

Ethanolamine Produced from Oleoylethanolamide Degradation Contributes to Acetylcholine/Dopamine Balance Modulating Eating Behavior

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▶ To cite this version:

Ilario Mennella, Gaëlle Boudry, David Val-Laillet. Ethanolamine Produced from Oleoylethanolamide Degradation Contributes to Acetylcholine/Dopamine Balance Modulating Eating Behavior. Journal of Nutrition, 2019, 149 (3), pp.362-365. 10.1093/jn/nxy281. hal-02050393

HAL Id: hal-02050393 https://univ-rennes.hal.science/hal-02050393

Submitted on 10 Sep 2019

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⁵ Authors affiliation: INRA, INSERM, Univ Rennes, Nutrition Metabolisms and Cancer, NuMeCan, Rennes, St Gilles, France
⁶ Corresponding author: Mailing address: Ilario Mennella, INRA NuMeCan, 16 Le Clos, 35590, Saint-Gilles, France. Phone: +33 223485652, fax: +33 223485080, e-mail: ilariomennella@gmail.com

Names for PubMed indexing: Mennella I, Boudry G, Val-Laillet D

Word count: 1952

Number of figures: 1

Number of tables: 0

Supplementary data submitted: Supplemental Table 1

Running title: Ethanolamine in acetylcholine/dopamine balance

Keywords: dopaminergic system, N-acetylethanolamines, fatty acid amide hydrolase, gamma aminobutyric acid, endocannabinoids, neurotransmitters, neurons, neuromodulator

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¹ List of abbreviations: acetylcholine (ACh); dopamine (DA); Oleoylethanolamide (OEA); N-acetylethanolamines (NAEs); ethanolamine (EA); nucleus accumbens (NAc); gamma aminobutyric acid (GABA); proliferator-activated receptor- α (PPAR- α)

² **Financial support:** this publication has been written with the support of the AgreenSkills+ fellowship programme which has received funding from the EU's Seventh Framework Programme under grant agreement N° FP7- 609398 (099/2017).

³ **Conflict of Interest and Funding Disclosure:** Dr. Mennella reports grants from Brittany Region (SAD program, Stratégie d'Attractivité Durable), University Bretagne Loire, and from AgreenSkills+ during the conduct of the study. Gaëlle Boudry and David Val-Laillet declare no potential conflict of interest. The funding source had no involvement in decisions related to the research and publication of results.

Oleoylethanolamide (OEA) is a well-recognized anorectic compound (1). Recent investigations pointed out that, when administrated at a dose of 10mg/kg, it affects food reward by influencing the acetylcholine (ACh)/dopamine (DA) balance in the cholinergic system (1). Recent published data support the effect of OEA on both the homeostatic and non-homeostatic regulation of food intake through the activation of proliferator-activated receptor- α (PPAR- α) in the small intestine, which mediates satiety signals to the brain *via* the afferent vagal fibers (2).

7 Mammals have the enzymes to synthetize and degrade N-acetylethanolamines (NAEs), including

8 OEA. NAEs are bios ynthesiz ed "on -demand" from their pr ecu rsors N -acylated ethanolamine

9 phospholipids, through three different enzymatic pathways (3). The absolute concentration of NAEs
10 in tissues is very low, in the order of a few nmol/g of tissue, the highest concentration being present
11 in the brain and intestine (3).

12 NAEs, including OEA, can also be provided by certain foods, oatmeal, nuts, and cocoa powder

13 being the major food sources, but the amount per gram of OEA found in these foods is very low (<2

14 μ g/g) (4) compared to the amount used in clinical trials. OEA can also be synthetized upon

15 ingestion of precursor-containing foods, such as olive oil. For example, the intake of a meal 16 containing a high amount of oleic acid increased plasma concentration of OEA in rats and humans 17 (5, 6) reducing the following energy intakes in humans (6, for a summary of human studies 18 addressing the role of OEA on eating behavior see supplemental table1).

19 NAEs hydrolysis is catalyzed by the fatty acid amide hydrolase that has a strong activity in the 20 intestine, liver, brain, and at the blood-brain barrier level (7), resulting in the production of fatty 21 acids and ethanolamine (EA). After OEA is orally or intragastrically administrated in relatively 22 abundant doses, *i.e.* 10mg/kg, it is likely that following its degradation, a peak in oleate and EA, 23 occurs in several organs. The effect of oleic acid on the gut-brain-axis has been extensively investigated, whereas EA received scarce attention. One study (8) tested the effect of oral EA 24 (1.88mg/kg, the equivalent amount released by 10mg/kg of OEA) and found no effect on food 25 26 intake compared to a control group, in rats. However, in this study only food intake and no other aspects of eating behavior, such as food preferences for high sugar/fat foods that may also be influenced by the activation of brain areas related to food reward, were investigated. On the contrary, there are scattered evidences from old and new recent researches, which underline the influence of EA on the cholinergic system.

31 Here, we briefly describe OEA and EA effects on the cholinergic system, suggesting that EA

32 contributes to the overall balance between DA and ACh after OEA administration (9, 10).

33

34 The cholinergic system regulates food reward by interacting with the dopaminergic system

35 The cholinergic system modulates various brain functions through the release of the Ach (11). This

36 occurs as a result of depolarization of the axonal termination that induces

37 a rise in the cytosolic Ca²⁺ concentration and the fusion between the axoplasmic membrane and the vesicles containing ACh.

38 Projection neurons constitute the main brain structure of the cholinergic system that originates 39 mainly in subcortical regions, such as the basal forebrain, and extends into many brain regions, 40 including cortical areas and the hippocampus. Interneurons with cholinergic activity were also 41 identified in specific target areas of mesolimbic system, such as the nucleus accumbens (NAc) (11),

42 which are involved in pleasure and motivation, contributing to the hedonic dimension of eating.

In the regulation of eating behavior, the cholinergic system constantly interacts with, and regulates the activity of one of the main neurotransmitters involved in reward, DA (12). DA is synthesized by dopaminergic neurons in the midbrain (*i.e.* ventral tegmental area and substantia nigra). The dopaminergic mesolimbic pathway is a neuronal circuit that is activated in order to translate motivation into action in a variety of behaviors, including eating (12).

In human lean subjects, food cues stimulate DA release in the dorsal striatum (12) and during eating, DA response is positively correlated with the pleasantness of the food. These evidences indicate that, in lean subjects, DA may be considered as a biomarker of the hedonic value of foods.

51 Conversely, in obese subjects, responses to food cues and food ingestion stimuli are blunted (12).

52 The dopaminergic and the cholinergic systems have a similar anatomical connectivity with

numerous functional neuronal endings, such as in the NAc. Here, cholinergic interneurons, together with dopaminergic projections of the ventral tegmental area, modulate the activity of gamma aminobutyric acid (GABA) projections neurons (12). In the NAc, ACh/DA fluctuations modulate eating behavior. For example, when rats are exposed to an adverse conditioned stimulus, there is a concomitant ACh increase and DA decrease. In addition, fasted mice in a free feeding state stop eating when the ACh/DA ratio begins to increase (12).

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60 **OEA influences cholinergic-mediated food-reward regulation**

Some investigations also suggested that OEA influences the food-reward system by modulating DA 61 62 release. High-fat-diet chronic consumption in mice produces a significant reduction in intestinal 63 OEA synthesis, but not in other tissues, which is concomitant with a reduction of DA in the brain. In high-fat-diet-fed mice, infusion of OEA (10mg/kg) solution 25 min before intragastric lipid 64 emulsion infusion restored DA release. No difference was found in DA increase in response to the 65 66 calories content of the emulsion. In low-fat-diet-fed mice, OEA influenced the release of DA when 67 stimulated by intragastric infusion of a low-calorie emulsion. In addition, in low-fat-diet-fed mice, 68 the infusion of OEA decreased the intake of both low-calorie and high-calorie emulsions. Finally, 69 the authors demonstrated that these effects were abolished in sub-diaphragmatic bilaterally 70 vagotomised and in PPAR- α knockout mice (1). These evidences indicate that OEA interacts with 71 the mesolimbic system, probably *via* activation of the PPAR- α on vagal afferent nerves. In contrast, 72 another investigation provides evidences that OEA can regulate DA release through central 73 mechanisms as well. In rats, when injected in the lateral hypothalamus, OEA increased extracellular 74 DA levels in NAc (9). The molecular mechanisms of OEA–DA interaction have not been fully 75 clarified yet.

76

Ethanolamine, a downstream metabolite of OEA, may serve as neuromodulator stimulating
acetylcholine release in the brain

79 The quantitative degradation of 10mg of OEA may produce 1.88mg of EA and this is particularly 80 interesting because it is a small molecule with a rather good ability to pass the blood-brain barrier 81 and influence the activity of diverse brain areas (13). Several pioneering studies demonstrated that 82 EA can be released from different brain regions following electrical or chemical depolarization (14). 83 A more recent *in vitro* study demonstrated that, when synaptosomes and synaptoneurosomes were 84 incubated with EA, this was stored in intracellular compartments, and released by classical exocytosis following depolarization (15). The authors pointed out that the concentration of EA 85 86 released was low compared to a classical neurotransmitter, but they did not exclude the possibility 87 that a discrete receptor exists post-synaptically for EA (15). Although EA cannot be considered as a 88 pure neurotransmitter, all in all, these studies support the role of EA as a neuromodulator, a 89 molecule that is not directly excitatory or inhibitory, but that can sustain both activities depending 90 on the site and the time frame of its release. Therefore, it is convincing that the EA released from 91 the degradation of NAEs in the intestine or at the blood-brain barrier level may play a role in the 92 brain. In fact, it is known that EA can modulate neurotransmitters. It was shown that EA stimulates 93 the release of aspartic and glutamic acids in the anterior hippocampus of rabbits (16), and it is also 94 known to inhibit the enzyme GABA aminotransferase with the consequence of reducing GABA 95 breakdown and increasing its concentration in the brain (17).

Some investigations demonstrated that, similarly to OEA, EA could influence the cholinergic 96 97 system. A first suggestion came from the neuronal cells' ability to use EA as a precursor of ACh. In 98 chicken neuronal cultures, it was proved that EA is rapidly phosphorylated to phosphoethanolamine 99 and converted to phosphatidylethanolamine that may also be methylated to phosphocholine (18). In 100 vivo, intraventricular injections of ³H-EA showed that the rat brain has the capacity to synthetize 101 choline by stepwise methylation of EA, phosphoethanolamine free de novo and 102 phosphatidylethanolamine (19). In the human cell line neuroblastoma LA-N-2, it was demonstrated 103 that the same pathway exists and that the choline thus produced is a source of *de novo* synthesis of 104 ACh (20). In contrast, Ansell and Spanner (21) described that after injection of labeled EA in the

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phosphatidylcholine was found. Similar results were obtained by Browning and Schulman (22) in slices of cerebral cortex of rats. They showed that labeled ACh was formed in the presence of labeled choline, whereas ACh was not labeled if choline was substituted with labeled EA, serine or methionine. Therefore it is accepted that, although the conversion of EA in ACh is possible, *in vivo* and in the presence of normal plasma choline levels, EA is not used for choline and ACh synthesis.

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111 However, other investigations found that EA can increase ACh extracellular concentration in the brain by indirect mechanisms. In cultured explants of the medial septal nucleus from rat brains, EA 112 113 enhances the ability to develop cholinergic neurons to utilize choline for the production of ACh (23). EA has been shown to increase extracellular levels of ACh in rat hippocampus slices during 114 115 continuous infusion in a depolarizing KCl buffer. This increase was not mediated by a greater synthesis of ACh, but by a higher cellular release that would be stimulated by amino-alcohols such 116 117 as EA (24). In a subsequent study, they suggested that the mechanism through which amino-118 alcohols facilitate ACh release in the hippocampus may involve the activation of calcium channels 119 (25). Interestingly, this effect appears to be specific to cholinergic endings, since other 120 neurotransmitters, such as norepinephrine and DA, were not affected. A similar result was obtained 121 by Khairy et al. (10) who have shown that the application of EA on dorsal root ganglion (DRG) neurons cells, with a threshold of 10 nM, increases the amplitude and duration of the transport of 122 KCl-stimulated Ca^{2+} release. Furthermore, EA stimulated voltage-activated K⁺ currents 123 independently of the activation of Ca^{2+} channels. In another study, Liao et al. (17) in accordance 124 125 Bostwick, also found that EA increased neurotransmitters release from brain synaptosomes, but they proposed different mechanisms of release. They demonstrated that synaptosomes incubated with 126 EA, at a concentration from 0.31mM to 5mM, increased their capability to release 127 128 neurotransmitters, i.e. [3H]-D-aspartic acid. They suggested that EA permeated into synaptic 129 vesicles and increasing their filling capability. The enhanced capability of synaptic vesicles to load 130 neurotransmitters explains how EA influence may regulate synaptic transmission. The 131 concentrations used in the study were within physiological levels of EA in the brain, *i.e.* between
132 197–870 nmol/g (17).

Therefore, even if the mechanisms are still not clear, there are strong evidences to support that EA influences neurotransmitters release in the brain. This is of great interest especially when the source of EA is OEA, because this NAE has itself an action on the cholinergic/dopaminergic system. It is also possible that there are differences in the overall effect on the brain between the administration of pure EA, as investigated by Nielsen et al (8) and EA released following OEA degradation, since this EA can keep acting on neurotransmitters previously stimulated by OEA. In conclusion, these evidences support the hypothesis that OEA and its metabolite, EA, can interact

in conclusion, these evidences support the hypothesis that OEA and its inetabolite, EA, can interact in the regulation of the overall ACh/DA balance influencing brain areas involved in food-reward (**Figure 1**). Taking into account this perspective, further studies should investigate the kinetic of EA release following OEA administration and whether it can impact the cholinergic regulation of dopamine.

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145 Statement of authors' contributions to manuscript: IM, GB and DV wrote the article, IM 146 designed research and had primary responsibility for final content. All authors have read and 147 approved the final manuscript.

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Figure Legends

Figure 1. Schematic representation of oleoylethanolamide (OEA) and ethanolamine (EA) effects on acetylcholine/dopamine (ACh/DA) balance. From intestine, OEA communicates *via* vagal afferent fiber with brain areas regulating DA release. OEA is then degraded by the enzyme fatty acid amide hydrolase in oleic acid and EA, which pass into the bloodstream and can in part penetrate the blood brain barrier. In the brain, EA acts as a neuromodulator being able to stimulate cholinergic neurons ACh release. ACh/DA fluctuations modulate eating behavior.



