PS255
Epigenetic alterations of BDNF DNA-methylation associated with Posttraumatic Stress Disorder: findings from the Korean Combat Veterans of the Vietnam War
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Abstract
Objective: Complex interactions between genes and environmental events may contribute to the development of post-traumatic stress disorder (PTSD). The aim of this study was to investigate whether epigenetic regulation of the brain-derived neurotrophic factor (BDNF) gene is a resilience marker of PTSD among veterans exposed to Vietnam War.

Methods: A total of 253 Korean combat veterans of the Vietnam War were included. The Clinician-Administered PTSD Scale (CAPS) and Combat Exposure Scale (CES) were assessed. BDNF DNA methylation levels at 4 CpG sites within the promoter region were quantified in the peripheral blood using the pyrosequencing. The effects of BDNF methylation levels and clinical variables on the diagnosis of PTSD were tested using binary logistic regression analysis.

Results: Using the CAPS interview, combat veterans were grouped into those with (n = 127) and without (n = 126) PTSD. Subjects with PTSD showed a significant higher DNA methylation of 4 CpG sites at the BDNF promoter compared to those without PTSD. High BDNF methylation status at the 4th CpG site, high CES and alcohol use predicted significantly PTSD diagnosis.

Conclusions: The present study demonstrated an association between higher DNA methylation of the BDNF promoter region and PTSD. Our findings suggest that altered epigenetic programming of the BDNF gene is related to the pathophysiology of PTSD and stress resilience after trauma exposure.

PS256
New drug therapy and the effect mechanism that the central anticholinergic drug Trihexylphenidyl reduces flashback in PTSD. The second report
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Abstract
Objective: We developed the acetylcholine hypothesis to explain the cause of PTSD flashbacks; it states that PTSD flashbacks are caused by the hyperexcitability of the memory-related circuit of the acetylcholine (ACh) nervous system (ACH-MRC), comprising ACh1 (medial septal nucleus) and ACh2 (diagonal band of Broca) to hippocampus and ACh4 (Nucleus basalis of Meynert) to amygdala paths, i.e., by the abnormal secretion of ACh. Based on this hypothesis, we would like to report a new drug therapy using the central anticholinergic agent trihexylphenidyl (TP) and its action mechanism. At the 12th World Congress of Biological Psychiatry held in Athens (June 2015), we reported favorable results of a clinical trial of TP conducted in seven subjects. Since then, we administered TP to 16 subjects presenting with flashbacks (FB) and would like to report our findings.

Methods: We administered TP to 23 patients experiencing flashbacks. TP was mainly administered once at 2mg in the form of draught when flashbacks occurred. The dose was adjusted according to severity; for a serious case, TP was administered thrice at 2mg/day for several weeks. However, no side effects have been reported.

Informed consent was obtained from all patients. This study was approved by the Ethical Committee of Warakukai.

Results: PTSD was diagnosed and evaluated using DSM-5, IES-R, and CAPS assessment. These evaluations showed an extremely beneficial effect. Twenty three cases were evaluated; 7 cases up to 2012 and 16 cases from 2012 to 2015. Of these, 65.2% (15/23) showed complete remission (CR), and 34.8% (8/23) showed partial remission (PR), indicating a favorable outcome. CR(65.2%)+PR(34.8%)=100%.

Conclusion: Thus far, no drug with definite effects on PTSD has been identified. Furthermore, the mechanism of action of drugs believed to have effects on PTSD is unclear. TP exhibited an excellent effect on PTSD flashbacks. The effect was rapid and could be seen in 1 to 1.5 hours after dosing, with the effect lasting for 5 to 6 hours. FB is an important, central symptom of PTSD, and improvements in FB lead to improvement of other PTSD symptoms. We primarily used single doses of the drug. For severe cases, we administered 3T (1T=2 mg) once daily for 1 to 2 months, with close monitoring of side effects. Thus far, no patients have shown any side effects.

Disclosure: There are no financial conflicts of interest.

PS257
The effects of brain-derived neurotrophic factor (BDNF) micro-infusion on the impaired fear extinction of the animal model of PTSD.
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Abstract
Although the impaired extinction of fear memory (Ext) is one of the hallmark symptoms of posttraumatic stress disorder (PTSD), the underlying mechanisms of impaired Ext are unclear and effective pharmacological interventions have not yet been developed. Recently, the neuronal plasticity induced by brain-derived neurotrophic factor (BDNF) in infralimbic (IL) prefrontal cortex and/or hippocampus were shown to be crucial for the Ext of naïve rats.

We used a single prolonged stress (SPS) paradigm, which mimic the pathophysiological abnormalities and behavioral characteristics of PTSD including the impaired Ext. The current study was conducted to investigate the expression of BDNF in the brain of SPS rats, as well as the therapeutic efficacy of intracranial BDNF micro-infusion for the impaired Ext of SPS. Either the sacrifice of rats for BDNF quantification or the micro-infusions for the behavioral study were conducted just before Ext training session (24 hr after fear conditioning), and the micro-infusions were targeted to the IL, or Prelimbic (PL) prefrontal cortex, or ventral hippocampus (vHPC).

The mature BDNF protein expression of SPS rats were significantly reduced just before Ext training in both the medial prefrontal cortex and the hippocampus. In addition, the micro-infusion of BDNF into IL or into vHPC, but not into PL, induced the significant reduction of freezing behavior at the Ext training session and Ext test session (24 hr after Ext training), and these effects were commonly seen in the naïve rats and the SPS rats.

Our results indicates that the neuronal plasticity induced by BDNF in IL and vHPC are, at least in part, involved in the
mechanism of the Ext even in the PTSD. At the meeting, we will show the changes in the phosphorylation of TrkB (an receptor of BDNF) after the micro-infusion of BDNF.

**PS258**
AMPK signaling in the dorsal hippocampus negatively regulates contextual fear memory formation
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**Abstract**
Both the formation of long-term memory (LTM) and dendritic spine growth that serves as a physical basis for the long-term storage of information require de novo protein synthesis. Memory formation also critically depends on transcription. Adenosine monophosphate-activated protein kinase (AMPK) is a transcriptional regulator that has emerged as a major energy sensor that maintains cellular energy homeostasis. However, still unknown is its role in memory formation. In the present study, we found that AMPK is primarily expressed in neurons in the hippocampus, and then we demonstrated a time-dependent decrease in AMPK activity and increase in mammalian target of rapamycin complex 1 (mTORC1) activity after contextual fear conditioning in the CA1 but not CA3 area of the dorsal hippocampus. Using pharmacological methods and adenovirus gene transfer to bidirectionally regulate AMPK activity, we found that increasing AMPK activity in the CA1 impaired the formation of long-term fear memory, and decreasing AMPK activity enhanced fear memory formation. These findings were associated with changes in the phosphorylation of AMPK and p70s6k and expression of BDNF and membrane GluR1 and GluR2 in the CA1. Furthermore, the prior administration of an mTORC1 inhibitor blocked the enhancing effect of AMPK inhibition on fear memory formation, suggesting that this negative regulation of contextual fear memory by AMPK in the CA1 depends on the mTORC1 signaling pathway. Finally, we found that AMPK activity regulated hippocampal spine growth associated with memory formation. In summary, our results indicate that AMPK is a key negative regulator of plasticity and fear memory formation.

**Keywords:** AMPK; fear memory; formation; mTORC1

**PS259**
TNF-α from hippocampal microglia induces working memory deficits by acute stress in mice.
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**Abstract**
The role of microglia in stress responses has recently been highlighted, yet the underlying mechanisms of action remain unresolved. The present study examined disruption in working memory due to acute stress using the water-immersion resistant stress (WIRS) test in mice. Mice were subjected to acute WIRS, and biochemical, immunohistochemical, and behavioral assessments were conducted. Spontaneous alternations (working memory) significantly decreased after exposure to acute WIRS for 2h. We employed a 3D morphological analysis and site- and microglia-specific gene analysis techniques to detect microglial activity. Morphological changes in hippocampal microglia were not observed after acute stress, even when assessing ramification ratios and cell soma volumes. Interestingly, hippocampal tumor necrosis factor (TNF)-α levels were significantly elevated after acute stress, and acute stress-induced TNF-α was produced by hippocampal-ramified microglia. Conversely, plasma concentrations of TNF-α were not elevated after acute stress. Etanercept (TNF-α inhibitor) recovered working memory deficits in accordance with hippocampal TNF-α reductions. Overall, results suggest that TNF-α from hippocampal microglia is a key contributor to early-stage stress-to-mental responses.

**PS260**
A Roadmap to Golden hour intervention for Posttraumatic Stress Disorder
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**Abstract**
**Objective:** Currently there are several compounds that are used in preclinical studies to target systems or receptors which are fundamental for consolidation and reconsolidation. While this offers an important opportunity to target these emotional memories and the expression of fear, and there is some validation from clinical studies, there is currently a need for roadmap that will assist in identification and evaluation of these compounds in their efficacy in treatment for PTSD. (This work is part of the Traumatic Stress Network of the European College of Neuropsychopharmacology).

**Method:** Two windows of opportunity that can be defined as ‘golden hours’ for treatment of PTSD can be identified: i) event-based golden hours and ii) exposure-based golden hours. The first are defined by the traumatic event, and subsequent consolidation of the traumatic event. The second is determined by the setting in which exposure as a therapeutic tool is introduced and the subsequent reconsolidation phase.

**Summary:** First we will provide an overview of the current knowledge of compounds – based on preclinical and clinical work - which are potentially interesting to target emotional memory processing in PTSD. We will discuss applied dosages, population, timing of treatment and exposure. Second, we will use this knowledge to define pertinent questions for future and novel developments to target PTSD. We identified the following potential compounds: Propranolol, Cortisol, D-Cycloserin, Ketamine, Oxytocine and MDMA (XTC).

**Conclusions:** We conclude that reconsolidation presents an interesting opportunity to modify or alter fear and fear-related memories. Several compounds are being used off label in augmentation of psychotherapy for PTSD. Following a roadmap will assist in moving the field forward in terms of design, dosage as well as effectiveness as augmentation strategies for treatment of PTSD.

**PS261**
Heart Rate Variability of Posttraumatic Stress Disorder in Korean Veterans
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**Abstract**
**Objective:** Heart rate variability (HRV) is reported to reflect the autonomic nervous system. Generally, patients with conditions such as posttraumatic stress disorder (PTSD) showed lower HRV,