Oxytocin Release during the Meditation of Altruism and Appreciation (Arigato-Zen)

Sohou Machida, Masataka Sunagawa, Toku Takahashi

ABSTRACT
A number of modern researchers have studied the relationship between religion and health. In every religious tradition, virtues such as self-sacrifice, altruism, and appreciation have been considered quite essential. Arigato-Zen (AZ) (Gratitude Zen) is an easy and simple voice-meditation developed by Dr. Machida, which motivates the feeling of altruism and appreciation. By performing AZ, one is able to cleanse the negative subconscious memories that underlie problematic phenomena. Hypothalamic oxytocin (OT) plays an important role in the ability to form social attachments, including parental care and pair binding. It has been shown that social interaction is important to adapt to our daily life stress via the OT expression. It is hypothesized that OT is stimulated via the AZ meditation of altruism and appreciation. Using saliva samples, OT levels were measured in 32 participants before and after AZ. Salivary OT levels were significantly increased after AZ from 66.3 ± 6.7 pg/mL to 90.6 ± 18.7 pg/mL (M ± SE, n = 32, p = 0.028). AZ practice is a quite unique type of meditation, which combines voice-vibration system and traditional Zen meditation in order to synergistically stimulate OT system.

INTRODUCTION
(1) Religious tradition and well being
In every religious tradition, virtues such as self-sacrifice, altruism, and appreciation have been considered quite essential. “Do to others as you would have them do to you (Luke 6: 31)” is quite well known even outside Christian community and so-called Golden Rule stands in the same line with this kind of biblical messages.

Religious traditions have also taught us to be grateful for what they have been given as divine blessing or grace. Diverse religious rituals and services such as Thanksgiving Day in Christian tradition have been formed in a principle of appreciation.

Key words: Buddhism; Dopamine; Loving-Kindness Meditation (LKM); Religion; Social Attachment; Serotonin

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between religion and health. Majority of those studies indicate that believers who regularly commute to religious facilities have longer life spans than those who do not believe in any religion.

“If you want others to be happy, practice compassion. If you want to be happy, practice compassion.” (Dalai Lama). Buddhist traditions have emphasized the importance of cultivating connection and love toward others through techniques such as loving-kindness meditation (LKM). A randomized clinical research has demonstrated that the practice of LKM decreased chronic low back pain, psychological distress, and anger\[9\]. Thus, performance of religious thoughts provided the evidence that cultivating connection and social interaction are key factors to maintain our well being. However, the biological and/or neural mechanisms of beneficial effects of LKM are not well understood\[2,3\].

(2) Arigato-Zen (AZ)
Arigato-Zen (AZ) (Gratitude Zen) is an easy and simple voice-zen meditation developed by Dr. Machida based on his decades of Zen training and scholarship on comparative religion. In AZ meditation, the practitioners are forced to vocalize ‘A—Ri—Ga—To—U—’ by the long and deep abdominal breathing. In addition, the practitioners are asked to meditate the feeling of altruism and appreciation. Arigato means “thank you” in Japanese. By performing AZ, one is able to cleanse the negative unconscious memories that Underlie problematic phenomena\[4\].

With the motto “mental change through physical practice,” Dr. Machida’s revolutionary method of meditation consists of four pillars (Arigato Breathing, Gratitude Zen, Joyful Zen, and Nirvana Zen), as shown in Methods. After this one-hour voice-meditation (AZ), one should feel a great change mentally and physically. Following the repetition of AZ practice several times, many people were released from the mental disorders, such as panic, anxiety and depression\[6\].

(3) Anti-stress effects of oxytocin (OT)
A growing body of evidence suggests that stress stimuli, both acute and chronic, promote different physiological mechanims and neuroendocrine responses. OT is mainly synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Besides female reproductive functions, OT is also known for its anti-stress and anti-anxiety effects\[9\]. OT attenuates hypothalamic–pituitary–adrenal (HPA) axis in response to stress\[6,7\].

Anti-stress and anti-anxiety effects of OT are mediated by its inhibitory effect on corticotropin releasing factor (CRF) expression\[9\]. The inhibitory effect of OT on CRF expression may not have a direct effect on CRF neurons, because the majority of neuronal responses to OT are excitatory. GABAergic neurons are located in the immediate surroundings of the PVN (peri-PVN). These GABA-projecting neurons into the PVN have been shown to inhibit CRF synthesis via GABA\(_A\) receptors\[9\]. We have previously demonstrated that the inhibitory effect of OT on CRF mRNA expression is mediated by GABA\(_A\) receptors at the PVN\[9\]. In the central amygdala, others also showed that OT enhances GABAergic transmission through an increased frequency of the inhibitory postsynaptic currents\[9\].

(4) Social attachment of OT
Social activity is related to beneficial effects on the cardiovascular, endocrine, and immune systems. Social connectedness may have stress-buffering effects. It is important for us to feel connected, to be trusted and loved. Feeling connected to others increases psychological and physical well being and decreases the risk of depression and physical ailments. People with a higher quality of social relationships show a lower risk of death, while social isolation has been shown as a major risk factor for mortality\[11,12\]. Unfortunately, our society is becoming increasingly isolated and distrustful.

OT has been implicated in a number of social behaviors, including maternal care, affiliation and social attachment. In humans, intranasal administration of OT increases trust behavior\[13\]. The social interaction of daily life as well as a positive environment continuously activates the system of OT release in both males and females\[14\]. We have shown that social interaction is important to adapt to our daily life stress via the hypothalamic OT expression\[15,16\].

A positive social interaction is bidirectional, involving both giving and receiving empathy. A recent study showed that affiliative behavior toward others attenuates stress responses via up-regulating hypothalamic OT expression\[17\]. This suggests that giving affection and empathy to others may be a key in upregulating hypothalamic OT expression. As OT is linked to health promoting cardiovascular, analgesic, and anti-stress effects, upregulated OT expression would help to maintain our mental and physical health.

Although kindness-based contemplative practices are increasingly employed by clinicians and cognitive researchers to enhance prosocial emotions, social cognitive skills, and well being, the mechanisms by which kindness-based meditation may alter the brain and body still remains unknown.

It is hypothesized that hypothalamic OT is stimulated via the AZ meditation of altruism and appreciation. It has been shown that salivary and plasma OT levels are highly correlated. Subjects with higher plasma OT also demonstrated higher salivary OT\[17\]. To study if AZ may affect the OT levels, saliva was collected form each subjects before and after AZ.

MATERIALS AND METHODS

(1) Subjects
Thirty two healthy subjects (10 males and 22 females, mean age; 46.4) were recruited for AZ. The first study was performed in the afternoon (2-3 pm) of November 2016 (14 participants) and the second study was performed in the afternoon (2-3 pm) 3 months later (18 participants).

The number of previous experience of AZ and the characteristics of the participants were summarized in Table 1. Especially, ten of 32 participants had no previous experience of AZ and none of them learned AZ before starting the study. All participants voluntarily signed consent forms and the study was approved by the local Ethical Committee.

(2) Practice of AZ

1. Arigato Breathing; During the first 15 minutes, in sync with diaphragm breathing, one internally recite the words “I am sorry” and “Thank you” toward to our own inner child. People today have the tendency to breathe in short, shallow breaths; this is one of the causes

Table 1 The characteristics of the participants of AZ meditation (n = 32).

<table>
<thead>
<tr>
<th>Times of previous experiences of AZ</th>
<th>Number of participants</th>
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of our daily stress. Simply by breathing long and deep, one feels calmer and more relaxed.

2. Gratitude Zen: During the next 15 minutes, while feeling the vibrations of own voice, one vocalize ‘A---Ri---Ga---To---U---’. It is especially important for everyone to think of those who were supportive and kind to us in the past, and express our profound gratefulness to them. This serves the purpose of introspective healing, and helps to relieve stiffness of the mind. While practicing Gratitude Zen, the head is turned slightly upwards, allowing one to chant merrily and sonorously. Tears may flow as a sign of the clarity of the unconsciousness.

3. Joyful Zen: In the next 15 minutes of Joyful Zen, everyone continue to vocalize ‘A-Ri-Ga-To-U’ in the same manner. However, in this part of meditation, we concentrate in remembering happy and joyful events in our life. No matter what small incidents they were, it does not matter. This is one of the best ways to delete traumatic memories in the past.

4. Nirvana Zen: In the last 15 minutes everyone lie down on the floor face up, continuing to chant the same words. It is important to let one become fully absorbed in the sounds of ‘A---Ri---Ga---To---U---’. The sound waves that are created by saying the vowel words together as a group have healing effects on the body and mind. As a result of this steady chanting technique, one is able to enter the mental state known as “non-self”.

Generally, the eyes are kept half-closed or closed. Sleepiness is a sign that the body and mind are entering a relaxed state – waking refocuses one’s concentration.

(3) OT assay
Before starting AZ, each subject was asked to collect the saliva (0.5 mL). AZ was performed for 60 min in total. Immediately after finishing the AZ meditation, another sample of saliva was collected to the tubes. These tubes were then immediately kept at -20°C until the assay.

OT levels in saliva were measured by enzyme-linked immunosorbent assay (ELISA) using the OT ELISA kit (ADI-900-153A, Enzo Life Sciences, Farmingdale, NY), in which the endogenous OT competes for the OT antibody-binding sites with added OT linked to alkaline phosphatase. After the overnight incubation at 4°C, the excess reagents were washed away and the bound OT phosphatase was incubated with substrate. After 1 h, this enzyme reaction (which generates a yellow color) was stopped and the optical density (OD) was read on a microplate reader (Model 680XR, Bio-Rad, Hercules, CA) at 405 nm. The intensity of the color is inversely proportional to the concentration of OT in the sample. The content (in pg/ml) was determined by plotting the OD of each sample against a standard curve. The lower limit of sensitivity was 15.6 pg/mL. Statistical analysis was performed by Wilcoxon t test.

RESULTS

(1) Changes of salivary OT following AZ
OT levels were increased in 23 out of 32 participants following AZ meditation. Minor increase (less than 10 percent increase form the pretreatment levels) was observed in 4 participants, and major increase (more than 20 percent increase from the pretreatment levels) was observed in 15 participants.

On the other hand, 9 participants showed the reduction of OT levels following AZ meditation. Minor reduction (less than 10 percent reduction form the pretreatment levels) was observed in 3 participants, and major reduction (more than 20 percent reduction from the pretreatment levels) was observed in 6 participants (Figure 1).

In total, the concentration of OT in saliva before AZ was 66.3 ± 6.7 pg/mL (M ± SE, n = 32). Salivary OT levels were significantly increased after AZ to 90.6 ± 18.7 pg/mL (M ± SE, n = 32, p = 0.028) (Figure 2).
DISCUSSION

(1) OT concentration of the body fluid

It has been demonstrated that plasma OT levels do not correlate with the OT concentrations in the cerebrospinal fluid. In contrast, others showed that plasma OT could reflect coordinated release from hypothalamic OT neurons. The neurons that project to the pituitary are known to release OT somato-dendrically. Such activation may result in release from terminals in the pituitary and some dendrites or collaterals that project into the brain. Various stimuli cause OT releases in the brain, which then directly modifies activity of socially-relevant brain circuits. Thus, it is conceivable that the plasma and/or salivary OT levels correlate strongly with the relevant brain levels of the peptide.

Plasma OT responses can occur extremely rapidly, and may be most evident within 90 seconds of a triggering event. In contrast, time series analyses suggest a delay in the transfer of hormones from plasma to saliva, and hormonal peaks in saliva have been documented to occur within 10 min after those in blood. Our current study showed a significant increase of salivary OT levels following one-hour practice of AZ meditation.

(2) OT and the altruism

Studies of humans and other social animals showed widespread evidence of the beneficial effects of prosocial and altruistic behavior. Numerous studies reveal protective effects of volunteering on mental and physical health. Both consistency of volunteering over time and diversity of participation are significantly related to well being and self-reported health. Participation in clubs and volunteer activities had a significant protective effect on mortality. Piliavin insisted, “One does well by doing good.”

It has been suggested that a positive social interaction, which upregulates hypothalamic OT expression, is an important factor to overcome daily life stress and various symptoms. Intranasal OT administration reduces behavioral and endocrine responses to social stress, mediates social buffering, attenuates the hyperactivity of amygdala to fearful stimuli and improves social cognition and empathy. Activation of OT signaling via social interactions can ameliorate the effects of stress on GI function. Amygdala is stimulated by emotional arousal, such as affective words. Amygdala is a source of efferent projections to the ventromedial hypothalamic nucleus. Outputs of the Amygdala to the basal forebrain and hypothalamus orchestrate the behavioral, autonomic, and neuroendocrine responses to conspecifics. Received prosocial or trust behaviors may activate efferent output from the amygdala to OTeric systems.

(3) OT release induced by somatosensory stimulation

Various types of somatosensory stimulation (massage, acupuncture, thermal stimulation, vibration, and afferent sciatic nerve stimulation) can increase OT levels in plasma and cerebrospinal fluid in anesthetized rats. Malfunction of GI tract induced by chronic hypertrophy stress is significantly improved by transcutaneous electrical nerve stimulation (TENS) in rats, which is abolished by icv-injection of OT antagonists. TENS increased the number of OT-immunopositive cells and decreased CRF-immunopositive cells at the PVN following chronic hypertrophic stress. These suggest that sensory nerve stimulation activates hypothalamic OT neurons via the spinothalamic pathway. Activated OT neurons inhibit CRF expression, resulting in the attenuation of stress responses of GI tract.

(4) OT release induced by Yoga and mindfulness meditation

Yoga as a therapy has proven to be effective as a sole or additional intervention in psychiatric disorders such as depression and anxiety. Research reports have demonstrated the feasibility and efficacy of yoga as an add-on therapy in schizophrenia, particularly in improving negative symptomatology and social cognition. The yoga therapy group showed a significant improvement in socio-occupational functioning and plasma increase in OT levels in schizophrenia, as compared with the waitlist group.

Cancer survivors experience high levels of distress, associated with a host of negative psychological states, including anxiety, depression, and fear of recurrence, which often lead to sleep problems and reduction in quality of life (QOL) and well being. OT levels were significantly larger in the mindfulness meditation group as compared with control. OT levels were negatively associated with sleep problems and depression and positively associated with cancer-related QOL and well being.

(5) OT release induced by AZ meditation

Honen (1,133 –1,212 DC) was the religious reformer and founder of the first independent branch of Japanese Pure Land Buddhism, called Jodo Buddhism (Jodo-shu). Honen sought an approach to Buddhism that anyone could follow. He undertook the teaching of rebirth in the pure land of Amitabha through nianfo. “Only repeat the name of Amitabha with all your heart. Whether walking or standing, sitting or lying, never cease the practice of it even for a moment.” This nianfo, called Nembutsu in Japanese, was all one needed to enter Amitabha’s pure land. Nembutsu is pronounced as “Na Ma Ami Da Bu Tsu” in Japanese.

Repetition of Nembutsu is the most fundamental practice of Jodo Buddhism, which derives from the primordial vow of Amitabha. In home practice, or in temple liturgy, Nembutsu may be recited in any number of styles. Practitioners are encouraged to engage in auxiliary practices, such as observing the Buddha’s teaching, meditation, chanting of sutras and other good conduct. As Jodo Buddhism stresses that the compassion of Amitabha is extended to all beings that recite Nembutsu, so how one observes auxiliary practices is left to the individual to decide.

It seems that calling Nembutsu may stimulate vocal sensory system and meditation during Nembutsu may stimulate prefrontal cortex (PFC), both of which may activate hypothalamic OT system. Thus, Nembutsu promotes altruism and peaceful feeling, which Honen recognized as pure land of Amitabha.

Instead of calling Nembutsu, Dr. Machida insisted to call “Arigato (Thank you in Japanese)” in order to exclude any religious matters. Our current study showed that AZ surely activated hypothalamic OT system, probably due to the activation of vocal sensory system and mindfulness meditation (Figure 3).

The mechanism of increased OT following AZ remains unclear. It has been shown that noradrenergic and serotonergic transmission plays important roles in neuroendocrine stress responses. Hypothalamic magnocellular neurons receive input from A1 and A2 noradrenergic neurons in the medulla oblongata. A variety of stressful stimuli activate medullary noradrenergic neurons, which stimulate OT release from the PVN via alpha, adrenergic receptors.

Dopaminergic (DAergic) fibers regulate OT release from the PVN. As there are no direct projections from the amygdala to the PVN, it is likely that DAergic neurons are involved in relaying between amygdala and PVN-OT neurons. D1 receptors in the nucleus accumbens are important for the mediation of social attachments in female voles. Based on these observations, it is likely that...
Social buffering may activate the hypothalamic OT neurons via the Amygdala - DAergic pathways and D_2 receptors. The meditation as mind-body training group showed higher scores on positive affect and lower scores on stress compared with the control group. Plasma DA levels were higher in the meditation than in the control group. The control group demonstrated a negative correlation between stress and positive affects, whereas this correlation was not observed in the meditation group. These results suggest that meditation is associated with lower stress, higher positive affect and higher plasma DA levels.

Using ^11^C-raclopride positron emission tomography (PET), increased endogenous DA release was shown in the ventral striatum during Yoga meditation. The increased DA was significantly correlated with a concomitant increase in Electroencephalography (EEG) theta activity.

Serotonergic (5-HT) neurons originating in the dorsal and median raphe nucleus also project to the PVN. 5-HT is able to stimulate OT neurons via 5-HT_1A, 5-HT_2A and 5-HT_3A receptors. The level of oxygenated hemoglobin in the anterior PFC was significantly increased during Zen meditation, accompanied by a reduction in feelings of negative mood, compared to before the meditation session. EEG revealed increased alpha band activity and decreased theta band activity following Zen meditation. EEG changes were correlated with a significant increase in whole blood 5-HT levels. These results suggest that activation of the anterior PFC and 5-HT system may be responsible for the improvement of negative mood and EEG signal changes observed during Zen meditation.

The characteristic feature of AZ is vocal sound-vibration practice combined with altruism and appreciation meditation. Somatic afferents from the sensory organs are involved in the control of various autonomic functions in humans. It has been well demonstrated that various types of somatosensory stimulation including vibration can increase OT levels in plasma and cerebrospinal fluid in anesthetized rats. The spinal-supraspinal pathways responsible for somatosensory stimulation mainly comprise the posterior column pathway and spinothalamic pathway. These impulses are further relayed to the thalamus, and ultimately sent to the primary somatosensory cortex. In addition, these impulses are also relayed to other brain areas, including hypothalamus, via collateral connections. These raise the possibility that vocal sound-vibration of AZ may stimulate somatosensory pathway and spinal-supraspinal pathways (bottom-up pathway).

In contrast, altruism and appreciation meditation of AZ may stimulate PFC and its network (top-down pathway). Both pathways synergistically activate OT system at the hypothalamus (Figure 3). Further studies are needed to study whether up-regulation of OT following AZ is mediated via DAergic, serotonergic, or other receptors.

During the process of maintaining the positive social interaction, both of giving and receiving empathy, OT system is upregulated in our brain. Especially, thinking about helpless people and giving sympathy to them may upregulate hypothalamic OT expression, which promotes mental and physical health on the givers. It is important to reconsider Buddhist traditions of cultivating connection and compassion towards others in order to maintain our well being.

Our current study showed that OT levels were increased in 23 participants, while 9 participants showed the reduction of OT levels following AZ meditation. Minor reduction (less than 10 percent reduction from the pretreatment level) was observed in 3 participants, and major reduction (more than 30 percent reduction from the pretreatment level) was observed in 6 participants. These reductions were not correlated with the previous experience of AZ, age or sex.

As the major deduction was observed among 3 subjects who have a previous experience of AZ meditation (more than 5 times of previous AZ practice). We cannot exclude the possibility that quick increase of OT responses may occur in the early phases of one-hr AZ, followed by the reduction of OT levels in these subjects.

It remains unknown how soon the mindfulness induced by AZ meditation affects salivary OT levels. Multiple assays are needed to study the time course of OT responses in the early phases and late phases during one-hr AZ meditation.

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