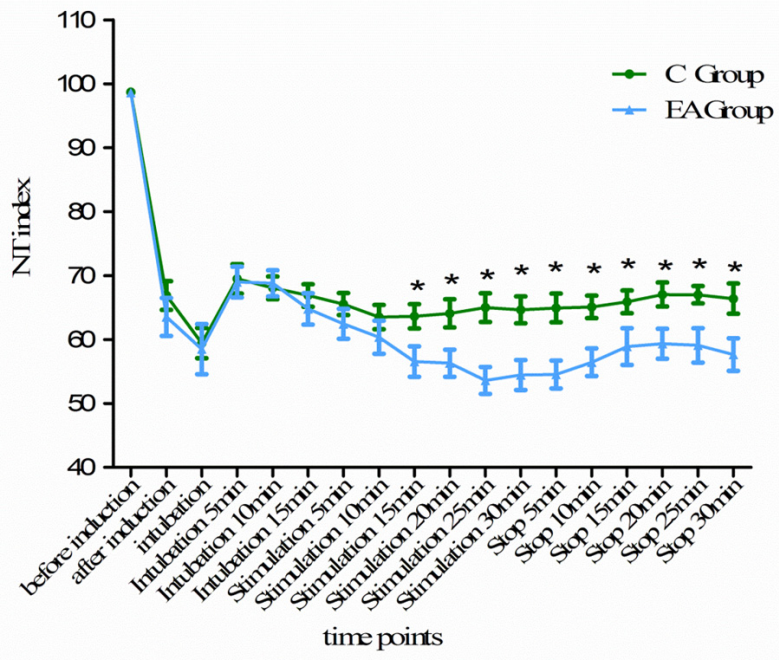


**Figure 2.** Comparison of Narcotrend index (NT index) values between EA group and C group. EA, electro-acupuncture. *: *P*<0.05.

## Results

The general characteristics including the age, sex, height, weight were similar among the groups, as shown in **Table 1**.

*EA at bilateral ST6 and PC6 increased the depth of general anesthesia*

After 15 minutes of EA stimulation, EA group showed significantly decreased NT indices compared with C group (*P*<0.05). And the NT index were significantly lower than the equilibrium value of EA at 15 min onward, as shown in **Table 2** and **Figure 2**.

*Flumazenil has no impact on the effect of EA in deepening anesthesia*

As expected, there were no statistically differences in NT index between C group and F group, after administering flumazenil, as shown in **Table 3** and **Figure 3**. Comparing with EA group, the NT index values in EA + F group showed no significant differences, after administering flumazenil (*P*>0.05), and the NT index values were not restored. The NT index were still lower than the equilibrium value (*P*< 0.05), as shown in **Table 4** and **Figure 4**. The HR and MAP between the two groups showed no clinical significance as shown in **Table 5**.

## Discussion

Flumazenil is a specific antagonist of benzodiazepine, which can competitive inhibit GABA receptors. It is widely used for reversing the over sedation of midazolam and accelerating the recovery of

**Table 3.** The NT Indices at different time points in F & C groups ($\bar{x} \pm$ SE) (n=20 in each group)

| Time Points | F Group | *p* (F)[1] | C Group | *p* (C)[2] | *p* (F vs C)[3] |
|---|---|---|---|---|---|
| Before induction | 98.00 ± 0.241 | | 98.70 ± 0.128 | | 0.02* |
| After induction | 63.00 ± 2.757 | | 66.90 ± 2.235 | | 0.28 |
| Intubation | 61.30 ± 2.636 | | 59.45 ± 2.354 | | 0.60 |
| Intubation 5 min | 69.35 ± 1.769 | | 69.50 ± 2.294 | | 0.96 |
| Intubation 10 min | 66.65 ± 1.482 | | 68.10 ± 1.783 | | 0.54 |
| Intubation 15 min | 64.30 ± 1.543 | | 66.90 ± 1.765 | | 0.27 |
| Equilibrium Value | 65.65 ± 1.435 | | 67.65 ± 1.572 | | |
| Stimulation 5 min | 64.40 ± 1.677 | 0.57 | 65.55 ± 1.730 | 0.37 | 0.64 |
| Stimulation 10 min | 62.90 ± 1.739 | 0.23 | 63.50 ± 1.902 | 0.10 | 0.82 |
| Stimulation 15 min | 63.50 ± 1.618 | 0.33 | 63.65 ± 1.907 | 0.11 | 0.95 |
| Stimulation 20 min | 63.00 ± 1.588 | 0.22 | 64.10 ± 2.211 | 0.20 | 0.69 |
| Stimulation 25 min | 62.45 ± 1.608 | 0.15 | 65.00 ± 2.255 | 0.34 | 0.36 |
| Stimulation 30 min | 64.10 ± 1.490 | 0.46 | 64.65 ± 2.112 | 0.26 | 0.83 |
| | | | | | 0.33[4] |
| Stop 5 min | 62.20 ± 1.651 | 0.12 | 64.95 ± 2.257 | 0.33 | 0.64 |
| Stop 10 min | 63.15 ± 1.840 | 0.29 | 65.10 ± 1.762 | 0.29 | 0.45 |
| Stop 15min | 63.00 ± 1.750 | 0.25 | 65.90 ± 1.775 | 0.46 | 0.25 |
| Stop 20 min | 63.45 ± 1.916 | 0.36 | 67.05 ± 1.882 | 0.80 | 0.18 |
| Stop 25 min | 64.25 ± 1.675 | 0.53 | 67.00 ± 1.355 | 0.76 | 0.21 |
| Stop 30 min | 64.60 ± 1.550 | 0.62 | 66.40 ± 2.353 | 0.66 | 0.53 |

1. Comparing NT index values with the equilibrium value in F group by t test. 2. Comparing NT index values with the equilibrium value in C group by t test. 3. Comparing NT index values at 18 time points between two groups by one-way ANOVA. 4. Comparing NT index values at time points after administering flumazenil between two groups by GLM with repeated measures. *: $P<0.05$ for the statistical significance.

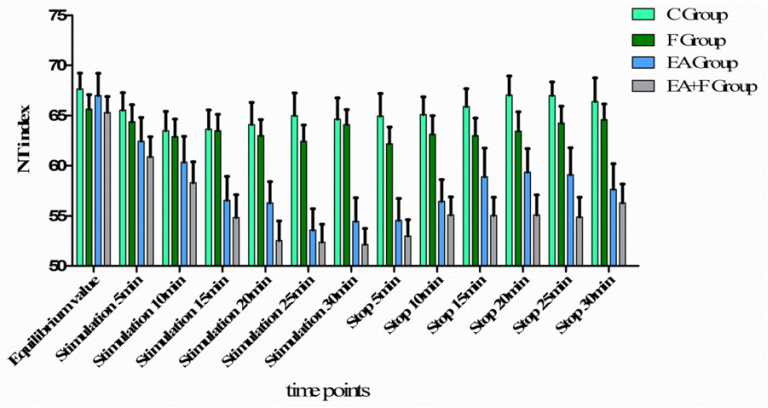


**Figure 3.** Comparison of Narcotrend index (NT index) values among four groups. EA, electro-acupuncture. F, flumazenil.

patients from deep anesthesia [9]. Recently, flumazenil was also found to be effective in treatment of idiopathic hypersomnia, which was considered to be related to the production of a molecule that facilitates the binding of GABA to the GABA receptors [11]. So flumazenil may be a worthy candidate to study the sedative effects of EA.

Weinbroum found that intravenous bolus injections of flumazenil 0.1 to 0.3 mg are effective in the diagnosis and treatment of pure benzodiazepine overdose [12]. In Other trails, the dosage of flumazenil was chosen at 0.3-0.5 mg as an antagonist [13, 14]. Considering the patients difference in weight, we chose 0.01 mg/kg for a pilot study. In that study, 5 patients were maintained in an anesthetic state by continuous infusion of midazolam. After the NT index was stabilized for 30 min, the patients received 0.01 mg/kg of flumazenil. Within 4 minutes, the NT index significantly increased (data not shown), accompanied by increased heart rate and blood pressure, which suggests that 0.01 mg/kg of flumazenil was a sufficient dose under these experimental conditions.

In our present study, Narcotrend index was used as a main indicator to observe the impact of flumazenil on the EA stimulation in deepening anesthesia. The Narcotrend is an EEG monitor designed to measure the depth of anesthesia, which includes a dimensionless Narcotrend index from 100 (awake) to 0 (electrical silence). It is approved by the US Food and Drug Administration. Kreuer *et al* [15] found that Narcotrend index detected differences in EEG dynamics as well as BIS. Otto *et al* [16] concluded that in consideration of sensitivity (71.4%) and specificity (97.44%), Narcotrend index seems to

**Table 4.** The NT Indices at different time points in EA & EA + F groups ($\bar{x}$ ± SE) (n=20 in each group)

| Time Points | EA Group | *p* (EA)[1] | EA + F Group | *p* (EA + F)[2] | *p* (EA vs EA + F)[3] |
|---|---|---|---|---|---|
| Before induction | 98.60 ± 0.112 | | 98.40 ± 0.169 | | 0.33 |
| After induction | 63.55 ± 2.969 | | 59.00 ± 2.598 | | 0.26 |
| Intubation | 58.50 ± 3.927 | | 57.00 ± 2.762 | | 0.76 |
| Intubation 5 min | 69.00 ± 2.420 | | 67.65 ± 1.935 | | 0.67 |
| Intubation 10 min | 68.80 ± 2.032 | | 66.25 ± 1.624 | | 0.33 |
| Intubation 15 min | 64.80 ± 2.439 | | 63.90 ± 1.679 | | 0.76 |
| Equilibrium Value | 67.00 ± 2.209 | | 65.30 ± 1.591 | | |
| Stimulation 5 min | 62.45 ± 2.343 | 0.17 | 60.90 ± 1.997 | 0.09 | 0.62 |
| Stimulation 10 min | 60.35 ± 2.580 | 0.06 | 58.30 ± 2.095 | 0.01* | 0.54 |
| Stimulation 15 min | 56.55 ± 2.389 | 0.00* | 54.85 ± 2.252 | 0.00* | 0.61 |
| Stimulation 20 min | 56.30 ± 2.111 | 0.00* | 52.55 ± 1.930 | 0.00* | 0.20 |
| Stimulation 25 min | 53.60 ± 2.104 | 0.00* | 52.40 ± 1.770 | 0.00* | 0.66 |
| Stimulation 30 min | 54.45 ± 2.348 | 0.00* | 52.15 ± 1.598 | 0.00* | 0.42 |
| | | | | | 0.31[4] |
| Stop 5 min | 54.55 ± 2.178 | 0.00* | 53.00 ± 1.619 | 0.00* | 0.57 |
| Stop 10 min | 56.45 ± 2.154 | 0.00* | 55.10 ± 1.786 | 0.00* | 0.63 |
| Stop 15 min | 58.90 ± 2.864 | 0.03* | 55.05 ± 1.809 | 0.00* | 0.26 |
| Stop 20 min | 59.35 ± 2.355 | 0.02* | 55.10 ± 1.993 | 0.00* | 0.19 |
| Stop 25 min | 59.10 ± 2.694 | 0.02* | 54.90 ± 1.961 | 0.00* | 0.22 |
| Stop 30 min | 57.65 ± 2.554 | 0.00* | 56.30 ± 1.874 | 0.00* | 0.67 |

1. Comparing NT index values with the equilibrium value in EA group by t test. 2. Comparing NT index values with the equilibrium value in EA + F group by t test. 3. Comparing NT index values at 18 time points between two groups by one-way ANOVA. 4. Comparing NT index values at time points after administering flumazenil between two groups by GLM with repeated measures. *: $P<0.05$ represents statistical significance.

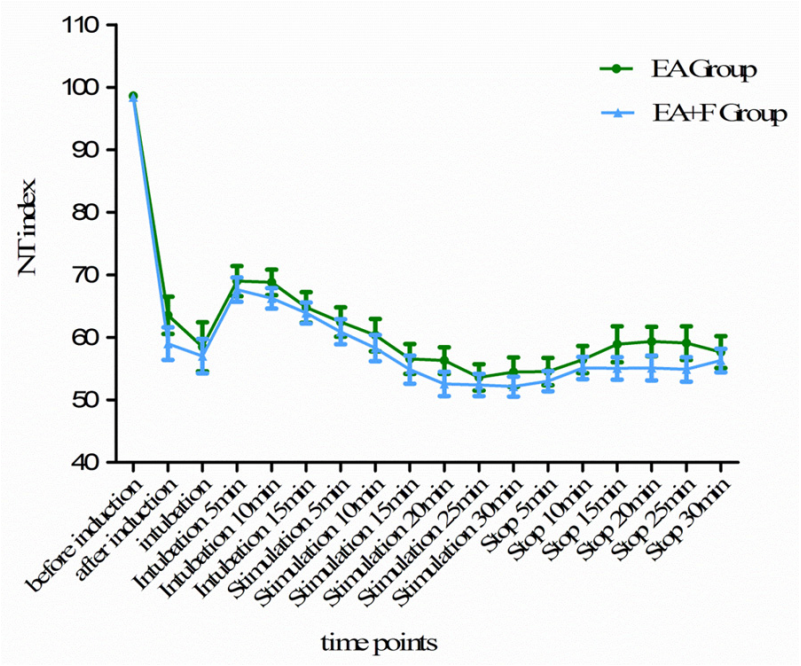


**Figure 4.** Comparison of Narcotrend index (NT index) values between EA group and EA + F group. EA, electro-acupuncture. F, flumazenil.

be the most appropriate EEG descriptor to assure adequate depth of anesthesia in isoflurane-anaesthetized sheep. Comparing with other EEG parameters, Grouven U [17] found Narcotrend Index showed the highest correlation with the propofol effect-site concentration and the lowest variability of individual correlation values. This study also showed that only the Narcotrend Index had a monophasic behavior during the analysis period. Liu SH [18] *et al* also found the values of Narcotrend index had a good linear correlation with OAA/S in Chinese people (R=0.938). The data above demonstrated and fully support that Narcotrend index can coherently reflect the sedative level of anesthesia in our study.

We used TCI of propofol with Ce 1.5 ug/ml to maintain anesthesia. The time point for administering flumazenil was 45 min after intubation. This time interval has exceeded the half-life of fentanyl, therefore the amount of fentanyl in the patient's body can be neglected. In addition, opioids in the usual clinical doses showed no effects on BIS monitoring [14], so fentanil would scarcely affect the result. In addition, flumazenil was thought to have limited intrinsic activities. Previous study shows that, 0.3 mg of flumazenil administered after midazolam led to a prompt restoration of acoustical and somatosensory-evoked cortical responses in volunteers. But flumazenil ad-

**Table 5.** Comparison of the HR and MAP of EA& EA + F groups at different time points ($\bar{x}$ ± SE) (n=20 in each group)

| Time Points | HR (bmp) | | | MAP (mmHg) | | |
|---|---|---|---|---|---|---|
| | EA Group | EA + F Group | *p* (HR)[1] | EA Group | EA + F Group | *p* (MAP)[2] |
| Before induction | 86.70 ± 3.235 | 90.80 ± 2.184 | 0.30 | 101.5 ± 2.569 | 90.80 ± 2.184 | 0.00* |
| After induction | 73.65 ± 3.026 | 78.40 ± 3.016 | 0.27 | 85.45 ± 2.817 | 78.40 ± 3.016 | 0.10 |
| Intubation | 95.70 ± 3.475 | 94.65 ± 3.877 | 0.84 | 101.6 ± 4.566 | 94.65 ± 3.877 | 0.26 |
| intubation 5 min | 77.50 ± 3.041 | 69.55 ± 1.636 | 0.03* | 77.20 ± 2.026 | 69.55 ± 1.636 | 0.01* |
| intubation 10 min | 71.60 ± 2.605 | 68.40 ± 1.463 | 0.29 | 72.65 ± 1.666 | 68.40 ± 1.463 | 0.06 |
| intubation 15 min | 69.95 ± 2.469 | 67.40 ± 1.510 | 0.39 | 73.05 ± 1.999 | 67.40 ± 1.510 | 0.03* |
| Stimulation 5 min | 68.95 ± 2.499 | 68.40 ± 1.597 | 0.85 | 72.50 ± 2.620 | 68.40 ± 1.597 | 0.19 |
| Stimulation 10 min | 67.60 ± 2.561 | 69.00 ± 1.503 | 0.64 | 70.40 ± 2.557 | 69.00 ± 1.503 | 0.64 |
| Stimulation 15 min | 67.70 ± 2.365 | 71.80 ± 1.456 | 0.15 | 71.55 ± 2.752 | 71.80 ± 1.456 | 0.94 |
| Stimulation 20 min | 68.05 ± 2.222 | 70.40 ± 1.598 | 0.40 | 71.05 ± 2.874 | 70.40 ± 1.598 | 0.85 |
| Stimulation 25 min | 68.10±2.222 | 70.55 ± 1.360 | 0.35 | 70.20 ± 2.464 | 70.55 ± 1.360 | 0.90 |
| Stimulation 30 min | 69.85±2.682 | 72.45 ± 1.413 | 0.40 | 69.25 ± 2.468 | 72.45 ± 1.413 | 0.27 |
| | | | 0.51[3] | | | 0.35[4] |
| Stop 5 min | 70.75 ± 2.021 | 70.50 ± 1.385 | 0.92 | 75.45 ± 3.448 | 70.50 ± 1.385 | 0.20 |
| Stop 10 min | 70.05 ± 2.058 | 73.00 ± 1.635 | 0.27 | 75.00 ± 2.007 | 73.00 ± 1.635 | 0.44 |
| Stop 15 min | 70.95 ± 1.966 | 73.30 ± 1.786 | 0.38 | 75.70 ± 1.898 | 73.30 ± 1.786 | 0.36 |
| Stop 20 min | 70.15 ± 2.019 | 72.55 ± 1.806 | 0.38 | 74.95 ± 1.598 | 72.55 ± 1.806 | 0.33 |
| Stop 25 min | 70.60 ± 1.864 | 72.00 ± 1.732 | 0.59 | 74.65 ± 1.931 | 72.00 ± 1.732 | 0.31 |
| Stop 30 min | 70.85 ± 1.783 | 73.40 ± 1.977 | 0.34 | 76.10 ± 1.778 | 73.40 ± 1.977 | 0.32 |

1. Comparing HR at 18 time points between two groups by one-way ANOVA. 2. Comparing MAP at 18 time points between two groups by one-way ANOVA. 3. Comparing HR at time points after administering flumazenil between two groups by GLM with repeated measures. 4. Comparing MAP at time points after administering flumazenil between two groups by GLM with repeated measures. *: $P<0.05$ represents statistical significance.

ministered without benzodiazepines did not change acoustical or somatosensory-evoked cortical responses [19]. On the other hand, Ashraf A [14] proposed that, flumazenil will raise the BIS index in propofol treated patients. That study, however, was quite different compared with our current study. Their experimental data was collected during surgery, in which the sedation levels were quite different, and the interference by the surgery procedures, and other anesthetics would considerably affect the results. On the other hand, our study was conducted before the surgery, and only used propofol TCI to maintain a stable sedation, so our results would have less variables and therefore more reliable. According to our observations, the NT index value with or without flumazenil between F group and C group showed no significant differences, which excluded the influence of flumazenil on propofol, and made the comparison between EA and EA + F group more reliable.

We hypothesized that EA induced sedation was a result of GABA receptor activation. However, to our surprise, in the current study flumazenil didn't reverse the EA induced deep sedation during general anesthesia. In other words, EA was still able to induce deep anesthesia in the presence of flumazenil. This supports the idea that the mechanism of EA on sedation does not depends on the GABA-benzodiazepines signalling pathway.

Other neurotransmitters may be involved in the sedative effects of EA including adrenergic pathways and opioid related pathways. Studies demonstrated that norepinephrine in CNS exert wake-promoting actions via β- and α1-receptors. Blockade of β- and α1-receptors or suppression of norepinephrine release led to profound sedation [20]. Another study concluded that the sedation effect of acupuncture at GV20 and Yintang acupoint may be partially related to the α2-adrenergic system, because of the remarkable increase of SEF95 (electroencephalographic spectral edge frequency) after administration of atipamezole, an α2-receptor antagonist [21]. Studies also suggested the opioidergic neurotransmissions may play an

important role in maintaining sleep. Chen [22] reported that EA of Anmian acupoints increased non-rapid eye movement sleep. And it may be partially mediated by cholinergic activation and stimulation of the opiodergic neurons to secret β-endorphins which regulate sleep via μ-opioid receptors. Li *et al* [23] also showed EA at Zusanli (ST36) and Sanyinjiao (SP6) was effective in normalizing the Non-rapid eye movement sleep, rapid eye movement sleep and total sleep time in morphine withdrawal rats. And they presumed the mechanism was related to the facilitated release of endogenous opioids by EA. However, the exact mechanism of EA in sedation and deepening anesthesia has not been established, and further research are still required.

In conclusion, flumazenil showed no impact on the effect of EA induced deep anesthesia. The decreasing of NT index which induced by EA at *Zusanli* (ST 36) and *Neiguan* (PC 6) bilaterally couldn't be reversed after administration of flumazenil. This supports the idea that the effect of EA in sedation or deep anaesthesia may not be mediated by the benzodiazepines-GABA signalling pathway. Further studies are still required to promote the application of EA in perioperative care.

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## Disclosure of conflict of interest

None.

## Authors' contribution

SZ: conceived and designed the study, read and analyzed the documents, collected data and drafted the paper. HZ: conceived and designed the study, collected data and revised the manuscript. YT: advised the study, read and analyzed documents. YC: conducted the document search, read and analyzed the documents, edited the paper and given final approval of the version to be published. YL: advised the study, conducted the document search, read and analyzed the documents, edited the paper and given final approval of the version to be published. All authors take responsibility for the content of the paper.

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