PERSPECTIVE

Intracerebral interplay and neurotransmitter systems involvement in animal models of neurodegenerative disorders: EEG approach expectations

An imbalance between activities of different structures and neurotransmitter systems in the brain is suggested to be the main cause of its abnormal functioning in neurodegenerative pathologies. Electroencephalogram (EEG) registered from areas specifically linked with a disease in combination with pharmacological testing of involved mediatory systems allows discovery of its progression and mechanism(s). This, in turn, potentiates development of perspective approaches for early diagnostic and effective treatment of neurodegenerative disorders.

The neurodegenerative disorders are characterized by pronounced disturbances in the brain functioning, which are associated with considerable damage or destruction of the cerebral cells and/or connections between them. The most prominent effects of the neurodegeneration are observed when it affects the main neurotransmitter systems in the brain: dopaminergic (DA), cholinergic (ACh), γ-aminobutyric acid (GABA), and glutamatergic (Glu). In Parkinson's disease (PD), Alzheimer's disease (AD), and temporal lobe epilepsy (TLE), close associations with disturbances in these systems were revealed (see, *e.g.*, *J Pharmacol Exp Ther* 1993, 265:1001; *Neuropharmacology* 2013, 64:108; *Front Aging Neurosci* 2014, 25:252; *Brain Res Mol Brain Res* 2003, 113:107; *Neuropharmacology* 1992, 31:469). On the other hand, the brain areas/structures involved in the mechanisms of learning/memory, attention and locomotion (the hippocampus, cortex, and basal ganglia) are well known to be specifically sensitive to detrimental influence of the neurodegeneration on the neurotransmitter systems as well (Xu et al., 2012). An imbalance between functional/electrical activities of different brain structures and their synchronized interaction is hypothesized to be the main cause of abnormal functioning of the diseased brain because of failed computation across neuronal populations (Oswal et al., 2013). To clarify this, we analyzed the frequency composition of EEG registered from those brain areas which have been expected to be predominantly suffered from neurodegeneration in rat models of PD, AD, and TLE. The main objectives were associated with revelation of both imbalanced interplay between these areas and disturbances in their neurotransmitter mediation.

In patients with AD, alterations in the brain EEG asymmetry and deficits in interhemispheric integration of information are well known. However, no direct evidence of an association between EEG asymmetry, morphological markers in the brain, and cognition was found either in AD patients or in AD models. To clarify this, we performed experiments in rats with preliminarily removed olfactory bulbs (OBX rats), as a model of AD (Bobkova et al., 2008). We tested both learning/memory ability of OBX rats, amyloid-beta peptides (Aβ) level (as an AD marker) in their brains, and EEG frequency spectra (0.5–30 Hz) in symmetrical frontal and occipital cortices. Close associations between impaired memory, increased Aβ in the cortex-hippocampus samples, and both eliminated beta rhythms (14–30 Hz) and enhanced theta oscillations (4-8 Hz) in the right frontal cortex were revealed in OBX rats. These contrasted with non-specific effects in the occipital brain areas, corroborating well-known role of frontal cortex in mental processing. Given the data pointing at involvement of ACh system in both AD and EEG asymmetry in frontal cortex of naïve rats (Vorobyov and Ahmetova, 1998), the EEG approach might be considered as a potentially effective tool for analysis of the brain neurotransmitter and compensatory/adaptive mechanisms in AD progression (Bobkova and Vorobyov, 2015).

Microinjections of Aβ peptides into the hippocampus allow investigation of their direct effects on the brain, revealing, in turn, symptomatic and pathophysiological similarities of this model to AD. Recently, we have studied differences in frequency spectra of EEG from frontal cortex and dorsal hippocampus (the main brain areas involved in learning/memory mechanisms) in rats intrahippocampally infused with $A\beta_{1-42}$ (Vorobyov et al., 2015). Two weeks after $A\beta_{1-42}$ injection, predominance of both hippocampal theta and cortical beta, observed in baseline EEG of control rats, was significantly diminished. DA has been shown to be involved in neuronal plasticity and Aβ transformation in different ways. To clarify DA involvement in the cortex-hippocampus interplay in Aβ model of AD, we used peripheral injection of a DA agonist, apomorphine (APO). In rats infused with $A\beta_{1-42}$ alone, APO attenuated the cortical beta predominance. Interestingly, pretreatment (30 minutes apart) with dispersed fullerene C_{60} nanoparticles, namely hydrated fullerene C_{60} (C_{60}HyFn), protected the intracerebral interplay from $A\beta_{1-42}$ detrimental influence on both baseline EEG and APO-produced EEG effects. These correlated with results of morphological and histochemical evaluation of neuronal viability in the hippocampus. We suggested that presynaptic DA mediation plays an important role in the C_{60}HyF effects and that C_{60}HyF has neuroprotective potential in the Aβ model of AD (Vorobyov et al., 2015).

Some movement disorders in patients with PD have been suggested to be associated with an imbalance between cortical and striatal network activities with several frequency modes of oscillation (*Mov Disorder* 2003,18:357). This imbalance is supposedly linked with the brain DA level depletion and reversed by treatment either with APO or with traditionally used DA precursor, L-DOPA. In the 6-hydroxydopamine (6-OHDA) rat model of PD, we studied the frequency spectra of EEG from frontal cortex and the striatum before and after injection of APO alone or in combination with the NMDA antagonist, MK-801 (Vorobyov et al., 2003; Vorobyov and Sengpiel, 2008). 6-OHDA intoxication produced disparity in baseline EEG spectra through both suppressed striatal alpha (7.6–12.5 Hz) and enhanced cortical beta. In control rats, APO evoked long-lasting suppression of alpha activity, predominantly in the cortex, whereas in 6-OHDA rats, even larger suppressive effect was observed in the beta range, again significantly more pronounced in the cortex than in the striatum. In 6-OHDA rats, pretreatment with MK-801 eliminated the APO-induced cortex-striatum difference in the beta range, inversed the effect in the alpha range, and intensified delta activity (0.5–3.5 Hz) stronger in the striatum than in the cortex. We conclude that frequency-dependent differences in EEG power between the cortex and striatum may be involved in DA treatment of PD and mediated, at least in part, through NMDA receptors (Vorobyov and Sengpiel, 2008). Furthermore, we evaluated DA receptors sensitization provoked by repetitive APO injections, which is considered as a basis of the complications observed in chronic treatment of PD by DA agonists. To study this so-called "priming" phenomenon, we used specific D_1 agonist, SKF 38393, on the third day after APO (Vorobyov et al., 2003). In 6-OHDA-lesioned rats, SKF 38393

decreased $beta_1$ (12.6–17.5 Hz) activities in both the cortex and the striatum. The effect of SKF 38393 was enhanced in APO-primed rats pretreated with MK-801, particularly in the striatum. We suggest that long-term changes in cortical-striatal EEG interplay after priming might contribute to the development of the behavioral complications observed in chronic treatment of PD with DA agonists.

Experiments on kainic acid (KA)-injected rodents, as a model of TLE, have demonstrated that the neuronal circuitries of the cortex and the hippocampus as well as the major neurotransmitter systems undergo fundamental degenerative modifications (see, *e.g.*, Buckmaster and Dudek, 1997; *Brain Res* 1980, 191:387; *Brain Res Mol Brain Res* 2003, 113:107; *Neuropharmacology* 1992, 31:469). To clarify the extent of different neurotransmitter systems involvement in epilepsy-associated alterations in the brain, we recorded EEG from the cortex and hippocampus before and after intracerebroventricular (i.c.v.) infusions of agonists at NMDA, α_2-adrenegic, $GABA_a$, and $GABA_b$ receptors (NMDA, clonidine, muscimol, and baclofen, respectively) in rats intraperitoneally injected with KA. Since the development of KA-induced TLE is known to comprise acute, delayed, and chronic stages of neurodegeneration and compensatory remodeling, EEG effects of the neurotransmitters were studied 2, 5, and 9 weeks after KA injection alone or in combination with basic fibroblast growth factor (bFGF, i.c.v.), used as a neuroprotective agent (Vorobyov et al., 2005). Within the first 5 weeks of KA injection, the EEG power shifted towards the lower frequency range, the EEG responses to NMDA and clonidine were potentiated, whereas the effects mediated by $GABA_a$ and $GABA_b$ receptors remained largely unaffected. In KA rats, bFGF potently mitigated abnormally increased NMDA sensitivity. Thus, we have showed how the neuroprotective action of bFGF, and its beneficial effects on subsequent neuronal and mediatory systems remodeling, are reflected in frequency spectra of the brain activity.

We developed this approach in our further study associated with analysis of mediatory mechanisms underlying well-known "bidirectional" relationship between attention-deficit hyperactivity disorder (ADHD) and epilepsy (see, *e.g.*, Hamoda et al., 2009). In spontaneously hypertensive (SH) rats, as a model of ADHD, the baseline EEG showed increased delta and $beta_2$ activity (17.8–26.5 Hz) in the hippocampus and decreased alpha-$beta_1$ activity in both the hippocampus and frontal cortex (Vorobyov et al., 2011). In KA rats, these effects were observed 2 weeks after KA injection, while the $beta_2$ activity increase occurred after 5 weeks in the hippocampus and, largely, after 9 weeks in both brain areas. In SH rats, NMDA increased delta and decreased alpha-$beta_1$ activity, similar to KA rats after five post-injection weeks. In SH rats, clonidine augmented theta-$beta_2$ increase in the cortex and alpha suppression in both brain areas, in parallel with induction of $beta_2$ activity in the hippocampus. These $beta_2$ effects were observed 5 and 9 weeks after KA injection. In SH rats, baclofen produced robust delta-theta enhancement and alpha-$beta_1$ suppression in both brain areas, with additional $beta_2$ activity increase in the hippocampus, while muscimol was ineffective in both groups of rats. In KA rats, EEG responses to GABA agonists were similar to those in control. Our results demonstrate sensitization of NMDA receptors and $alpha_2$-adrenoceptors in SH and KA rats, and that of $GABA_b$ receptors specifically in SH rats.

Thus, EEG registration from brain areas associated with different types of neurodegenerative pathology, in combination with pharmacological testing of involved neurotransmitter systems, allows discovery of both progression and mechanisms of these disorders that, in turn, potentiates development of perspective approaches for their early diagnostic and further effective treatment.

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