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Review Article

Alterations of Endocannabinoid System in Depression in Adolescent Population: Results of Experiments in Early-Life Stress Model and Clinical Studies

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Abstract

As major depression among children and adolescents becomes a growing issue in our society, a lot of effort has been made to broaden our knowledge about this disease in order to improve its diagnostics and treatment options. Traditional theory about neurotransmitter deficits, even though it was a huge step forward in understanding pathophysiology of depression, seems not to discuss the topic at length. With growing incidence of marijuana consumption among youngsters and its psychiatric implications, a lot of attention has been paid to endocannabinoid system and its role in pathophysiology of depressive disorders. An Early Life Stress is a preclinical developmental model of depressive-like disorders in rodents that has been exploited to explore alterations in endocannabinoid system. This protocol has however different modifications with miscellaneous outcomes. For understandable bioethical reasons there is significantly lower number of clinical studies that describe endocannabinoid variations in adolescent humans. Even though some reviews that discuss alterations in endocannabinoid system in result of early life stress already

exist, none of them puts these data together with information from human adolescent population. This reviewdescribes possible changes in the mentioned system in early life stress presented in recent papers and compares them with limited data we have from clinical studies performed among adolescent population.

Abbreviations

MDD- Major depression disorder; ECS- Endocannabinoid system; ELS- Early life stress; MS- Maternal separation; MSEW- Maternal Separation Early-Weaning; HPA- Hypothalamic-pituitary-adrenal; CNS- Central nervous system; PND- Postnatal day; AEA- Anandamide; DER- Denied Expected Reward; DEA- N-Docosatetraenoylethanolamine; DHEA- N-Docosahexaenoylethanolamine; 2-AG- 2-Arachidonyloglicerol; 2-LG-2-Linoleoylglycerol; 2-OG-oleoylglycerol; PFC - Prefrontal cortex; DLPFC-Dorsolateral prefrontal cortex: NAcc- Nucleus accumbens; CB1R- Cannabinoid receptor type 1; CB2R- Cannabinoid receptor type 2; SEA- Stearoylethanolamide; LEA- N Linoleoylethanolamine; OEA- Oleoylethanolamide; PEA-Palmitoylethanolamide; P-Gly- *N*-palmitoyl glycine; D-Gly- *N*-docosahexaenoyl glycine; PGF2α - Prostaglandin F2α; A-Taur- Arachidonoyl taurine; AA- Arachidonic acid; MAGL- Monoacylglycerol lipase; FAAH- Fatty acid amine hydrolase; NAPE-PLD- N-acyl phosphatidyl-ethanolamine phospholipase D; DAGLα- Diacylglycerol lipase alpha; CPu- Caudate-putamen or striatum; CA3- Dorsal field 3 of Ammon's horn; DG- Dentate gyrus; BLA- Basolateral nucleus of amygdala; CeA- Central amygdaloid nucleus; SA- Attempted suicide; PC- Psychiatric controls

1. Introduction

Major depression disorder (MDD) is one of the most common diagnosis in children and adolescents with incidence ranging up to 12% [1] and is associated with significant morbidity and suicidality in this population. Being considered a major public health care problem, it is connected with increased demand on medical services and higher disability rates [2]. Sadly, unlike other fields of clinical medicine, in psychiatry we lack substantial diagnostic tools and laboratory indicators [3]. Psychiatric diagnosis has to rely completely on physical examination, which of course when performed by an experienced physician is an invaluable instrument, but it also has its limitations like lack of repeatability of examination or wide array of external stressors (culture, language, literacy etc.) that may distort the results [4]. Especially in pediatric population, diagnosis of major depression might be tricky, because its symptoms differ from the ones we know from adults' population. As a result of that fact there is a high risk of under diagnosis and undertreatment of MDD [1]. That is why there exist an urgent need to search for an easily measurable biomarker that may indicate existence of the disease as well as estimate the severity of MDD. Even though no-one should question the traditional theory about the etiology of major depression that aroused in mid-1960s of last century, it has been proven that it demands some supplement. Apart from monoamine levels and its activity shortage other grounds for depression have been raised e.g., altered activity of hypothalamic-pituitary-adrenal (HPA) axis, reduced hippocampal volume, modifications of the immune system, infections, genetic background, or variations in endocannabinoid system (ECS) [5-7].

In this review, I would like to gather and describe possible changes in endocannabinoid system that occur in animal model of depression in adolescent population and compare them with limited clinical data that we have from population of human youth.

2. Endocannabinoid system

Endocannabinoid system (ECS) is a modulatory lipid signaling system present in central nervous system (CNS). Main components of it are anandamide (AEA) and 2-arachidonyloglicerol (2-AG) being ligands of cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R). Moreover, intrinsic part of this system are enzymes that play a crucial role in the synthesis and inactivation of the endocannabinoids. Fatty acid amine hydrolase (FAAH) is responsible for degradation of AEA, whereas monoacylglycerol lipase (MAGL) is the enzyme metabolizing 2-AG [8-10]. Cannabinoid type 1 receptors are a G protein-coupled structures that are mainly found across synaptic junctions in axon terminals of GABAergic, glutamatergic, monoaminergic and neuropeptidergic neurons in different brain areas including anterior cingulate and prefrontal cortex, amygdala, hippocampus, basal ganglia, and cerebellum [11, 12]. Their main function covers inhibition of neurotransmitter release from the presynaptic neuron. At the same time cannabinoid type 2 receptors are much less widespread across the CNS, being found mostly in microglia, endothelial cells, and the peripheral immune system [10, 13]. Recently, there have been some data suggesting that CB2 receptors could also play some role in central nervous system signaling, but this needs further exploration [14]. Main function of ECS is keeping balance and internal homeostasis of central nervous system. This system is involved in regulation of mood, reward processes and neural development. It also plays an important role as a controller of hypothalamic-pituitary-adrenal (HPA) stress axis being under the influence of stress itself.

2.1 Early Life Stress as an animal model of depression

As depressive-like disorders are becoming more and more frequent in modern society worldwide, many scientists tried to perform an accurate animal model of this disorder [15]. Especially in pediatric population, because of its fragility and legal issues, it is hard to perform proper clinical study without having strong preclinical premises. A lot of research has been performed to propose an animal model that fully corresponds with actual progress of depression in humans. First experiments involving primates to investigate nature of psychiatric disorders date to 1940s, when Harlow and his team used some macaques for this purpose. Nowadays, usage of monkeys, because of their resemblance towards humans, is source of ethical controversies. At the same time rodents are much more socially acceptable as experimental animals. To induce depressive-like behavior in rodent model of depression different methods are being used, for example: Chronic Mild Unpredictable Stress (CUMS), Single Prolonged Stress (SPS) (which might be used as a developmental method as well [16], Chronic Social Defeat Stress (CSDS), as well as some developmental models, which include both pre- as postnatal stress. This article focuses on Early Life Stress (ELS) model, which belongs to postnatal stress models and is frequently used to simulate depressive-like syndromes in adolescent rodents. [17].

Early life stress model is based on the developmental origins of depressive disorders. It has been proven that stressful events during early childhood and adolescence may remain

profound imprint in neuronal circuits of an individual. It may increase its vulnerability towards developing psychiatric disorders in adulthood [18-20]. Early life stress protocol has already been widely used as a laboratory tool to induce depressive-like behaviors in rodents. Nevertheless, the actual protocol is not homogenous – different studies alter it according to the conditions of experiment. Different protocols have a bit different outcome in terms of effect on rat's behavior. For example, one of studies revealed that rats exposed to two different lengths of maternal separation formed two different psychiatric disorders profiles. Animals that were taken away from their mother for 4h a day in postnatal days (PND) 1-21 tended to suffer from anxiety-like disorders, which became rather long-lasting and remained until adulthood. At the same time, rats exposed to maternal separation for 4h a day in postnatal days 1-10 suffered from emotional and associative problems, but only in adolescence. [21] However, the most typical ELS protocol include separation in postpartum days 2-14 and happens once a day for 3 hours [22]. Some researchers tend to prolong this protocol until day 21, because postnatal days 2-14 typically overlap with stress hyporesponsive period which usually occur between days 4-14 [18, 23].

The actual duration of the protocol is subject to many discussions, because as some studies suggest sustained maternal separation may surprisingly lead to higher level of stress tolerance in adulthood. Animals that underwent such experiments featured better spatial learning and less anxiety-like behaviors [24, 25]. Even though typical ELS (2-14 PND) overlaps, as mentioned above, most of the stress hyporesponsive period (4-14 PND), maternal separation (MS) protocols that are prolonged until PND21 tend to enter the period where the HPA-axis is already mature and therefore desensitized towards impact of external stressors. [25]. Actually, it is also supposed that also dams tend to catch up with increased maternal care after such prolonged maternal separation period [26]. Another approach to ELS protocol is a single, 24 hours long separation of pups from their mother, usually occurring at postnatal day 9. [27] Nevertheless, this model is used occasionally and has limited significance [28].

In some publications one can read about Denied Expected Reward (DER), which is another variety of maternal separation. This model is based on the experiment with T-maze, where pups between 10^{th} and 13^{th} postnatal day are placed. Their task is to look for the location of their mother placed in a cage at the end of one arm. Once they find her they are being rewarded with opportunity to experience maternal care and contact. Experiment contains two group of pups: the first one gets the expected reward, whereas the other one's contact with mother is denied. As the result of this test DER rats showed decreased sucrose preference – signal of anhedonia, increased immobility in FST, reduced locomotion and time spent in the centre of the area in open field test. All the behavioral tests were conducted when rats were 4 months old. Cleary, the behavioral phenotype of these animals responds to depression-like and anxiety disorders [29].

ELS protocol might be extended to Multimodal Early Life Stress, where pups in the age of PND 2-21 undergo Maternal Separation protocol simultaneously with or following some other physical and sometimes psychological stressors. Examples of stressors used in this protocol are peer separation (social isolation), shaking and exposure to cold

[20]. Some data suggest that it is not duration of the single stress period, but cumulative effect of more stressful situations throughout the whole life that is responsible for final depressive phenotype. This approach resulted in "two-hit" model of ELS protocol. This model is based on assumption that first episode of stress does not induce depressive-like behavior itself but creates some kind of susceptibility towards further stressors [30].

There is no need to emphasize proper control group in any research. To perform ELS protocol properly planned control group must be appropriate as well. Intuitively, it may seem reasonable to regard non-handled animals that are left with their mother for the whole time as a control group. Nonetheless, those animals are raised being impoverished in a social and environmental way. Because of that, leaving non-handled offspring as a control group may distort results. That is why, sometimes researchers use rats exposed to short periods of maternal separation (e.g. 15 minutes a day) to be a control group for animals who underwent the full version of protocol [28, 31]. It has been proven that limited maternal separation imitates the natural cycle of maternal contact, when it is common for lactating dam to leave the whole litter for some short amount of time, usually not longer than one hour [28]. It seems reasonable also to leave the control group with their mother for the whole time, but to provide to them the same amount of handling as to the separated animals [27]. This idea evolved into procedure called Animal Facility Rearing (AFR), a protocol that involves natural-like housing condition and limited short contact of the pups with researcher hands, mostly during switching cages. However, because of different customs and slightly different environmental condition in every laboratory this protocol seems to be possible source of study bias [28]. For this reason, some scientists prefer Maternal Care Deprivation Protocol. Unlike the previous ones it consists of removing mother from pups instead of displacing young ones. This type of protocol avoids any manipulation of the pups and thus none of the groups experience stress connected with handling [32, 33]. Regardless, if we prefer Maternal Care Deprivation Protocol or typical Maternal Separation, it is crucial to maintain comfort and safety (factors like light, temperature, bedding etc.) for both of the groups, so that the results would not be biased with some other stressors than lack of maternal protection.

Different Maternal Separation protocols have different attitude towards type of separation: if pup should be kept individually or together with littermates, if the separation should take place in special dedicated cage, causing higher level of stress or isolated pups should remain in their homecage. Scientists did not agree on one, universal solution so far [28].

Also, sex of the animal should be taken into consideration, but because of the fact that majority of researches includes male pups only it still remains rather unclear, which animal model remains the best to perform studies on depression in females [34]. So far different papers revealed that response to ELS protocol is sex dependent [35-38]. As women are twice more likely to suffer from depression, finding a proper female depression animal model might remain an important point of further research. Another disturbing factor might be the origin of given animal. A few research studies already revealed that rodents belonging to various strains react to MS in a different way [39, 40]. These data suggest that direct collation across different studies might be tricky, because of different study conditions and other variables.

Some concerns have been placed in terms of exact human maltreatment type that maternal separation corresponds to. It has not been acknowledged yet if maternal separation can be compared with child abuse, child neglect, domestic violence, or sexual abuse. The classification of human mishandling that exact rodent maternal separation model refers to might be important in order to establish its translational value [30]. For this reason, many sorts of ELS protocols are being formed. Another one, worth mentioning, that corresponds issues mentioned above is to disable maternal care by providing an environment lacking in components that are crucial to build up a proper, functional nest. Unfortunately, even though in primary research this experiment showed increased anxiety-like and depressive-like behaviors in rodents that underwent such maternal care impairment, further studies did not confirm it. Yet again, further studies are necessary in this matter.

Regardless of the fact that stress in early period of life has mostly negative impact on litter's further development, some studies reveal that to some extent it may present some positive consequences. Rats that underwent MS-protocol followed by social interaction test, partially baited radial arm maze test, 5-choice serial reaction time task showed better spatial learning and memory, also their attentional capabilities were improved [24]. Of course, those benefits do not balance all the harmful effects of early life stress. Further studies are necessary to assess possibility of enhancing positive results of these experiences with simultaneous softening of the negative consequences. Perhaps once it will be possible to project such training and therapeutic strategies for neglected children to provide them a better quality of life.

2.2 Alterations in endocannabinoid system provoked by early life stress in rodents

Papers reviewing changes in ECS in rodent models of depression already exist. [i.e. Smaga et al., 2014]. However, from that time on several new studies examined the exact impact of ELS on ECS activity and expression in rodent's brain. Examples of these alterations in some of the recent papers are visualized in Table 1. Not all the results are coherent, what might be an outcome of different study condition including wide array of factors like different species of animals, sex, early life stress protocol used or age of rodents at which assays of ECS activity has been executed. Also, wide variety of brain regions was examined and non-identical parameters of ECS were taken into consideration, so comparing them with each other seems difficult.

Up until now, depression has been associated with reduced activity of ECS with reduced levels of AEA detected in different CNS structures in animal models of depression [7]. This has been confirmed in research conducted by Portero-Tressera et al [41], where levels of AEA, DEA and DHEA in striatum of MSEW (Maternal Separation Early-Weaning) mice were reduced. Also, levels of AEA in amygdala and hippocampus tend to be significantly lower in animal subjected to stress in their early life [9].

Results are more unequivocal when it comes to measurement of 2-AG levels. In previous reviews direction of changes was dependent on number of factors like brain structure tested and type of ELS procedure used. However, most of the research suggest increase in the levels of 2-AG in frontal cortex, hypothalamus, hippocampus, and nucleus accumbens [7]. This was confirmed to some extent in newer

papers, which indicated not only lower levels of 2-AG in prefrontal cortex, amygdala and interpositum of cerebellum, but also some differences in concentration of it between sexes. The results however are not unanimous, so should be interpreted with caution.

Data regarding endocannabinoids degradation enzymes seem to demand supplement as well. So far, limited and incoherent data suggested raised amount of FAAH, an enzyme deteriorating AEA, in frontal cortex and hippocampus, what would be an excellent justification for reduced concentration of AEA [7]. Unfortunately, [42] in their paper do not support this theory, revealing unaltered levels of FAAH in frontal cortex and decreased in nucleus accumbens. This might be explained by very late assay of this parameter, which corresponds more to adulthood than adolescence. According to that study, also quantity of MAGL, a membrane enzyme involved in degradation of 2-AG, is either lessend (e.g., in nucleus accumbens) or remains unaltered (in frontal cortex).

Abundantly deployed across brain structures associated with pathophysiology of MDD (e.g., prefrontal cortex, hippocampus, cerebellum) CB1 receptors play a vital role in modulating stress response and neurotransmission, thus their contribution to formation of mood disorders has long been postulated. Both genetic deletion and pharmacological blockade of CB1 receptor cause depressive-like phenotype in rodents. Also, previous animals' studies revealed reduced density of CB1 receptors in variety of brain areas including midbrain, hippocampus, hypothalamus, and ventral striatum [7]. This stands in contradiction to the results of Amancio-Belmont et al. 2020 [4], who discovered increased levels of CB1 receptors in nucleus accumbens of MS rats. On the other side, Hill et al. reveals lowered CB1 receptor binding site density in prefrontal cortex, amygdala, and hippocampus in adolescent and adult rats, who underwent MS procedure in their early life period. What is also worth noting is the dynamism of CB1 during lifetime. Vangopoulou et al [43], showed in their paper that CB1 receptors' mRNA decreases with age in most of brain structured regardless of age and the presence of handling.

2.3 Possible changes in endocannabinoid system in population of adolescent humans

Changes in endocannabinoid system were long forecasted as a possible pathophysiological ground in different mood disorders. Obviously, for bioethical reasons and difficult tissue collection, so far clinical data is limited. Some publications about post-mortem examinations of suicide victims cerebrums exist, but those represent mostly population of severe depression, thus this can be also a source of possible bias in results. According to the study performed by [11] expression of CB1 receptor in prefrontal cortex (PFC) of a healthy (without prior psychiatric history) human being decreases constantly from the moment of birth to the age of 50. Simultaneously, PFC level of CB2 receptor remains fixed at the same time. Levels of CB1 receptor in the population suffering from mood disorders were significantly higher than levels in non-depressive individuals at the same age. The dynamics of decrease in expression of CB1 receptor during lifetime remained unchanged keeping stable difference between control group. What is interesting, in population suffering from bipolar disease this regularity was not detected. Neither in depressive nor bipolar population levels of CB2 receptors differed significantly from the proper age-correlated control group. A significant limitation of this study was the fact that

only mRNA levels of above receptors were analyzed and those does not necessarily correspond to the actual protein levels. Also, further study is needed to identify exact anatomical structures in PFC where changes in ECS occur [11].

Similar difference in expression of CB1 receptors as in the paragraph above was also found in research conducted by Vinod et al. During this study brain samples from dorsolateral prefrontal cortex (DLPFC) were obtained from population of suicidal victims suffering from chronic alcohol disease and alcoholics who died by other causes. Significant rise in levels of CB1 receptors in patients who were suicide victims was confirmed in Western Blot analysis. What is interesting is that CB1 receptors excess is probably not an effect of up-regulation due to lack to endogenous cannabinoids, because DLPFC levels of AEA and 2-AG in the suicidal group were higher than in control group [44]. Still, a fact that has to be mentioned is that population in this paper [44] was heterogenous with age of subjects ranging from 16 to 70. However, samples from both control and investigated group were bondly adjusted according to different factors including age and no statistical significance between different groups was observed. Another similar study conducted by [45] revealed coherent results. CB1 receptor density in DLPFC was significantly higher in group of depressed victims of suicide compared with control group comprising people who died by other causes, but were matched with tested group for age, sex, postmortem interval (PMI) and ethnicity. Likewise, to the above, tested group did not contain only adolescents, but was a composite of people aged between 13 to 79. Also, here, previously mentioned age-related decrease in expression of CB1 in DLPFC was noticed. Moreover, in this paper the increase of CB1 receptor-stimulated [35S] GTPgS binding turned out to be 45% higher in depressed suicide group. CB1 receptor agonist, CP-55,940 was employed to define the binding efficiency between a receptor and its G-protein. Another paper by [46] examined changes in ECS of ventral striatum connected with alcohol disease and suicidal behavior. Three groups were tested and compared: alcohol-dependent non-suicides (CA), alcohol-dependent suicides (AS) and group of people without prior psychiatric history who died by other causes. Again, it is crucial to keep in mind the fact that even though suicidal behavior might be the consequence of those, it is not identical with depression and mood disorders. Moreover, suicidal behavior corresponds usually to only the most severe cases of major depression. Also, this study is not based entirely on adolescent population, but the test group consists of patients between 15 and 67 years old. However, this research revealed no significant effect of age on expression of CB1 receptor or G-protein binding among three tested groups. When it comes to statistical evaluation of results it turns out that levels of CB1 receptor were lower in group of alcohol suicides and chronic alcoholics than in control group. Simultaneously, this level in CA group was notably lower than in AS group. In regard to CB1 receptor agonist-stimulated [35S] GTP S binding levels, present study showed a decreased extent of it in group of chronic alcoholics, whereas no statistical relevance was found between groups of AS and controls. Another measured parameters: levels and activity of FAAH revealed significant dissimilarities among different age groups. Nevertheless, exact course of these changes has not been described. Regardless of the effect of age, evaluation of FAAH activity indicated decreased level in AS and CA groups compared to the benchmark group. However, a

marked higher level of activity of FAAH occurred in AS group compared to CA group. Also, it is worth mentioning that suicide may be the result of major depression as well as chronic alcohol consumption itself. Moreover, major depression is a well-known risk factor that increases susceptibility towards alcohol abuse [41], so further study is necessary to confirm those alterations in purely depressed population.

One of the older papers by [6] examined serum levels of AEA and 2-AG in group of female patients with diagnosis of minor and major depression. Tested group was aged between 17 and 39 years old and every patient had her demographically and ethnically matched control. In population of women with major depression serum levels of 2-AG were significantly higher and serum levels of AEA did not reveal any remarkable differences compared with age matched controls. On the contrary, in the population of patients with minor depression an outstanding divergence in serum levels of AEA was found, whereas serum levels of 2-AG were higher than in control group, but without statistical importance. However, the relationship between serum levels of ECS and its amount and activity in central nervous system has not been determined yet [6]. Interestingly, quite opposite results were achieved by [47]. In their study serum levels of four endocannabinoids (AEA, 2-AG, PEA and OEA) were measured in group of people who were admitted to psychiatric ward for other reasons (PC) and in patients who attempted suicide (SA). Eventually, the only statistically significant parameters were AEA and PEA levels, which were elevated in SA group compared to PC group. This research had interesting methodology though: first blood was collected from all the patients; serum levels of EC was estimated, and statistical evaluation was carried out. Then urine tests for presence of cannabis metabolites were performed and eventually, patients who were tested positive to cannabis use were excluded from final results. Even though no statistical variation was found after narrowing the tested group in this paper, the fact of potential cannabis consumption should be taken into consideration when designing future studies.

Even though hair samples analysis seems promising as a simple and easily accessible diagnostic test in mental disorders, because of its non-invasive character, the results of a recent paper by [48] revealed only limited positive correlation between depression in population of Middle Eastern refugee minors and levels of 1-AG/2-AG (measured as one variable because of the fact of easy isomerising of 2-AG into 1-AG and therefore difficulties in extraction of pure 2-AG) in their hair samples. Also, self-efficacy and prosocial behavior were related to higher levels of oleoylethanolamide (OEA), stearoylethanolamide (SEA), and palmitoylethanolamide (PEA) in this population. The similar idea of exploring hair endocannabinoids concentration was principle of another research by Koenig et al [49], Pregnant women exposed to childhood maltreatment in the past tended to present higher levels of 1-AG and decreased amounts of SEA in their hair analysis compared to control group, which were pregnant women without previous history of childhood maltreatment. Moreover, newborn babies of mistreated women demonstrated elevated levels of 1-AG and OEA. Worth noting is also the fact of correlation between severity of childhood maltreatment and low SEA levels in mothers and high OEA levels in neonates. However, it still remains unclear what is the relation between hair concentration of ECS components to blood EC levels and brain EC activity.

Study	**Animal species**	**Sex of animals**	**Type of ELS**	**Age of assay**	**Alterations in ECS**
Portero-Tresserra et al. [41]	C57BL/6 mice	male only	MSEW	N/A	↓AEA, ↓DEA , ↓DHEA in striatum;↓2-AG, ↓2-LG, ↓2-OG in PFC
Atsak et al. [50]	Sprague-Dawley rats	male only	Limited nesting early-life stress	~ 12 weeks	↔2-AG, ↑AEA in hippocampus; ↔2-AG, ↑AEA in PFC; ↓2-AG, ↑AEA in amygdala;
Amancio-Belmont et al. [51]	Wistar rats	male only	MS (3h per day, PND2-PND15) + social isolation	PND 64	↑CB1R in NAcc ↔ CB2R in NAcc
Moussa-Tooks et al. [52]	Long-Evans rats	Male and female	Limited bedding	PND70	Crus I of the cerebellum: ↓SEA, ↓LEA, ↓ OEA, ↑P-Gly, ↑D-Gly, ↑2-AG, ↑PGF2α in control females compared to control males. ↓D-Gly,↓ 2-AG, ↑P-Gly, ↑2-LG, ↑linoleic acid in stressed females Interpositum of the cerebellum:↓LEA, ↓A-Taur, ↓linoleic acid, ↓AA, ↓PGE2 in control females compared to control males. ↓2-AG, ↓AA in stressed males. ↓LEA, ↓A-Taur, ↑2-AG in stressed females. Dorsal hippocampus: ↓2-LG, ↓2-AG in control females compared to control males.

Romano-López et al. [42]	Wistar rats	Male only	MS (2x3h per day, PND2-PND15)	PND100	↓FAAH, ↓MAGL, ↔NAPE-PLD, ↔DAGLα in NAcc of stressed animals. ↔FAAH, ↔MAGL, ↔NAPE-PLD, ↔DAGLα in FC of stressed animals
Vangopoulou et al. [43]	Wistar rats	Male and female	Neonatal handling (PND1-PND21, 15min/day)	PND39-40 (adolescent); PND89-90 (adult)	↑[3 H]CP55,940 binding levels in prelimbic cortex and medial orbital cortex of handled rats in P40 compared to control group ↓[3 H]CP55,940 binding levels in nucleus accumbens, caudate putamen and basolateral amygdala of handled rats in P90 compared to control group ↓[3 H]CP55,940 binding levels in female handled rats in CPu, NAcc, CA3, DG, BLA compared to control group ↑[3 H]CP55,940 binding levels in female non-handled rats compared to male non-handled animals ↑[3 H]CP55,940 binding levels in both sexes non-handled rats from adolescence to adulthood in Nac, PrL, MO ↓[3 H]CP55,940 binding levels in both sexes handled rats BLA and CPu from adolescence to adulthood ↓CB1 mRNA levels of male handled rats in CPu and BLA from adolescence to adulthood ↓CB1 mRNA levels of female handled rats in CeA and BLA from adolescence to adulthood ↓CB1 mRNA levels of male non-handled rats in BLA from adolescence to adulthood

					↓CB1 mRNA levels of female non-handled rats in CPu from adolescence to adulthood ↑CB1 mRNA levels of male non-handled rats in CPu from adolescence to adulthood ↑CB1 mRNA levels of male rats in PFC from adolescence to adulthood ↓CB1 mRNA levels of female rats in PFC from adolescence to adulthood
Hill et al. [9]	Sprague-Dawley rats	Male only	MS (3h/day, PND2-PND12)	PND2 PND12 PND14 PND40 PND70	Prefrontal cortex: No effect of MS. ↑AEA in P40 and P70 compared to P2-P14 ↑2-AG in P12-P14 compared to P2, P40 and P70 ↓CB1 receptor binding site density in P40-P70 in MS rats compared to control group Amygdala: ↓AEA in P2 compared to P12-P70 ↓AEA in P12-P14 compared to P40-P70 ↓AEA in P12-P14 in MS rats compared to control group ↑2-AG in P12-P14 compared to P2, P40 and P70 ↑2-AG in P12-P14 in MS rats compared to control group ↓CB1 receptor binding site density in P40-P70 in MS rats compared to control group Hippocampus: ↑AEA in P40 and P70 compared to P2-P14

					↓AEA in all ages in MS rats compared to control group ↑2-AG in P40-P70 compared to P2-P14 ↓2-AG in P2 in MS rats compared to control group ↑2-AG in P12 in MS rats compared to control group ↓2-AG in P40-P70 in MS rats compared to control group ↓CB1 receptor binding site density in P70 in MS rats compared to control group ↓binding affinity of the CB1 receptor in P40-P70 in MS rats compared to control group ↑Bmax of the CB1 receptor in P70 compared to P40 in control group.

Table 1: Studies examined the exact impact of ELS on ECS activity and expression in rodent's brain.

3. Conclusions

As childhood adversity induces long-lasting behavioral effects in humans we might use it to provoke such changes in rodents as well. Even though this animal model corresponds very well with the actual human disease, there are a lot of factors we need to take into account to avoid unplanned bias of our results. First of all, it must be noted that psychiatric disorders have some genetic background. With inherited predisposition towards depressive-like behaviors varying from individual to individual, MS protocol might not induce those changes in every litter. For this reason, it is vital for the proper study to use different litters and sometimes perform multiple experiments so that our experiment would be reliable.

Especially now, during COVID-19 pandemic and thus growing incidence of social isolation and loneliness, which in long perspective may lead to wide array of health issues including cardiovascular diseases, altered immune responses and depressive disorders [53], proper screening and precise diagnosing of MDD, especially in pediatric and adolescent population, should be a dominant task of public health systems [54]. Apart from epidemic situation, growing incidence of mood disturbances and suicide rates among adolescents is bondly connected with extending popularity of social media and daily screen time spent using them [55]. Also, as cannabis is the most wide-spread drug worldwide with year-to-year rising popularity and with its proved influence on causing depressive disorders, especially among adolescents, it might turn out to be very useful to find biomarkers and new grip points for anti-depressive drugs among ECS.

When we try to compare results from animal studies with limited clinical data we described in previous chapter, we might get a bit confused. For instance, whereas CB1R levels in most of the brain structures in MS animals tended to be decreased, in human studies this tendency was completely reversed. The reason for that might be incorrect „human model of depression", which based on the most severe cases that resulted in suicide. Also, the possible source of distortion might have been the ground for depression, which was usually chronic alcohol disease, whereas in animal model MDD was cause by traumatic experiences from early life.

To sum up, as clinical data in this field is restricted until now, conclusions that we draw might prove to be a little far-fetched, so they should be interpreted with caution. Also, that is why further investigation in this topic is crucial.

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