Accepted Manuscript

Title: The effects of acupuncture on serotonin metabolism

Author: Eun Jin Lee Sherry Warden

PII: S1876-3820(16)30124-X
DOI: http://dx.doi.org/doi:10.1016/j.eujim.2016.06.022
Reference: EUJIM 572

To appear in:

Received date: 24-8-2015
Revised date: 30-6-2016
Accepted date: 30-6-2016

Please cite this article as: Lee Eun Jin, Warden Sherry.The effects of acupuncture on serotonin metabolism.*European Journal of Integrative Medicine* http://dx.doi.org/10.1016/j.eujim.2016.06.022

The effects of acupuncture on serotonin metabolism

Eun Jin Lee, PhD, Sherry Warden, PhD

Sherry Warden

University of Kentucky, 413 Cochran Rd. Lexington, KY USA 40502 lsward00@uky.edu

Correspondence should be addressed to Eun Jin Lee: peace-peace@hanmail.net

Inha University, Nursing Department, Inharo 100, Namgu, Incheon, South Korea 22212

Phone: 8232-860-8214, FAX: 82-32-874-5880

ABSTRACT

Introduction The release of the neurotransmitter, serotonin is associated with different diseases and symptoms and is thought to enhance mood. As acupuncture may effect serotonin metabolism, this paper aimed to review the evidence for changes in serotonin associated with the use of acupuncture.

Methods PubMed, Scopus, RISS, and Web of Science for articles were searched, together with manual searches for articles published between 1974 to 2015 using the key words "acupuncture" with "5-hydroxytryptamine (5-HT)," "tryptophan," "5-hydroxytryptophan (5-HTP)," or "5-hydroxyindole acetaldehyde (5-HIAA)."

Results Of the 61 studies identified, 37 (n=27 animal and n=10 human studies) were included in which investigators used serotonin or serotonin antagonists/agonists to examine the mechanisms of acupuncture for various symptoms. Investigators in 32 studies concluded that acupuncture improved symptoms, and of the 24 studies measuring serotonin, it was concluded that acupuncture changed the serotonergic mechanism. Acupuncture improves pain, which might be associated with increased levels of serum or plasma serotonin or tissue serotonin in the colon or the trigeminal nucleus caudalis, and decreased levels of platelet serotonin. Receptors 5-HT_1, 5-HT_2, and $5HT_3$ and subtypes 5-HT_{1A} and 5-HT_{2A} are related to the mechanisms of acupuncture on pain. Acupuncture improves anxiety/depression, but the evidence for change in the serotonergic mechanism is conflicting. Acupuncture improves diarrhoea, which might be associated with reduced 5-HT expression in the colon and appeared to improve obesity, which might be associated with increased levels of tissue serotonin at the raphe nuclei.

Conclusion Acupuncture improves symptoms and conditions such as pain, obesity, and depression. Those symptoms might be associated with changes in 5-HT.

Keywords: acupuncture, serotonin, review, mechanism, 5-HT

INTRODUCTION

For more than 5,000 years, acupuncture has been used to treat diseases. Acupuncture uses needles to stimulate acupuncture points. Electro-acupuncture (EA) uses needles with electric stimulation. A variety of explanations have been provided for how acupuncture works.

In Oriental Medicine, the meridian theory suggests that a life force called qi flows through the body along certain channels (meridians). If the movement of qi is blocked, illness can occur and acupuncture stimulation at precise locations along those channels is thought to unblock the flow of qi, relieving pain and restoring health [1]. In Western models, the gate control theory and neurophysiologic theories might explain how acupuncture works. According to the gate control theory, acupuncture closes the gate to pain and reduces pain perception in the brain through stimulation of large nerve fibres [2]. Neurophysiological theories suggest that acupuncture activates receptors and the secretion of neurotransmitters such as endorphins, serotonin, and norepinephrine [3]. For example, EA with low frequency activates the release of beta-endorphin, encephalin, and endomorphin, which in turn stimulates the mu- and delta-opioid receptors, whereas EA with high frequency activates dynorphin, which stimulates the kappa-opioid receptor [4].

For 40 years, Western methods have measured neurotransmitters and hormones, including endorphins, dopamine, serotonin, epinephrine, norepinephrine, and cortisol, to investigate the physiological changes associated with acupuncture [4, 5]. Although numerous clinical trials to examine the mechanisms of acupuncture have been published, reviews of how acupuncture effects changes in specific neurotransmitters are few. Serotonin is an important neurotransmitter related to symptoms such as pain, gastrointestinal functions, sleep, and emotions [6, 7]. More

than 1.5 billion people worldwide suffer from chronic pain[8]. Estimates also suggest that 9–23% of people worldwide suffer from irritable bowel syndrome and that it is the most common problem of gastrointestinal problems [9]. In addition, 10-15% of people worldwide suffer from insomnia[10] anddepression is the 4th leading cause of disability worldwide[11]. Acupuncture improves pain, diarrhoea, insomnia, and depression and that might be related to abnormal levels of serotonin[12-15]. Serotonin can be measured in urine, platelets, serum, tissues, and plasma.It is synthesized from its precursor amino acid tryptophan and metabolized into 5-hydroxyindole acetaldehyde (5-HIAA) [16] (Figure 1). This exploratory review summarizes the effects of acupuncture on various symptoms and mechanisms related to serotonin metabolism.

METHODS

Search Strategy and Selection Criteria

Using online literature search engines and manual searches of references articles were selected for review. PubMed, Scopus, Web of Science, and RISS were searched for articles published from 1974 to 2015 using key words, “acupuncture” with “5-hydroxytryptamine (5-HT/5HT),” “tryptophan,” “5-hydroxytryptophan (5-HTP),” or “5-hydroxyindole acetaldehyde (5-HIAA).” The search was limited with the key phrases “clinical trial, human, English” or “experimental, animal, English.” Clinical trials and experimental studies were included if they examined the mechanisms of acupuncture on serotonin metabolism and if they were published in English. Exclusions were if the full text was unavailable; if they did not provide serotonin levels; if they did not investigate serotonin metabolism; if they used auricular or hand acupuncture with different acupuncture-point names than body acupuncture; if they used moxibustion, bee venom, far-infrared rays, or lasers; if they did not use acupuncture needles; or if they did not use an experimental design.

The effect size was calculated using Hedges' g for each trial using differences in the means of serotonin levels and symptoms in the acupuncture and control groups [17]. Hedges' g is the correction for Cohen's d [18], and provides a superior estimate of the standardized mean difference with small samples [19]. Descriptive data synthesis was employed together with the Cochrane group's tool to assess the risk of bias when evaluating clinical studies [20]. This tool consists of six domains: sequence generation; allocation concealment; blinding of participants, personnel, and outcomes; incomplete outcome data; selective outcome reporting; and other sources of bias. Experimental studies were evaluated for the presence of each domain and awarded one point for each domain present. Revised standards for reporting interventions in clinical trials of acupuncture (STRICTA) was used to evaluate quality of reporting [21]. STRICTA consists of the acupuncture rationale, details of needling, treatment regimen, other components of treatment, practitioner background, and control or comparator interventions.

RESULTS

A total of 47 articles were identified in PubMed, 15 articles in Scopus, 33 articles in Web of Science, 34 articles in RISS, and one article was identified by a manual search in articles' references. After removing duplicate articles, we had identified 61 studies using the keywords. Of these 23 were excluded based on the following criteria: serotonin was not measured (9), acupuncture needles were not used (9), the investigators did not use an experimental design (4), or the full text was unavailable in English [22]. Thirty-seven studies remained for evaluation (Figure 2).

The probable mechanisms for the effects of acupuncture on serotonin metabolism have been proposed for humans and animals. The 37 studies used 713 patients and 2,378 animals to examine the serotonergic mechanisms of acupuncture on pain, anxiety/depression, hot flashes,

diarrhoea, nausea/vomiting, obesity, and psychological symptoms. In the studies covered in this literature review, acupuncture was administered from once to up to six weeks, the number of sessions varied from one to 36, and the total treatment time varied from 30 to 1,620 minutes. The number of acupuncture points used ranged from one to nine. The frequency of EA varied from 2 Hz to 100 Hz, and the intensity of EA varied from 0.07 mA to 10 mA. The experimental protocols were summarized in Table 1. The WHO's (World Health Organization) definition of standard acupuncture points was used to describe the points used in these studies. The risk of bias was summarized in Table 2. The effect sizes of acupuncture on serotonin metabolism are summarized in Table 3. The effect sizes of acupuncture on symptoms are summarized in Table 4.

Quality of studies

Investigators in 16 of the 37 studies used a randomized control trial (RCT) design, and investigators in the other 21 studies used a quasi-experimental design (Table 1). Investigators in five studies described the random sequence generation for their randomization [12, 13, 23-25]. Investigators in three studies explained the blinding of participants, personnel, and outcomes [26-28]. None of the investigators in the 16 RCT-based studies explained allocation concealment. Investigators in two studies did not explain the experimental protocol [29] or acupuncture points [30]. Authors in six studies reported attrition rates and reasons for attrition. Authors in three studies reported that acupuncture had no side effects, whereas authors in three other studies reported adverse reactions to acupuncture such as nausea, vomiting [31], fatigue, mood change, sweating, somnolence, and dry mouth [32]. Yuan and his colleagues did not describe side effects [25]. Investigators in 34 studies did not report whether side effects occurred. None of investigators described qualification or professional affiliation, years in acupuncture practice, other relevant experience of participating acupuncturists (Table 5). Investigators in 3 studies

reported diameter, length, manufacturer, and materials of needles, while investigators in 18 studies reported partially. Authors did not define their style of acupuncture. Styles of acupuncture were assumed from first author's nationality. Authors in 20 studies used traditional Chinese medicine, 9 studies used western medical acupuncture, 5 studies used Korean acupuncture, and 3 studies used Japanese acupuncture. Authors in only 5 studies described response sought such as de qi.

Description of studies

Pain

Investigators in 14 studies examined the serotonergic metabolism of acupuncture on pain [12, 23, 28-30, 33-41].

Human studies

EA with 2 and20 Hz at PC4 and PC6 decreased postoperative pain ($p < .05$) and plasma 5-HT levels ($p < .01$) in comparison with the placebo group, which received acupuncture at PC4 and PC6 without stimulation, in 60 patients with thoracic oesophagectomy. Acupuncture was performed once 30 minutes before general anesthesia and during operative time which was average 222 minutes [23].

In a study of 275 patients, EA with 100 Hz at GB40 for 30 minutes for five sessions improved migraine headaches in comparison with the control group, which received EA at ST25 [33]. The visual analogue scale (VAS) scores of migraine patients did not differ significantly immediately after acupuncture; however, a significant difference in the VAS scores of patients in the acupuncture group emerged four weeks after acupuncture in comparison to the control group ($p < .05$). Acupuncture increased plasma 5-HT levels in the treatment group in comparison to the control group ($p < .01$) [33].

EA with 2and100Hz at LI4 and SP6 was performed for 20 minutes at the first stage of labour, then for another 20 minutes at 7–8 cm dilatation in 38 women with labour pain [12]. EA decreased labour pain and increased serum serotonin levels in comparison to the control group, which did not receive acupuncture for labour pain (all $p < .05$) [12].

Mao and colleagues compared the effects of low-intensity and high-intensity EA on chronic pain [30]. The authors did not describe the exact electrical parameters. Authors used cross-over design that half of subjects were treated with high-intensity acupuncture for seven 45-min sessions; then they were treated with low-intensity acupuncture for the same period. High-intensity EA decreased pain and increased platelet 5-HT levels in 26 patients with chronic pain, compared to low-intensity acupuncture group and the normal control group who did not have pain and did not receive acupuncture ($p < .01$) [30].

Sprott and colleagues reported that acupuncture improved pain in 29 patients with fibromyalgia and increased serum 5-HT levels and decreasing platelet 5-HT levels ($p < .01$). This study did not use any control group. Acupuncture was performed once per week for 6 weeks. Acupuncture points were individualized but exact acupuncture points were not reported [29].

Animal studies

Animals have been used for several studies using EA for pain. EA with 2 Hz or100Hz, 1 mA at ST25 and ST37 for 20 minutes daily for seven days decreased chronic visceral hypersensitivity ($p < .05$) and increased tissue serotonin levels in the colon ($p < .01$) in 24 rats with irritable bowel syndrome in comparison to the control group of rats, which did not receive acupuncture [34].

EA with 2 Hz at ST36 and SP6 for 30 minutes decreased chronic visceral hypersensitivity ($p< .01$) and increased tissue serotonin levels in the colon ($p< .05$) in 12 rats with irritable bowel syndrome in comparison to the control group, which did not receive acupuncture [35].

EA with 2 Hz, 0.07 mA, 0.3 ms at ST36 for 30 min improved pain in rats with collagen-induced arthritis compared to the control group that did not receive acupuncture [41]. The analgesic effect of EA was blocked by spiroxatrine (5-HT1a receptor antagonist, 1 mg/kg i.p.), ondansetron (5-HT3 receptor antagonist, 0.5 mg/kg i.p.), and atropine (muscarinic cholinergic receptor antagonist, 1 mg/kg i.p.), but not by ketanserin (5-HT2 receptor antagonist, 1 mg/kg i.p.) [41].

In a study of 110 rabbits with tooth pulp stimulation-induced pain, EA with 2 Hz at ST 36 for 40 min increased tissue 5-HT in the trigeminal nucleus caudalis compared to the control group, that did not receive acupuncture. The authors did not evaluate pain symptoms [36].

EA with 4 and60Hz at ST36 and BL60 for 20 min increased pain threshold, 5-HT, and 5-HIAA compared to the control group that received normal saline ($p< .01$). EA with fenfluramine (5-HT releaser) increased pain threshold more than EA alone (EA 140±11 vs. fenfluramine+EA 226 ±30, $p<. 01$) [40].

EA with 2, 10, and 100 Hz at ST36 for 5 min decreased pain in 120 mice with formalin-induced pain compared to the control group that did not receive acupuncture [37]. The 5-HT_{1A} and 5-HT_3 receptor antagonists (pindobind-5-HT_{1A} and LY-278584, respectively) blocked the effects of EA on pain with three different frequencies, but the 5-HT_{2A} receptor antagonist (ketanserin) increased the effects of EA with 100 Hz [37].

In a study of 12 rabbits with tooth pulp stimulation-induced pain, EA with 2 Hz, 7–15 mA at ST36 for 40 min decreased pain compared to the control group that did not receive acupuncture

[38]. The 5-$HT_{1,}$ 5-HT_2, and 5-HT_3 receptor antagonists blocked the effects of EA on pain, and 5-HT_{1A} and 5-HT_{2A} receptors antagonists increased the effects of EA [38].

EA with 2 and100 Hz at ST36 and PC6 was performed for 20 minutes on 572 rats with heat-induced pain [39], and 360 rats (63%) responded [39]. Cannulas were implanted at the dorsal or ventral anterior pretectal nucleus (APtN) of the rats that responded to EA. EA at ST36 and PC6 decreased pain in comparison to the control group, which received acupuncture without stimulation at ST36 and PC6. Non-selective antagonists of serotonergic, muscarinic, and opioid receptors and selective antagonists against μ, δ, or κ, $5HT_1$, $5HT_2$, or $5HT_3$ and $GABA_A$ receptors were used to examine the mechanism of EA at ST36 and PC6 in the dorsal or ventral APtN of rats. The effects of EA with 2 Hz were blocked by muscarinic, μ-opioid, $GABA_A$, and 5-HT_1 antagonists in the dorsal APtN and by μ-opioid and 5-HT_1 antagonists in the ventral APtN. The effects of EA with 100Hz were blocked by μ-opioid and 5-HT_1 antagonists in the ventral APtN, but the effects were not blocked by them in the dorsal APtN [39].

EA with 10 Hz, 3 mA at GB30 was performed twice for 20 minutes in 148 rats with inflammatory hyperalgesia [28]. EA decreased pain in comparison to the control group, which received acupuncture without stimulation ($p< .01$). The effects of EA were blocked by 5-HT_{1A} receptor antagonists [28].

In summary, serotonin appeared to increase the pain threshold in subjects, although abnormally high levels of serotonin can induce pain [34]. Acupuncture decreases pain and inhibits 5-HT_{1A} inflammatory mediators [23]. Receptors 5-HT_1, 5-HT_2, and $5HT_3$ and subtypes 5-HT_{1A} and 5-HT_{2A} are related to the mechanisms of acupuncture on pain. Acupuncture improves pain and increases levels of serum or plasma serotonin and tissue serotonin at the colon or the trigeminal nucleus caudalis and decreases platelet serotonin levels.

Unconsciousness

Investigators in two studies examined the effects of acupuncture on unconsciousness [42, 43].

Animal studies

EA with 2, 15, and 50 Hz for 45 min reduced anesthesia-induced sleeping time in 240 rabbits in comparison to the control group, which received acupuncture without stimulation ($p < .001$) [42]. Para-chlorophenylalanine (p-CPA; a 5-HT depleter) and naloxone, (an opiate receptor antagonist,) did not block the effects of EA at GV26 (Jen-chung), whereas prazosin (an adrenergic receptors blocker) and guanethidine (a noradrenergic nerve terminals blocker) did block the effects of EA at GV26 [42]. Serotonin was not related to the anesthesia-reducing actions of EA at GV26. EA at GV26 might reduce the activity of anaesthetics via an activation of central noradrenergic neurotransmission in rabbits.

Acupuncture at GV20 for 40 min reduced sleeping time in 44 rats with hexobarbital-induced anesthesia compared with the control group that received acupuncture at the same points with 5 mm less depth [43]. The effect of acupuncture was enhanced by deprenyl (MAO-B inhibitor) but was not changed by naloxone (opioid antagonist), a-methyl-p-tyrosine (tyrosine hydroxylase enzyme inhibitor), or apomorphine (non-selective dopamine agonist), whereas the effect of acupuncture was eliminated by p-CPA (endogenous 5-HT depleter) and 5-hydroxytryptophan (5-HT precursor). Thus, 5-HT is related to the mechanism of acupuncture at GV20 to reduce anesthesia effects in rats [43].

Mental health

Investigators in four studies examined the effects of acupuncture on serotonin metabolism in association with anxiety and depression [14, 25, 44, 45].

Human studies

Yuan and colleagues examined the serotonergic mechanism of acupuncture in 86 patients with anxiety [25]. Acupuncture was performed at PC6, HT7, and SP6 for 45 minutes per day, seven days a week, for six weeks. The authors compared the effects of acupuncture (n=29), selective serotonin reuptake inhibitors (SSRI) and/or benzodiazepine (n=29), and a combination of acupuncture and medicine (n=28). Acupuncture and SSRI and/or benzodiazepine both decreased platelet serotonin, but in neither case was the difference significant. The stress index decreased for all three groups in this study, but the efficacy was best for the acupuncture only group ($p<0.01$) [25].

Animal studies

In 42 mice with depression-like behaviour, acupuncture at HT7 or SP6 decreased immobility time in comparison to the control group, which received saline injections. Acupuncture was performed for 30 seconds daily for 7 days. Acupuncture did not change 5-HT levels or the 5-HIAA/5-HT ratio, but it did decrease the kynurenine/tryptophan ratio and increased the serum kynurenic acid/3-hydroxykynurenine ratio [44] (Figure 1).

A study on the use of acupuncture points HT7 and ST36 in maternally separated rat pups examined behaviour associated with depression. Acupuncture was performed for 1 min daily for 16 days. Rat pups were assigned to a normal group (n=6), the HT7 group (n=6), or the ST36 group (n=6). No groups experienced changes in 5-HT levels. Acupuncture at HT7 or ST 36 improved depression as measured by immobility time ($p< .01$), but it did not change tissue serotonin levels, 5-HIAA levels, or the 5-HIAA/5-HT ratio at the hippocampus and the prefrontal cortex in comparison to the maternally separated control group that did not receive acupuncture[45].

Maternally separated rat pups were again studied for the effects of acupuncture at HT7 on depression-like behaviour and levels of serotonin in the prefrontal cortex. Rats were assigned to a normal group (n=6), a maternal separation group (n=6), a maternal separation + HT7 group (n=6), or a maternal separation + ST36 group (n=6). Acupuncture was performed for 30 seconds daily for 7 days. Acupuncture at HT7 improved depression and increased tissue serotonin levels at the prefrontal cortex in comparison to the maternally separated control group that did not receive acupuncture (all $p < .01$) [14].

In summary, acupuncture improves anxiety/depression, but changes in the serotonin mechanism show conflicting results.

Obesity

Investigators in two studies examined the effects of acupuncture on serotonin metabolism in a sample of rats with obesity [46, 47]. 5-HT manages appetite in the central nervous system. Low plasma 5-HT is related to obesity [48].

Animal studies

Liu and colleagues studied the effects of EA at ST36 and ST44 for 10 minutes, twice daily, for 12 days in obese rats. The rats were randomly assigned to three groups: an obese control group (n=22), an acupuncture group (n=22), and a normal control group (n=22). Acupuncture resulted in weight reduction in the obese group and a decrease in tissue 5-HIAA levels in the ventromedial nucleus of the hypothalamus (all $p < .05$), but tissue 5-HT levels and the 5-HT/5-HIAA ratio were not significantly changed compared to the control group, which did not receive acupuncture [47].

Wei and colleagues examined the effects of EA with 10 Hz at ST36 and ST44 for 10 minutes daily for 12 consecutive days in 44 rats with obesity [46]. That study involved three groups: a

normal group (n=15), an obese control group (n=15), and an obese with acupuncture group (n=14). Acupuncture resulted in a reduction in ingestion and weight ($p<0.01$) and increased levels of serotonin in the raphe nucleus of the brain stem in comparison to the obese control group, which did not receive acupuncture ($p<0.05$) [46].

In summary, acupuncture appears to have an effect on obesity, increasing levels of tissue serotonin at the raphe nuclei of the brain stem, and decreases levels of tissue serotonin at the ventromedial nucleus of the hypothalamus.

Spinal cord injury

Animal studies

The authors in two studies examined the effects of EA on spinal cord injury [26, 27].

Animal studies

Acupuncture at GV1, 2, 6, and 9 was performed for 20 min per day for 25 days. EA increased 5-HT positive nerve fibres in the transected spinal cord at thoracic cord 10 in comparison to the control group, which did not receive acupuncture, in 66 rats with spinal cord injuries ($p< .01$) [26]. Mobility was improved with 20 minutes of acupuncture every other day for seven weeks in comparison to the control group that did not receive acupuncture [26].

EA at GV1, 2, 6, and 9 increased 5-HT-positive nerve fibres at the rostral site near the injured spinal cord (T6-L1 segments) in comparison to the control group, which did not receive EA, in 36 rats with spinal cord injuries ($p< .05$). Mobility improved with 20 minutes of acupuncture every other day for 30 days in comparison to the control group that did not receive acupuncture ($p< .05$) [27].

In summary, acupuncture improves mobility following a spinal cord injury and increases 5-HT-positive nerve fibres.

Gastrointestinal symptoms

Investigators in three studies examined the effects of acupuncture on serotonin metabolism in a sample of subjects with nausea/vomiting and diarrhoea [13, 24, 49]. Diarrhoea in irritable bowel syndrome is related to high mucosal 5-HT concentrations [50].

Human studies

Liu and colleagues examined the effects of acupuncture on diarrhoea in 71 patients with irritable bowel syndrome and compared the effects of acupuncture and moxibustion [13]. Acupuncture at ST37 and ST25 was performed for 30 minutes per treatment session for a total of 36 sessions. Moxibustion at ST37 and ST25 was performed for a total of 36 sessions. Both treatments decreased diarrhoea and abdominal distension and improved sleep among subjects. In one case, enteroscopic examination and immunohistochemical staining four months after acupuncture showed no abnormal membranes in the colon. Acupuncture decreased 5-HT expression in the colon membrane [13].

Acupuncture at PC5 and PC6 for one hour twice a day for three days decreased nausea and vomiting ($p < .01$) and serum 5-HT levels ($p < .05$) in comparison to the control group, which received EA at non-acupoints, in 72 patients with cancer [24].

Animal studies

In 140 rats with no symptoms, EA with 2 Hz, 1V at SP6 and ST36 for 30 min per day for three days increased gastric emptying in comparison to the control group, which received EA at non-acupoints. P-CPA, a serotonin inhibitor, blocked the effects of EA [49].

In summary, acupuncture showed improvement in symptoms of diarrhoea, reduced 5-HT expression in the colon,reduced nausea and vomiting and decreased serum 5-HT levels.

Hot flashes

Hot flashes are related to low oestrogen levels and a rise in core body temperature. Serotonin plays a role in the thermoregulatory centre [51].

Human studies

Beer and colleagues studied the effects of EA with 2 Hz at GB34, BL15, 23, 32, GV20, LR2, and SP6 for 30 minutes once or twice per week for 10 weeks in 22 male patients with prostate cancer [32]. Acupuncture resulted in a greater than 50% reduction in hot flashes in 12 of 22 patients (55%). Authors did not have the control group. Responders to acupuncture had a 27% decrease in 24-h urinary 5-HIAA in comparison to the baseline (n=12), and non-responders had a 29% increase in 24-h urinary 5-HIAA in comparison to the baseline (n=10) (all $p < .05$) [32]. In summary, acupuncture improves hot flashes and decreases urine 5-HIAA without altering serum 5-HT.

Memory impairment

Chang and colleagues examined the effects of acupuncture on memory impairment in rats [52]. Serotonin is involved in learning and memory.

Animal studies

Acupuncture at GV20 was administered for 15, 30, and 60 minutes, respectively, 15 minutes before electric shock was administered to rats [52]. Acupuncture administered 30 minutes before and after electric shock was not effective. Acupuncture in rats who had cycloheximide-induced memory impairment improved passive avoidance behaviour in comparison to the control group, which did not receive acupuncture ($p <. 01$). When researchers injected P-chloroamphetamine hydrochloride, a 5-HT releaser, the effects of acupuncture were decreased. The cholinergic receptor antagonist slightly blocked the effects of acupuncture [52]. In summary, effect of acupuncture at GV20 on memory impairment appears to be blocked by excessive 5-HT.

Mixed symptoms

Human studies

Riederer and colleagues examined the effects of acupuncture on serotonin metabolism in a sample of subjects with various diagnoses, including arthritis, mastitis, depression, and muscular dystrophy [31]. Pseudo-acupuncture for 20 minutes did not change urine 5-HIAA levels after three hours in seven healthy subjects. In contrast, acupuncture at LI3 alone or combined with acupuncture at ST36 for 20 minutes increased urine 5-HIAA levels in 19 patients (n=26, all $p< 0.05$) [31]. Fourteen of the 19 patients reported improvement in symptoms. The authors did not provide details regarding pseudo-acupuncture and did not use objective measures to evaluate symptom improvement [31]. In summary, acupuncture demonstrated some improvement in various symptoms and increased 5-HIAA levels.

Cardiovascular function

Authors in three studies examined the effects of acupuncture on cardiovascular function in animals with no symptoms [53-55].

Animal studies

EA with 2 Hz at PC5 and PC6 for 30 minutes decreased mean blood pressure and increased tissue serotonin levels in all three midline medullary nuclei in cats, especially in the nucleus raphe pallidus ($n = 6$), in comparison to cats using manual acupuncture ($n = 5$, all $p < 0.05$) [53].

EA with 2 and 4 Hz, 2–4 mA at PC5 and PC6 for 30 minutes lessened the increase in mean blood pressure compared with pre-acupuncture in 36 rats with bradykinin-induced high blood pressure ($p < .01$) [54]. The effects of acupuncture on blood pressure in the rostral ventrolateral medulla were blocked by 5-HT_{1A} receptor antagonists [54].

Acupuncture at ST36 for 20 minutes every day for seven days on exercised rats (n = 10) increased the time to exhaustion among rats for treadmill exercise and suppressed tissue serotonin levels at the dorsal raphe, in comparison to exercised rats (n = 10) that did not receive acupuncture (n = 60, all $p < 0.01$) [55].

In summary, acupuncture reduces the increase in mean arterial pressure by increasing tissue serotonin levels in all three midline medullary nuclei and increasing 5-HT_{1A}. Acupuncture appears to improve cardiovascular function and suppresses tissue serotonin levels at the dorsal raphe.

Adrenal atrophy

Animal studies

Investigators in one study examined the effect of acupuncture on serotonin in 87 rats with hydrocortisone-induced adrenal atrophy [56]. EA at BL23 for 15 minutes every other day for 9 days increased paired adrenal weight compared to the control group that did not receive acupuncture ($p < .01$). EA at BL23 for 15 minutes once increased 5-HT and 5-HIAA in diencephalon compared to the control group that received saline ($p < .05$) [56].

No symptoms

Investigators in three studies examined the effects of acupuncture on serotonin in animals with no symptoms [57-59].

Animal studies

In 40 rabbits, acupuncture at CV4 for 30 minutes released epinephrine, serotonin, and unknown substances in comparison to the placebo group, which received acupuncture at non-acupoints [58].

Yoshimoto and his colleagues examined the neuropharmacological mechanisms of acupuncture on serotonin in the brains of a sample of rats. When the BL23 acupoints were stimulated unilaterally (n=8) or bilaterally (n=8) for one hour, tissue serotonin levels at the nucleus accumbens increased in comparison to the placebo group, which received acupuncture at non-acupoints ($p < .05$) [57].

In 48 rats, single EA with 1.5–2.5 mA for one hour at GV20 and the Yintang increased cerebral 5-HT and 5-HIAA levels in comparison to the control group with no acupuncture ($p < .01$). Multi-EA for 30 minutes for 10 days increased cerebral 5-HT and 5-HIAA levels but not significantly compared to the control group that did not receive acupuncture [59].

DISCUSSION

In this review of findings about the effects of acupuncture on serotonin metabolism in association with multiple symptoms, acupuncture was shown to improve anxiety without changing platelet 5-HT levels [25]. However, results concerning the relationship between anxiety/depression and levels of platelet 5-HT are controversial and inconsistent. According to one study, platelet 5-HT levels do not differ significantly between premenopausal women with depressive symptoms and women without depressive symptoms [60]. Platelet 5-HT levels are similar in patients with post-traumatic stress disorder and healthy controls. However, patients with suicidal tendencies across different psychiatric diagnoses have low platelet 5-HT levels [61]. Anxious patients undergoing haemodialysis have higher levels of platelet 5-HT than patients without anxiety [62]. Further studies are needed to examine when platelet 5-HT levels are an appropriate metric to compare acupuncture effects.

According to further findings, acupuncture for fibromyalgia increases levels of serum 5-HT and decreases levels of platelet 5-HT [29], whereas acupuncture for chronic pain increases levels

of platelet 5-HT [30]. The reason for this could be that diseases differ in regard to levels of platelet 5-HT. Increased serotonin levels in serum, urine and platelets are associated with carcinoid tumors, while decreased serotonin levels are associated with CNS disorders such as autism, depression and suicidal schizophrenia[63]. Serum 5-HT and platelet 5-HT levels change in parallel in healthy individuals [64]. However, serum, plasma, and platelet 5-HT levels might not change in parallel in patients with diseases. A high level of plasma 5-HT in relation to serum 5-HT is related to pain and anxiety in patients with fibromyalgia [65]. Chronic daily headaches are related to low levels of platelet 5-HT, and fibromyalgia is related to high levels of platelet 5-HT [66, 67]. Even when different patients have the same disease, levels of 5-HT can vary with the stage of disease [68]. In their review, Markelova and colleagues found that acupuncture normalizes increased or decreased levels of platelet 5-HT [68].

Findings indicate that acupuncture for hot flashes decreases urine 5-HIAA but does not change levels of serum 5-HT [32], whereas acupuncture for various other symptoms increases urine 5-HIAA, but does not change levels of tryptophan [31]. The first reason for the conflicting findings of these two studies could be the use of different acupuncture points. Beer and colleagues [21] used GB34, BL15, 23, 32, GV20, LR2, and SP6, whereas Riederer and colleagues [20] used LI4, ST36, and/or LI3. The second reason could be that urine 5-HIAA levels vary in different diseases. Hot flashes are related to elevated urine 5-HIAA levels [69]. Even though the authors suggested that serum 5-HT is not sensitive enough to determine the effects of acupuncture on hot flashes, they did not assess important factors that influence hot flashes and 5-HT levels [32]. Hot flashes and 5-HT levels are influenced by many factors, such as exercise, food, stress, and medication [69-71]. The reason that tryptophan levels did not change in that particular study could be that the sample size is small(n=10) [31]. Finally, genetic differences might explain the

results. Chae and colleagues found that heredity influences the response to acupuncture in humans [72].

Chang and his colleagues found that excessive 5-HT reduced the effect of acupuncture on memory impairment [52]. Short-term memory loss is related to low 5-HT levels in serotonergic-neuron-rich regions of the murine brain [73]. Both excessive and insufficient 5-HT levels is related to impaired spatial working memory performance[74]. Acupuncture has bidirectional effects on serotonin release. Acupuncture increased serotonin levels in women with labour pain[12], while acupuncture decreased serum serotonin in cancer patients with nausea and vomiting[24]. Further studies are needed to examine bidirectional effects of acupuncture using the same acupuncture points.

The eight studies that used 5-HT_{1A} receptor antagonists to examine the mechanisms of acupuncture had conflicting results. The range of results indicates that 5-HT_{1A} receptor antagonists blocked the effects of acupuncture at GB30 or ST36 on pain in rats [28, 37], whereas 5-HT_{1A} receptor antagonists increased the effects of EA at ST36 in 12 rabbits with tooth pulp stimulation-induced pain [38]. The inconsistency could be caused by different subjects (rats vs. rabbits), different electrical parameters (2–100 Hz, 3 mA vs. 2 Hz 7–15 mA), or different kinds of pain (inflammatory pain, formalin-induced pain vs. tooth pulp stimulation). Further studies are needed to examine the function of 5-HT receptor subtypes related to acupuncture.

Our review could have been limited by our exclusion criteria (excluding papers not published in English). Indeed, some appropriate studies might have been omitted by our search strategy. We could not conduct a meta-analysis because of heterogeneity such as the different sources of serotonin, measures, and wide variability of samples. There might be the possible irrelevancy of evaluating holistic interventions on a single biomarker to alert readers of any incompatibility in

larger philosophical differences underlying modern and traditional medicines. The interpretation of this review was limited by not reflecting on complex aspects of serotonin. There are many different serotonin receptors that have different functions. There are different markers of serotonin metabolism that are used pertaining to different areas.

In conclusion, acupuncture appears to improve symptoms and conditions such as pain, hot flashes, spinal cord injury, obesity, gastric emptying, blood pressure, and diarrhoea. These symptoms might be associated with changes in 5-HT, but other mechanisms could be involved. Randomized controlled trials with large sample sizes are needed to examine this question further.

Authors: All research done by the authors

Financial support: This work was supported by a National Research Foundation of Korea grant funded by the Korean Government (2014002505).

Conflicts of interest: none

References

[1] National Center for Complementary and Alternative Medicine. An introduction to acupuncture. In: U.S. Department of Health and Human Services, editor. Bethesda, Maryland National Institutes of Health; 2008.
[2] A. Khadilkar, S. Milne, L. Brosseau, G. Wells, P. Tugwell, V. Robinson, et al. Transcutaneous electrical nerve stimulation for the treatment of chronic low back pain: a systematic review. Spine (Phila Pa 1976). 30(2005) 2657-2666.
[3] W. Hao, X. Zhu, H. Zhang, B. Tian. Study on a mechanical acupuncture instrument with computer aided controlled. Conf Proc IEEE Eng Med Biol Soc. 4(2005) 4259-4262.
[4] J. G. Lin, W. L. Chen. Acupuncture analgesia: a review of its mechanisms of actions. Am J Chin Med. 36(2008) 635-645.
[5] S. Ceniceros, G. R. Brown. Acupuncture: a review of its history, theories, and indications. South Med J. 91(1998) 1121-1125.
[6] M. T. Cabioglu, N. Ergene, U. Tan. Smoking cessation after acupuncture treatment. Int J Neurosci. 117(2007) 571-578.
[7] K. McCance, S. Huether. Pathophysiology 4th ed. St. Louise, MO: Mosby; 2002.
[8] D. Borsook. A future without chronic pain: neuroscience and clinical research. Cerebrum. 2012(2012) 7.
[9] International Foundation for Functional Gastrointestinal Disoders. Statistics. 2013.
[10] C. M. Morin, M. LeBlanc, M. Daley, J. P. Gregoire, C. Merette. Epidemiology of insomnia: prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors. Sleep Med. 7(2006) 123-130.
[11] R. C. Kessler, E. J. Bromet. The epidemiology of depression across cultures. Annu Rev Public Health. 34(2013) 119-138.
[12] F. Qu, J. Zhou. Electro-acupuncture in relieving labor pain. Evid Based Complement Alternat Med. 4(2007) 125-130.
[13] H.-R. Liu, Y. Y, H.-G. Wu. Clinical study on acupuncture in treating diarrhea-predominant irritable bowel syndrome. Journal of Acupuncture and Tuina Science. 6(2008) 360-362.
[14] H. Park, D. Yoo, S. Kwon, T. W. Yoo, H. J. Park, D. H. Hahm, et al. Acupuncture stimulation at HT7 alleviates depression-induced behavioral changes via regulation of the serotonin system in the prefrontal cortex of maternally-separated rat pups. J Physiol Sci. 62(2012) 351-357.
[15] Y. Feng, X. Y. Wang, S. D. Li, Y. Zhang, H. M. Wang, M. Li, et al. Clinical research of acupuncture on malignant tumor patients for improving depression and sleep quality. J Tradit Chin Med. 31(2011) 199-202.
[16] S. M. Stahl. Stahl's essential psychopharmacology. 3rd ed. New Yord, NY: Cambridge University Press; 2008.
[17] L. V. Hedges. Distribution theory for Glass's estimator of effect size and related estimators. Journal of Educational Statistics. 6(1981) 107-128.
[18] D. Lakens. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 4(2013) 1-11.
[19] C. O. Fritz, P. E. Morris, J. J. Richler. Effect size estimates: current use, calculations, and interpretation. J Exp Psychol Gen. 141(2012) 2-18.
[20] D. Moher, K. F. Schulz, D. G. Altman. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. J Am Podiatr Med Assoc. 91(2001) 437-442.

[21] H. MacPherson, D. G. Altman, R. Hammerschlag, L. Youping, W. Taixiang, A. White, et al. Revised STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA): Extending the CONSORT statement. J Evid Based Med. 3(2010) 140-155.
[22] M. T. A. Cabioğlu, R. B. Kutlu, C. D. Albayrak, K. B. Marakoğlu, N. C. Ergene. Changes in the Levels of Serum Beta Endorphin, Serotonin, Adrenaline, Noradrenaline and Dopamine During Smoking Cessation by Electroacupuncture and Nicotine Patch. Turkiye Klinikleri Cardiovascular Sciences. 24(2012) 95-101.
[23] Y. H. Xie, X. Q. Chai, Y. L. Wang, Y. C. Gao, J. Ma. Effect of electro-acupuncture stimulation of Ximen (PC4) and Neiguan (PC6) on remifentanil-induced breakthrough pain following thoracal esophagectomy. J Huazhong Univ Sci Technolog Med Sci. 34(2014) 569-574.
[24] X. Zhang, H. F. Jin, Y. H. Fan, B. Lu, L. N. Meng, J. D. Chen. Effects and mechanisms of transcutaneous electroacupuncture on chemotherapy-induced nausea and vomiting. Evid Based Complement Alternat Med. 2014(2014) 860631.
[25] Q. Yuan, J.-n. Li, B. Liu, Z.-f. Wu, R. Jin. Effect of Jin-3-needling therapy on plasma corticosteroid, adrenocorticotrophic hormone and platelet 5-HT levels in patients with generalized anxiety disorder. Chin J Integr Med. 13(2007) 264-268.
[26] Y. Ding, Q. Yan, J. W. Ruan, Y. Q. Zhang, W. J. Li, Y. J. Zhang, et al. Electro-acupuncture promotes survival, differentiation of the bone marrow mesenchymal stem cells as well as functional recovery in the spinal cord-transected rats. BMC Neurosci. 10(2009) 35.
[27] Q. Yan, J. W. Ruan, Y. Ding, W. J. Li, Y. Li, Y. S. Zeng. Electro-acupuncture promotes differentiation of mesenchymal stem cells, regeneration of nerve fibers and partial functional recovery after spinal cord injury. Exp Toxicol Pathol. 63(2011) 151-156.
[28] Y. Zhang, R. X. Zhang, M. Zhang, X. Y. Shen, A. Li, J. Xin, et al. Electroacupuncture inhibition of hyperalgesia in an inflammatory pain rat model: involvement of distinct spinal serotonin and norepinephrine receptor subtypes. Br J Anaesth. 109(2012) 245-252.
[29] H. Sprott, S. Franke, H. Kluge, G. Hein. Pain treatment of fibromyalgia by acupuncture. Rheumatol Int. 18(1998) 35-36.
[30] W. Mao, J. N. Ghia, D. S. Scott, G. H. Duncan, J. M. Gregg. High versus low intensity acupuncture analgesia for treatment of chronic pain: effects on platelet serotonin. Pain. 8(1980) 331-342.
[31] P. Riederer, H. Tenk, H. Werner, J. Bischko, A. Rett, H. Krisper. Manipulation of neurotransmitters by acupuncture (?) (A preliminary communication). J Neural Transm. 37(1975) 81-94.
[32] T. M. Beer, M. Benavides, S. L. Emmons, M. Hayes, G. Liu, M. Garzotto, et al. Acupuncture for hot flashes in patients with prostate cancer. Urology. 76(2010) 1182-1188.
[33] C.-s. Jia, X.-s. Ma, S. Jing, Y.-m. Wang, Y.-f. Li, Y. Jun, et al. Electroacupuncture at Qiuxu (GB 40) for treatment of migraine-— a clinical multicentral random controlled study. J Tradit Chin Med. 29(2009) 43-49.
[34] H. R. Liu, X. M. Wang, E. H. Zhou, Y. Shi, N. Li, L. S. Yuan, et al. Acupuncture at both ST25 and ST37 improves the pain threshold of chronic visceral hypersensitivity rats. Neurochem Res. 34(2009) 1914-1918.
[35] X. Y. Tian, Z. X. Bian, X. G. Hu, X. J. Zhang, L. Liu, H. Zhang. Electro-acupuncture attenuates stress-induced defecation in rats with chronic visceral hypersensitivity via serotonergic pathway. Brain Res. 1088(2006) 101-108.

[36] N. Yonehara. Influence of serotonin receptor antagonists on substance P and serotonin release evoked by tooth pulp stimulation with electro-acupuncture in the trigeminal nucleus cudalis of the rabbit. Neurosci Res. 40(2001) 45-51.
[37] F. C. Chang, H. Y. Tsai, M. C. Yu, P. L. Yi, J. G. Lin. The central serotonergic system mediates the analgesic effect of electroacupuncture on ZUSANLI (ST36) acupoints. J Biomed Sci. 11(2004) 179-185.
[38] J. Takagi, N. Yonehara. Serotonin receptor subtypes involved in modulation of electrical acupuncture. Jpn J Pharmacol. 78(1998) 511-514.
[39] J. R. Silva, M. L. Silva, W. A. Prado. Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the activation of different descending pain inhibitory mechanisms. J Pain. 12(2011) 51-60.
[40] X. Y. Li, C. B. Zhu, H. N. Chen, Y. H. Zhu, G. C. Wu, S. F. Xu. Effects of fenfluramine combined with electroacupuncture on monoamine release in periaqueductal gray of rat brain. Zhongguo Yao Li Xue Bao. 20(1999) 597-600.
[41] Y. H. Baek, D. Y. Choi, H. I. Yang, D. S. Park. Analgesic effect of electroacupuncture on inflammatory pain in the rat model of collagen-induced arthritis: mediation by cholinergic and serotonergic receptors. Brain Res. 1057(2005) 181-185.
[42] C. L. Chang, J. C. Lee, C. C. Tseng, Y. H. Chang, J. T. Cheng. Decrease of anesthetics activity by electroacupuncture on Jen-Chung point in rabbits. Neurosci Lett. 202(1995) 93-96.
[43] Y. H. Chang, M. T. Hsieh, J. T. Cheng. Increase of locomotor activity by acupuncture on Bai-Hui point in rats. Neurosci Lett. 211(1996) 121-124.
[44] S. Kwon, B. Lee, M. Yeom, B. J. Sur, M. Kim, S. T. Kim, et al. Modulatory effects of acupuncture on murine depression-like behavior following chronic systemic inflammation. Brain Res. 1472(2012) 149-160.
[45] S. Kwon, D. Kim, H. Park, D. Yoo, H. J. Park, D. H. Hahm, et al. Prefrontal-limbic change in dopamine turnover by acupuncture in maternally separated rat pups. Neurochem Res. 37(2012) 2092-2098.
[46] Q. Wei, Z. Liu. Effects of acupuncture on monoamine neurotransmitters in raphe nuclei in obese rats. J Tradit Chin Med. 23(2003) 147-150.
[47] Z. Liu, F. Sun, J. Su, M. Zhao, M. Zhu, Y. Wang, et al. Study on action of acupuncture on ventromedial nucleus of hypothalamus in obese rats. J Tradit Chin Med. 21(2001) 220-224.
[48] H. J. Park, S. E. Lee, J. H. Oh, K. W. Seo, K. H. Song. Leptin, adiponectin and serotonin levels in lean and obese dogs. BMC Vet Res. 10(2014) 113.
[49] G. C. Sugai, O. Freire Ade, A. Tabosa, Y. Yamamura, S. Tufik, L. E. Mello. Serotonin involvement in the electroacupuncture- and moxibustion-induced gastric emptying in rats. Physiol Behav. 82(2004) 855-861.
[50] J. Miwa, H. Echizen, K. Matsueda, N. Umeda. Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. Digestion. 63(2001) 188-194.
[51] R. R. Freedman, D. Norton, S. Woodward, G. Cornelissen. Core body temperature and circadian rhythm of hot flashes in menopausal women. J Clin Endocrinol Metab. 80(1995) 2354-2358.
[52] Y. H. Chang, M. T. Hiseh, C. R. Wu. Effects of acupuncture at pai-hui on the deficit of memory storage in rats. Am J Chin Med. 27(1999) 289-298.

[53] Z. L. Guo, A. R. Moazzami, A. L. S. Tjen, J. C. Longhurst. Responses of opioid and serotonin containing medullary raphe neurons to electroacupuncture. Brain Res. 1229(2008) 125-136.
[54] A. Moazzami, A. L. S. C. Tjen, Z. L. Guo, J. C. Longhurst. Serotonergic projection from nucleus raphe pallidus to rostral ventrolateral medulla modulates cardiovascular reflex responses during acupuncture. J Appl Physiol (1985). 108(2010) 1336-1346.
[55] S. H. Lee, S. H. Chung, J. S. Lee, S. S. Kim, H. D. Shin, B. V. Lim, et al. Effects of acupuncture on the 5-hydroxytryptamine synthesis and tryptophan hydroxylase expression in the dorsal raphe of exercised rats. Neurosci Lett. 332(2002) 17-20.
[56] X. J. Zhao, H. R. Fang, T. R. Shi, Z. S. Lu. Effect of electroacupuncture (EA) on the functional activity of the rat adrenal. Zhen Ci Yan Jiu. 11(1986) 308-311.
[57] K. Yoshimoto, F. Fukuda, M. Hori, B. Kato, H. Kato, H. Hattori, et al. Acupuncture stimulates the release of serotonin, but not dopamine, in the rat nucleus accumbens. Tohoku J Exp Med. 208(2006) 321-326.
[58] T. Luo, Y. Guo. Specificity study on concentration of monoamine transmitters at acupoint and effect of acupuncture on its distribution. Evid Based Complement Alternat Med. 2014(2014) 704507.
[59] Z. Wenhe, S. Yucun. Change in levels of monoamine neurotransmitters and their main metabolites of rat brain after electric acupuncture treatment. Int J Neurosci. 15(1981) 147-149.
[60] M. Flores-Ramos, J. Moreno, G. Heinze, R. Aguilera-Perez, F. Pellicer Graham. Gonadal hormone levels and platelet tryptophan and serotonin concentrations in perimenopausal women with or without depressive symptoms. Gynecol Endocrinol. 30(2014) 232-235.
[61] Z. Kovacic, N. Henigsberg, N. Pivac, G. Nedic, A. Borovecki. Platelet serotonin concentration and suicidal behavior in combat related posttraumatic stress disorder. Prog Neuropsychopharmacol Biol Psychiatry. 32(2008) 544-551.
[62] I. Barisic, N. Pivac, D. Muck-Seler, M. Jakovljevic, M. Sagud. Comorbid depression and platelet serotonin in hemodialysis patients. Nephron Clin Pract. 96(2004) c10-14.
[63] Immusmol. Serotonin ELISA kit - Serum, Urine, Platelets - Fast Track. https://www.immusmol.com/serotonin-5-ht-elisa-kit-serum-urine-platelets-fast-track-5-hydroxytryptamine.html#references(accessed 16.06.26).
[64] Abcam. Serotonin ELISA Kit ab133053. In: Abcam, editor.2015.
[65] M. Ernberg, U. Voog, P. Alstergren, T. Lundeberg, S. Kopp. Plasma and serum serotonin levels and their relationship to orofacial pain and anxiety in fibromyalgia. J Orofac Pain. 14(2000) 37-46.
[66] A. Srikiatkhachorn. Chronic daily headache: a scientist's perspective. Headache. 42(2002) 532-537.
[67] E. Legangneux, J. J. Mora, O. Spreux-Varoquaux, I. Thorin, M. Herrou, G. Alvado, et al. Cerebrospinal fluid biogenic amine metabolites, plasma-rich platelet serotonin and [3H]imipramine reuptake in the primary fibromyalgia syndrome. Rheumatology (Oxford). 40(2001) 290-296.
[68] V. F. Markelova, V. A. Vesnina, S. I. Malygina, L. A. Dubovskaia. [Changes in blood serotonin levels in patients with migraine headaches before and after a course of reflexotherapy]. Zh Nevropatol Psikhiatr Im S S Korsakova. 84(1984) 1313-1316.
[69] C. Nasr. Flushing. Cleveland Clinic; 2014.
[70] I. D. Hay, J. H. A. Wass. Clinical endocrine oncology. 2nd ed. Massachusetts, USA: Blackwell; 2008.

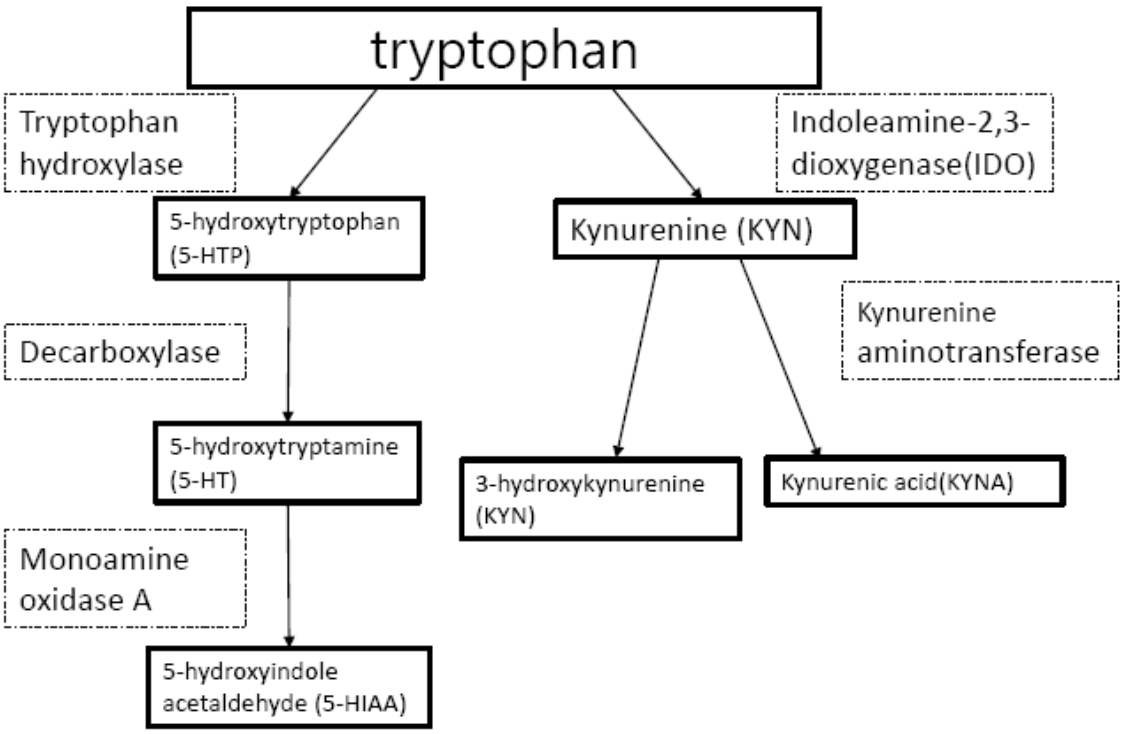
[71] J. K. Payne, J. Held, J. Thorpe, H. Shaw. Effect of exercise on biomarkers, fatigue, sleep disturbances, and depressive symptoms in older women with breast cancer receiving hormonal therapy. Oncol Nurs Forum. 35(2008) 635-642.
[72] Y. Chae, H. J. Park, D. H. Hahm, S. H. Yi, H. Lee. Individual differences of acupuncture analgesia in humans using cDNA microarray. J Physiol Sci. 56(2006) 425-431.
[73] S. Banik, T. Lahiri. Decrease in brain serotonin level and short term memory loss in mice: a preliminary study. Environ Toxicol Pharmacol. 19(2005) 367-370.
[74] M. Cano-Colino, R. Almeida, A. Compte. Serotonergic modulation of spatial working memory: predictions from a computational network model. Front Integr Neurosci. 7(2013) 71.

Figure Captions

Fig. 1. Tryptophan metabolism.

Figure 2. PRISMA flow diagram.

Fig. 1. Tryptophan metabolism.



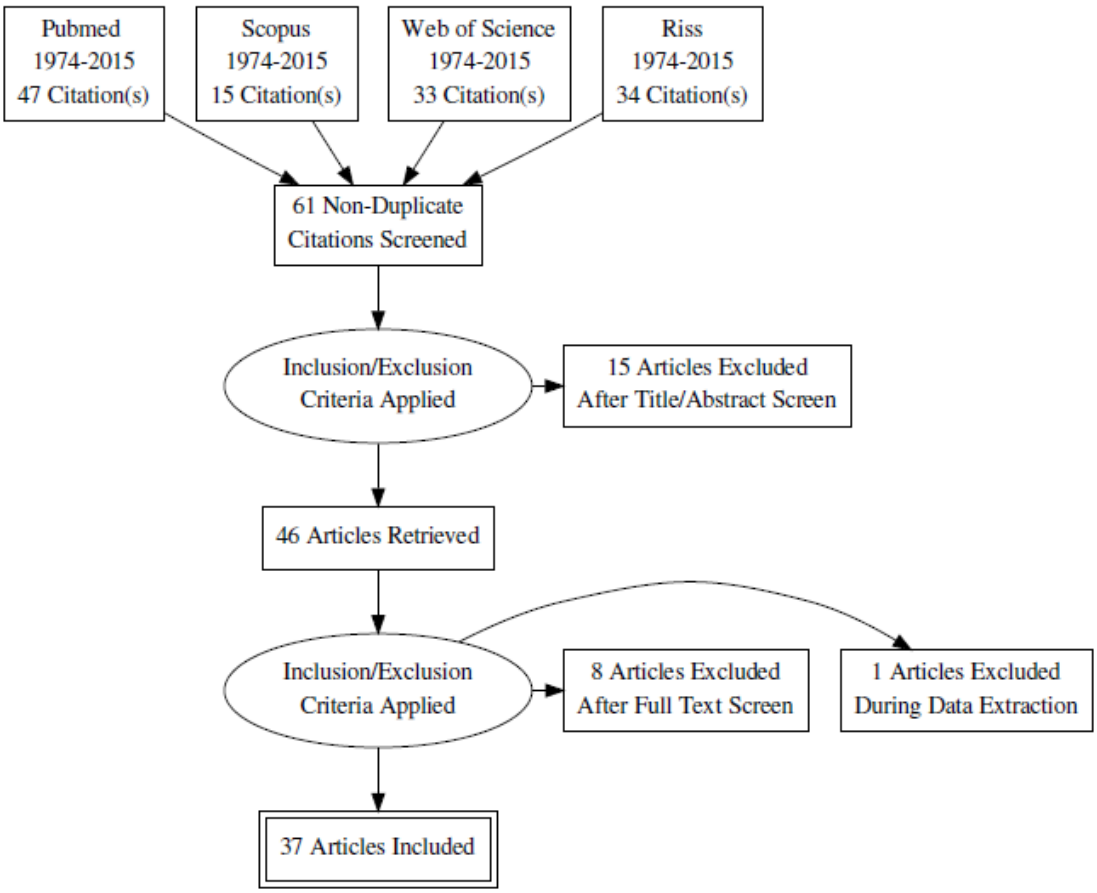


Figure 2. PRISMA flow diagram.

Table 1
Experimental protocols.

First author (year)	Sample	Symptom	n	Desig n	Intervention (depth of needle)	Intervention Total time	Acupuncture points	Placebo/Control
					Human			
Xie(2014)	Esophageal cancer	Postoperative pain	60	RCT	EA, 2-20Hz (1-2cm) D: 0.38mm/L: 40mm M: Huato, Suzhou Medical Supplies	Operative time(222 min)	PC4, PC6 with stimulation	PC4, PC6 with no stimulation
Jia (2009)	Adults	Migraine	275	RCT	EA, 100 Hz(1.5-2cm)	30 min × 5 = 150 min	GB 40	ST25
Qu (2007)	Females	Labor pain	36	RCT	EA, 2–100 Hz(1.5-2cm) D: 0.25mm/L: 50mm	20 min 1st stage labor, 20 min at 7–8 cm dilation = 40 min	LI4, SP6	Natural delivery
Sprott (1998)	25 females 4 males	Fibromyalgia	29	Quasi	Manual	One per week for 6 weeks	No data	None
Mao (1980)	Adults	Chronic pain	36	Quasi	EA, low, high	45 min × 7 days = 315 min	No data	None
Yuan (2007)	Anxiety	Anxiety	86	Quasi	Manual Jin 3 needling	45 min/day × 6 days/week × 6 weeks = 1620 min	PC6, HT7, SP6 plus individualized points	Antidepressant
Zhang (2014)	Cancer patients	Nausea /vomiting	72	RCT	EA, 20Hz, 10 mA	1 hrx2x3days=6hrs	PC5, PC6	EA on non-acupoint
Liu (2008)	Irritable bowel syndrome	Diarrhea	71	RCT	Manual	30 min × 6 sessions × 6 weeks = 1080 min	ST 25, 37	Moxibustion at ST 25, 37
Beer (2010)	Prostate cancer	Hot flashes	22	Quasi	EA, 2 Hz M: Seirin needles	30 min × 1–2 sessions/week × 10 weeks = 420 min	GB34, BL 15,23,32, GV20, HT 7, PC6, LR 2, SP 6	None
Riederer (1975)	12 children 14 adults	Various symptoms	26	Quasi	Manual	20 min* 1–12 sessions = 20–240 min	LI4, ST36, or/and LI3	Not described
					Animal			
Silva(2013)	Rat	Pain	572	Quasi	EA, 2, 100Hz (5mm) D: 0.3mm/L: 30 mm	20 min	ST36, SP6	Acupuncture without stimulation
Zhang (2012)	Rat	Inflammatory pain	148	Quasi	EA, 10Hz, 3mA(5mm) D: 0.25mm/L: 12.5 mm	20 min two times=40 min	GB30	Acupuncture without stimulation
Liu(2009)	Rat	Pain, IBS	24	RCT	EA, 2-100 Hz, 1 mA D: 0.25mm	20 min/day x 7 days = 140 min	ST25, ST37	No acupuncture
Tian(2006)	Rat	Pain	12	RCT	EA, 2 Hz, D: 0.25mm	30 min	ST36, SP6	No acupuncture
Baek	Rat	Inflammatory	100	Quasi	EA, 2 Hz, 0.07 mA, 0.3	30 min	ST36	No acupuncture

First author (year)	Sample	Symptom	n	Design	Intervention (depth of needle)	Intervention Total time	Acupuncture points	Placebo/Control
(2005)		pain			ms(?) D: 0.25mm/L: 50 mm M: stainless-steel			
Chang (2004)	Mice	Pain	120	Quasi	EA, 2,10,100Hz (3-5mm) 32-gauge steel needles	5 min	ST36	No acupuncture
Yonehara (2001)	Rabbit	Pain	110	Quasi	EA, 2 Hz(5mm) D: 0.2mm/L: 48 mm	40 min	ST36	None
Li(1999)	Rat	Pain	20		EA, 4-60Hz, <1mA(5mm)	20 min	ST36, BL60	None
Takagi (1998)	Rabbit	Pain	12	Quasi	EA, 2Hz, 7-15 mA(5mm) D: 0.2mm/L: 48 mm	40 min	ST36	None
Chang (1996)	Rat	Unconsciousness	44	Quasi	Manual(5mm)	120 min	GV20	Acupuncture at same point but less than 5mm deep
Chang (1995)	Rabbit	Unconsciousness	240	Quasi	EA, 2,15,50Hz (5mm)	45 min	GV26, ST36	Acupuncture without stimulation
Kwon (2012a)	Rat	Depression	24	RCT	Manual(3mm) D:0.16mm	30 sec x 7 days = 210 sec	ST36 or HT7	No acupuncture
Kwon (2012b)	Mice	Depression	42	Quasi	Manual(2mm) D: 0.18mm/L: 8 mm M: Dongbang Acupuncture, Inc., Sungnam, Korea	1 min x 16 days	SP6 or HT7	Saline injection
Park(2012)	Rat	Depression	48	RCT	Manual(3mm) D: 0.16mm	30 sec/day/1 week = 210 sec	HT7 or ST36	No acupuncture
Sugai (2004)	Rat	Gastric emptying	140	Quasi	EA, 2Hz, 1V D: 0.25mm/L: 20 mm	30 min for 3 days	ST36,SP6	EA on non-acupoint
Wei(2003)	Rat	Obesity	44	RCT	EA, 10 Hz D: 0.19mm/L: 10 mm	10 min/day x 12 days = 120 min	ST36, ST44	No acupuncture
Liu(2001)	Rat	Obesity	66	RCT	EA(2-4mm) D: 0.19mm/L: 10 mm	10 min 2x/day x 12 days = 240 min	ST36, ST44	No acupuncture
Yan(2011)	Rat	Spinal cord injury	36	RCT	EA, 2-60Hz,1mA Model G 6805-2/ M:Shanghai Medical Electronic Apparatus Company, China	20 min, every other day, for 30days=300 min	GV1, 2. 6, 9	No acupuncture
Ding(2009)	Rat	Spinal cord injury	66	Quasi	EA, 2-60Hz,1mA (5mm) D: 0.3mm/L: 50mm Stainless silver needles Suixin brand, Suzhou Hualun Medical Appliance Corporation	20min x 25 days=500 min	GV1, 2. 6, 9	No acupuncture
Chang (1999)	Rat	Memory deficit	224	RCT	Manual	15, 30, 60 min	GV20	No acupuncture

First author (year)	Sample	Symptom	n	Design	Intervention (depth of needle)	Intervention Total time	Acupuncture points	Placebo/Control
Zhao(1986)	Rat	Adrenal atrophy	87	Quasi	EA, 17Hz	15 min x 9 days=135min	BL23	Saline or none
Luo(2014)	Rabbit	No symptom	20	RCT	Manual(2mm)	Once, 30 min	CV4	Acupuncture at non-acupoints
Moazzami (2010)	Rat	No symptoms	36	Quasi	EA, 2-4Hz, 2-4mA(4mm)	Once, 30 min	PC5, PC6	Acupuncture without stimulation
Lee(2002)	Rat	No symptoms	60	RCT	Manual D: 0.3mm	20min/day x 7 days =140 min	ST36	No acupuncture
Guo(2008)	Cat	No symptoms	11	RCT	EA, 2 Hz, 2–5 V, 1–4 mA (3-4mm) D: 0.25mm/L: 25mm M:stainless steel Suzhou Medical Appliance Factory	30 min	PC5, PC6	No acupuncture
Yoshimoto (2006)	Rat	No symptoms	24	Quasi	Manual D: 0.19mm/L: 10 mm/M: Seirin Kei, Co. Ltd., Shizuoka	60 min	BL23	Acupuncture at non-acupoints
Wenhe (1981)	Rat	No symptoms	48	Quasi	EA, 1.5-2.5mA	60 min vs. 30 min*10 days	GV20, EX-HN3	No acupuncture

Quasi (quasi experimental design), RCT (randomized clinical trial), EA (electro acupuncture), TCM(traditional Chinese medicine), JP(Japanese), KR(Korean) WM (western medical), Conception vessel (CV), governing vessel (GV), heart (HT), pericardium (PC), lung (LU), spleen (SP), liver (LR), kidney (KI), small intestine (SI), triple energizer (TE), large intestine (LI), stomach (ST), gallbladder (GB), urinary bladder (BL), IBS(irritable bowel syndrome), D:diameter, L:length, M: manufacturer and material, ?=No data or not clear

Table 2
Risk of bias

First author (year)	Sequence generation	Allocation concealment	Blinding of participants, personnel, and outcomes	Incomplete outcome data	Selective outcome reporting	Other sources of bias	
						Attrition rate, fidelity	Adverse Reaction?
				Humans			
Xie (2014)	1	0	0	0(no SD)	0	0	0
Jia (2009)	0	0	0	1	1	0	0
Qu (2007)	1	0	0	1	1	1	1(No)
Sprott (1998)	0	0	0	1	1	0	0
Mao (1980)	0	0	0	0(no SD)	0	0	1(No)
Yuan (2007)	1	0	0	1	1	1	1(Yes:2/86, Not described)
Beer (2010)	0	0	0	0	0	1	1(Yes, total number?)
Zhang(2014)	1	0	0	0(no SD)	1	0	1(No)
Liu (2008)	1	0	0	1	1	1	0
Riederer (1975)	0	0	0	0	1	0	1(Yes :1/19)
				Animals			
Silva(2013)	0	0	0	0(no exact number)	1	0	0
Zhang(2012)	0	0	1	0(no exact n)	1	0	0
Liu(2009)	0	0	0	1	1	0	0
Tian(2006)	0	0	0	1	1	0	0
Baek (2005)	0	0	0	1	1	0	0
Chang(2004)	0	0	0	1	1	0	0
Yonehara(2001)	0	0	0	0	0	0	0
Li(1999)	0	0	0	1	1	0	0
Takagi(1998)	0	0	0	0	0	0	0
Chang(1996)	0	0	0	1	1	0	0
Chang(1995)	0	0	0	1	1	0	0
Kwon(2012a)	0	0	0	0(no SD)	1	0	0
Kwon(2012b)	0	0	0	0	1	0	0
Park(2012)	0	0	0	1	1	0	0
Sugai(2004)	0	0	0	0	1	0	0
Wei(2003)	0	0	0	1	1	0	0
Liu(2001)	0	0	0	1	1	0	0
Moazzami	0	0	0	1	0	0	0

(2010)							
Lee(2002)	0	0	0	1	1	0	0
Yan(2011)	0	0	1	0(no exact number)	1	0	0
Ding(2009)	0	0	1	0(no exact number)	1	0	0
Chang(1999)	0	0	1	1	1	0	0
Zhao(1986)	0	0	0	1	1	0	0
Luo(2014)	0	0	0	0(no mean, no SD)	0	0	0
Guo(2008)	0	0	0	1	1	0	0
Yoshimoto(2006)	0	0	0	0(no SD)	0	0	0
Wenhe(1981)	0	0	0	1	1	0	0

Note: SD (standard deviation), N=number, 1 = described, 0 = not described, ?=No data

Table 3

Effect size of serotonin metabolism change.

First author (year)	Location	Acupuncture			Placebo/control/post			Effect size	CI	P
		Mean	SD	n	Mean	SD	n			
			Human							
Xie(2014)	Plasma 5-HT [a]	154.66	52.49	20	225.28	82.03	20	1.02	0.35-1.66	<0.01
Jia (2009)	Plasma 5-HT [b]	5.46	0.77	133	4.25	0.5	130	1.85	1.57–2.14	<0.01
Qu (2007)	Serum 5-HT [c]	2501.3	890.2	18	2099.2	675.4	18	0.5	-0.17–1.16	<0.05
Sprott (1998)	Serum 5-HT (pre-post) [c]	134.0	14.3	29	171.2	14.6	29	2.54	1.85–3.24	<0.01
	Platelet 5-HT (pre-post) [d]	715.8	225.8	29	352.4	47.9	29	2.2	1.55–2.86	<0.01
Mao (1980)	Platelet 5-HT (pre-post) [e]	0.27	No data	13	0.45	No data	13			<0.01
Yuan (2007)	Platelet 5-HT (pre-post) [f]	511.6	195.4	27	502.7	203.9	27	0.04	-0.49–0.58	NS
Beer (2010)	Serum 5-HT [b]	No data								NS
	24-h urinary 5-HIAA (% of change)	0.73	0.2	12	1.29	0.20	10	2.69	1.54–3.85	<0.01
Zhang(2014)	Serum 5-HT(mean difference between pre-post) [d]	45	No data	38	10	No data	34			0.03
Liu (2008)	5-HT expression in colon	Dark stain			Light stain					
Riederer (1975)	Urine 5-HIAA (pre-post) [a]	5.8	1.7	19	13.2	4.55	19	2.11	1.32–2.90	<0.01
	Free tryptophan [a]	3.05	0.57	5	3.15	0.52	5	0.17	0.08–1.41	NS
	Protein-bound tryptophan [a]	6.75	1.15	5	6.65	1.08	5	0.08	-1.16–1.32	NS
			Animal							
Liu(2009)	Tissue 5-HT : colon [d]	64.5	13.2	8	82.1	14.8	8	1.19	0.12-2.25	<0.05
Tian(2006)	Tissue 5-HT: colon [c]	25.12	8.8	6	38.6	8.0	6	1.48	0.2-2.76	<0.05
Yonehara(2001)	Tissue 5-HT: trigeminal nucleus caudalis [i]	140.9	27.8	8	79.6	10.2	8	2.77	1.40-4.14	<0.01
Li(1999)	5-HT in in periaqueductal gray [l]	29.2	2.5	5	22	3	5	2.35	0.74-3.97	<0.01
	5-HIAA in in periaqueductal gray [l]	35	3	5	27	3	5	2.41	0.78-4.03	<0.01
Kwon(2012a)	Tissue 5-HT: prefrontal cortex [g]	120	20?	6	130	5?	6	0.63	-0.53-1.79	NS
	Tissue 5-HT: hippocampus [g]	110	10?	6	140	40?	6	0.96	-0.24-2.14	NS
	Tissue 5-HIAA: prefrontal cortex [g]	140	10?	6	140	20?	6	0	-1.13-1.13	NS
	Tissue 5-HIAA: hippocampus [g]	130	5?	6	130	3?	6	0	-1.13-1.13	NS
	5-HIAA/5-HT: prefrontal cortex	0.8	0.15?	6	0.7	0.15?	6	0.62	-0.54-1.77	NS
	5-HIAA/5-HT: hippocampus	1.25	0.2?	6	1.05	0.2?	6	0.92	-0.27-2.11	NS
Kwon(2012b)	Tissue 5-HT: prefrontal cortex [g]	40	2?	6	38	2?	5	0.92	-0.27-2.11	NS
	Tissue 5-HT: hippocampus [g]	180	15?	6	175	20?	5	0.26	-0.92-1.45	NS
	5-HIAA/5-HT: prefrontal cortex	0.4	0.07?	6	0.55	0.15?	5	1.22	-0.08-1.22	NS

	5-HIAA/5-HT: hippocampus	0.28	0.06?	6	0.23	0.02?	5	0.98	-0.28-2.53	NS
Park(2012)	Tissue 5-HT: prefrontal cortex [g]	112.8	4.4	12	98.3	3.9	12	3.4	1.72- 5.04	<0.01
Wei(2003)	Tissue 5-HT: raphe nuclei [g]	6.5	2.3	15	4.4	1.9	14	0.96	0.2-1.73	<0.05
Liu(2001)	Tissue 5-HT: VMH [j]	1.2	0.1	11	1.6	0.4	9	0.32	-0.26-0.93	NS
	Tissue 5-HIAA: VMH [j]	2	1.8	11	3.7	1.6	9	0.95	0.02-1.88	<0.05
	5-HT/5-HIAA:VMH	0.6	0.18	11	0.4	0.17	9	0.16	-0.72-1.04	NS
Yan(2011)	5-HT positive nerve fiber	7	1?	5	2.5	0.1?	5	3.41	1.47-5.36	<0.01
Ding (2009)	5-HT positive nerve fiber	27	3	?	9	1	?			<0.01
Lee(2002)	Tissue 5-HT: dorsal raphe [k]	63.8	3.15	10	46.4	3.0	10	5.44	3.54-7.34	<0.01
	Tissue tryptophan: dorsal raphe [k]	144.9	6.7	10	100.57	7.3	10	6.06	3.99-8.13	<0.01
Zhao(1986)	5-HT in the diencephalon [g]	1467	178	15	1107	135	15	2.2	1.31-3.13	<0.01
	5-HIAA in the diencephalon [g]	1509	157	15	1168	145	15	2.2	1.29-3.10	<0.01
Luo(2014)	Tissue fluid 5-HT [b]	0.15	No data	20	0.002	No data	20			
Guo(2008)	Tissue 5-HT: NRP [b]	60	3	6	46	6	5	2.79	1.13-4.46	<0.01
Yoshimoto (2006)	Tissue5-HT: nucleus accumbens (% of baseline)	175	No data	5	110	No data	8			<0.05
Wenhe(1981)	Cerebral 5-HT(single EA)[g]	705	10	12	609	10	12	9.27	6.53-12.01	<0.01
	Cerebral 5-HT(multi EA)[g]	479	29	12	426	31	11	1.7	0.75-2.66	<0.01
	Cerebral 5-HIAA (single EA)[g]	1152	52	12	930	40	12	4.62	3.09-6.15	<0.01
	Cerebral 5-HIAA(multi EA)[g]	614	31	9	560	36	8	1.53	0.45-2.62	<0.01

5-HT:5-hydroxytryptamine), 5-HIAA :5-hydroxyindole acetaldehyde, VMH: ventromedial nucleus of hypothalamus, NRP: nucleus raphe pallidus ,CI: confidence interval, SD: Standard deviation, NS: not significant, [a] μg/ml , [b] no unit, [c] ng/ml, [d] pg/mL, [d] μg/10^{12}plateltes, [e] μg/10^{9}plateltes, ng/L, [f] ng/10^{9} platelets, [g] ng/g wet tissue, [h] ng/mg protein, [I] pg/20 min, [j] μg/g, [k] Bregma-7.30 mm to –8.00 mm [l] μg /L^{-1}

Table 4
Effect size of symptom changes.

First author (year)	Symptom	Acupuncture			Placebo/control/post			Effect size	CI	p
		Mean	SD	n	Mean	SD	n			
			Human							
Xie(2014)	VAS for pain	2	No data	20	4	No data	20			<0.05
Jia (2009)	% of improvement on VAS for pain at week 4	0.7	0.2	138	0.58	0.2	137	0.6	0.36–0.84	<0.01
Qu(2007)	Labor pain measured by mean rank using 0–10 scale	14.33	No data	18	22.67	No data	18			<0.05
Sprott (1998)	VAS for pain (0–100)	64	3.4	29	34.5	4.3	29	7.51	6.05–8.97	<0.01
Mao(1980)	VAS for pain (0–100) (pre-post)	70	No data	13	44.2	No data	13			< 0.01
Yuan(2007)	Anxiety measured by efficacy scale among clinical global impression scale	3.64	1.17	27	2.03	1.18	26	1.37	0.75–1.95	< 0.01
Beer(2010)	Hot Flash Related Daily Interference Scale	1	0.2	22	0.55	0.2	22	2.21	1.46–2.96	<0.01
Zhang(2014)	Nausea	1.21	0.15	38	1.88	0.1	34	5.28	4.25-6.19	<0.01
Liu(1980)	% of patients who have diarrhea (pre-post)	1	0.2	71	0.57	0.2	71	2.14	1.73–2.55	<0.01
Riederer(1975)	% of patients who have symptoms	1	0.2	19	0.26	0.2	19	3.62	2.59–4.66	<0.01
			Animal							
Liu(2009)	Pain measured by Abdominal Withdrawal Reflex (AWR) Scores	3.5	0.4	8	4.0	0.1	8	1.71	-0.49-2.75	< 0.05
Silva(2011)	Pain threshold measured by tail-flick test (second)	4.8	0.2?	6	2.8	0.1?	6	11.67	6.87-16.48	< 0.05
Zhang(2012)	Pain measured by paw withdrawal latency test	6.89	0.26	8	5.13	0.39	6	5.1	2.94-7.27	<0.01
Tian(2006)	Pain measured by AWR score	2.7	0.4	6	3.7	0.4	6	2.5	0.85-3.77	<0.01
Baek(2005)	Pain threshold measured by tail-flick test (%)	39.2	5.2	6	0.8	2.1	6	8.9	5.19-12.69	<0.01
Chang(2004)	Pain measured by licking time	56.1	5.1	8	118.7	7.9	8	8.9	5.66-12.14	<0.01
Li(1999)	Pain threshold measured by tail-flick test (second)	140	11	5	36	9	5	9.3	5.06-13.62	<0.01
Takagi(1998)	Pain measured by electrodes (%)	39.7	No data	6	100	No data	6			
Chang(1996)	Sleeping time	36.2	1.2	22	45.6	1.6	22	6.5	5.04-8.01	<0.01
Chang(1995)	Sleeping time	76.1	8.4	8	243.6	6.1	8	21.56	14.03-29.11	<0.01
Kwon(2012a)	Depression measured by immobility	38	5.21	6	143.5	20.05	6	7.2	3.76-9.54	< 0.01

	time (second)									
Kwon(2012b)	Depression measured by immobility time (second)	165	10	6	210	5	6	4.86	2.51-7.21	< 0.01
Park(2012)	Depression measured by immobility	31.20	5.61	12	87.75	13.09	12	5.62	3.69-7.15	< 0.01
Sugai(2004)	Gastric emptying measured by plastic beads	1	4	10	12	3	10	2.98	1.71-4.25	< 0.01
Wei(2003)	Obesity measured by Lee's index	227.5	11	14	248.9	11.9	15	1.86	0.95-2.68	<0.01
Liu(2001)	Obesity measured by Lee's index	0.2394	0.01	22	0.2528	0.012	22	1.21	0.55-1.83	<0.05
Zhao(1986)	Adrenal function measured by adrenal weight (mg)	35.06	0.9	15	31.09	1.2	15	3.64	2.74-4.81	<0.01
Moazzami (2010)	Mean arterial blood pressure	22	4	5	41	4	5	4.29	2.04-6.54	<0.01
Lee(2002)	Endurance treadmill exercise (min)	60.91	2.84	10	40.92	3.29	10	6.5	4.11-8.35	<0.01
Yan(2011)	Open field locomotion test (0-21)	3	0.5?	5	1	1?	5	2.28	0.69-3.88	<0.05
Ding(2009)	Mobility by locomotor rating scale	4	1.8?	4	1.9	0.4?	3	1.25	-0.38-2.88	NS
Chang(1999)	Passive avoidance response(sec)	10.52	1.45	8	7.19	1.28	8	2.3	1.04-3.57	< 0.01
Guo(2008)	% of mean blood pressure change	0.5	0.2	6	0	0	5	3.35	1.32-4.81	< 0.01

Lee index: cube root of body weight (g) /nose-to-anus length (cm), VAS: Visual Analogue Scale

Table 5

Revised standards for reporting interventions in clinical trials of acupuncture.

First author (year)	1a	1b	1c	2a	2b	2c(mm)	2d	2e	2f(min)	2g	3a	3b	4a	4b	5	6a	6b
							Human										
Xie(2014)	TCM	Y	S	4	Y	10-20	Sore/expanding	E	222	Y	1	Y	N	N	N	Y	Y
Jia (2009)	TCM	Y	S	2	Y	15-20	N	E	30	N	5	Y	N	N	N	Y	Y
Qu (2007)	TCM	Y	S	4	Y	15-20	Sore distending sensation	E	20	P	2	Y	N	N	N	Y	Y
Sprott (1998)	WM	Y	I	N	N	N	N	M	N	N	N	N	N	N	N	N	X
Mao (1980)	WM	Y	N	N	N	N	Heaviness	E	45	N	7	Y	N	N	N	N	Y
Yuan (2007)	WM	Y	I	10	Y	N	N	M	45	N	36	Y	N	N	N	Y	Y
Zhang(2014)	TCM	Y	S	2?	Y	N	N	E	60	N	6	Y	N	N	N	Y	Y
Liu (2008)	TCM	Y	S	4	Y	N	N	M	30	N	36	Y	N	N	N	Y	Y
Beer (2010)	WM	Y	S	13	Y	N	N	E	30	P	10-20	Y	N	N	N	N	X
Riederer(1975)	WM	Y	I	2-6	Y	N	N	M	20	N	1-12	Y	N	N	N	N	N
							Animals										
Silva(2013)	WM	Y	S	4	Y	5	Muscle twitch	E	20	P	1	Y	N	N	N	Y	Y
Zhang(2012)	TCM	Y	S	2	Y	12	N	E	20	P	2	Y	N	N	N	Y	Y
Liu(2009)	TCM	Y	S	4	Y	5	N	E	20	P	7	Y	N	N	N	Y	Y
Tian(2006)	TCM	Y	S	4	Y	5	N	E	30	P	1	Y	N	N	N	Y	Y
Baek (2005)	KR	Y	S	2	Y	N	N	E	30	P	1	Y	N	N	N	Y	Y
Chang(2004)	TCM	Y	S	2	Y	3-5	N	E	40	P	1	Y	N	N	N	Y	Y
Yonehara(2001)	JP	Y	S	2	Y	5	N	E	40	P	1	Y	N	N	N	Y	X
Li(1999)	TCM	Y	S	2	Y	5	N	E	20	N	1	Y	N	N	N	N	X
Takagi(1998)	JP	Y	S	2	Y	5	N	E	40	P	1	Y	N	N	N	N	X
Chang(1996)	TCM	Y	S	1	Y	5	N	M	120	N	1	Y	N	N	N	Y	Y
Chang(1995)	TCM	Y	S	2	Y	5	N	E	45	N	1	Y	N	N	N	Y	Y

First author (year)	1a	1b	1c	2a	2b	2c(mm)	2d	2e	2f(min)	2g	3a	3b	4a	4b	5	6a	6b
Kwon(2012a)	KR	Y	S	2	Y	3	N	M	0.5	P	7	Y	N	N	N	Y	Y
Kwon(2012b)	KR	Y	S	4	Y	2	N	M	1	Y	16	Y	N	N	N	Y	Y
Park(2012)	KR	Y	S	4	Y	3	N	M	0.5	P	7	Y	N	N	N	Y	Y
Sugai(2004)	WM	Y	S	4	Y	N	N	E	30	P	3	Y	N	N	N	Y	Y
Wei(2003)	TCM	Y	S	4	Y	N	N	E	10	P	12	Y	N	N	N	Y	Y
Liu(2001)	TCM	Y	S	4	Y	2-4	N	E	10	P	12	Y	N	N	N	Y	Y
Yan(2011)	TCM	Y	S	8	Y	N	N	E	20	P	15	Y	N	N	N	Y	Y
Ding(2009)	TCM	Y	S	8	Y	5	A slight twitch of the hind limb	E	20	Y	25	Y	N	N	N	Y	Y
Chang(1999)	TCM	Y	S	1	Y	N	N	E	V	N	3	Y	N	N	N	Y	Y
Zhao(1986)	TCM	Y	S	2	Y	N	N	E	15	N	9	Y	N	N	N	Y	Y
Luo(2014)	TCM	N	S	2	Y	2	N	M	30	N	1	Y	N	N	N	Y	Y
Moazzami(2010)	WM	N	S	4	Y	4	N	E	30	N	1	Y	N	N	N	Y	Y
Lee(2002)	KR	N	S	2	Y	N	N	M	20	P	7	Y	N	N	N	Y	Y
Guo(2008)	WM	N	S	4	Y	N	Paw flexion in each forelimb	E	30	N	1	Y	N	N	N	Y	Y
Yoshimoto(2006)	JP	N	S	2	Y	N	N	M	60	Y	1	Y	N	N	N	Y	Y
Wenhe(1981)	TCM	N	S	2	Y	N	N	E	30-60	N	10	Y	N	N	N	Y	Y

1a) Style of acupuncture (e.g. Traditional Chinese Medicine, Japanese, Korean, Western medical, Five Element, ear acupuncture, etc)
1b) Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate
1c) Extent to which treatment was varied 2a) Number of needle insertions per subject per session (mean and range where relevant)
2b) Names (or location if no standard name) of points used (uni/bilateral) 2c) Depth of insertion, based on a specified unit of measurement, or on a particular tissue level
2d) Response sought (e.g. *de qi* or muscle twitch response) 2e) Needle stimulation (e.g. manual, electrical) 2f) Needle retention time
2g) Needle type (diameter, length, and manufacturer or material)3a) Number of treatment sessions 3b) Frequency and duration of treatment sessions
4a) Details of other interventions administered to the acupuncture group (e.g. moxibustion, cupping, herbs, exercises, lifestyle advice)
4b) Setting and context of treatment, including instructions to practitioners, and information and explanations to patients
5) Description of participating acupuncturists (qualification or professional affiliation, years in acupuncture practice, other relevant experience)
6a) Rationale for the control or comparator in the context of the research question, with sources that justify this choice
6b) Precise description of the control or comparator.
TCM: traditional Chinese medicine; JP: Japanese; KR: Korean; WM: western medical, Y: described, N: not described S: standard, I: individualized, E:electroacupuncture, M: manual acupuncture, V: various, X: no control, P: partially reported