REVIEW

The melanin-concentrating hormone receptors: neuronal and non-neuronal functions

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Melanin-concentrating hormone (MCH) is a cyclic peptide highly conserved in vertebrates and was originally identified as a skin-paling factor in Teleosts. In fishes, MCH also participates in the regulation of the stress-response and feeding behaviour. Mammalian MCH is a hypothalamic neuropeptide that displays multiple functions, mostly controlling feeding behaviour and energy homeostasis. Transgenic mouse models and pharmacological studies have shown the importance of the MCH system as a potential target in the treatment of appetite disorders and obesity as well as anxiety and psychiatric diseases. Two G-protein-coupled receptors (GPCRs) binding MCH have been characterized so far. The first, named MCH-R1 and also called SLC1, was identified through reverse pharmacology strategies by several groups as a cognate receptor of MCH. This receptor is expressed at high levels in many brain areas of rodents and primates and is also expressed in peripheral organs, albeit at a lower rate. A second receptor, designated MCH-R2, exhibited 38% identity to MCH-R1 and was identified by sequence analysis of the human genome. Interestingly, although MCH-R2 orthologues were also found in fishes, dogs, ferrets and non-human primates, this MCH receptor gene appeared either lacking or non-functional in rodents and lagomorphs. Both receptors are class I GPCRs, whose main roles are to mediate the actions of peptides and neurotransmitters in the central nervous system. However, examples of action of MCH on neuronal and non-neuronal cells are emerging that illustrate novel MCH functions. In particular, the functionality of endogenously expressed MCH-R1 has been explored in human neuroblastoma cells, SK-N-SH and SH-SY5Y cells, and in non-neuronal cell types such as the ependymocytes. Indeed, we have identified mitogen-activated protein kinase (MAPK)-dependent or calcium-dependent signalling cascades that ultimately contributed to neurite outgrowth in neuroblastoma cells or to modulation of ciliary beating in ependymal cells. The putative role of MCH on cellular shaping and plasticity on one side and volume transmission on the other must be now considered.

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Melanin-concentrating hormone (MCH) is a cyclic peptide that was first isolated from salmon pituitary glands¹ and subsequently characterized in the rat hypothalamus.² Brain and peripheral expression of MCH was then established in many mammalian species including humans (reviewed in Nahon *et al.*³). In the central nervous system, MCH mRNA and peptide syntheses were found predominantly in neurons of the lateral hypothalamus and zona incerta that projected throughout the brain^{4,5} (Figure 1, reviewed in Bittencourt⁶). Central MCH displayed a wide range of functions (reviewed in Nahon *et al.*,³ Griffond and Baker,⁷ Pissios *et al.*,⁸ Adamantis and de Lecea⁹), with a major role in energy maintenance by decreasing energy expenditure and stimulating feeding behaviour¹⁰ (see below). Two high-affinity receptors for MCH have been identified (reviewed in Hervieu¹¹ and Chung *et al.*¹²). The first receptor, named here MCH-R1, also called SLC1, was simultaneously identified through reverse pharmacology strategies by several groups as a cognate receptor of MCH.^{13–16} This receptor is expressed at high levels in many brain areas of rodents and primates^{13,17} and is also expressed in peripheral organs, albeit at a lower rate. A second receptor, designated here as MCH-R2, exhibits 38% identity to the MCH-R1 and was originally identified

in humans¹⁸ (reviewed in Boutin *et al.*¹⁹). Interestingly, although MCH-R2 orthologues were also found in fishes, dogs, ferrets and non-human primates, this MCH receptor gene appeared either lacking or non-functional in Glires species (including rodents and lagomorphs).^{20,21} Both receptors are class I G-protein-coupled receptors (GPCRs), whose main role is to mediate the actions of peptides and neurotransmitters in the central nervous system. We present here an overview of recent studies concerning the effect of MCH on feeding behaviour and energy storage, the *MCH* gene(s) and complex regulation in primates particularly and finally the MCH receptor family and functional characterization in cells expressing exogenous or endogenous MCH-R1 or MCH-R2.

MCH IS INVOLVED IN ENERGY HOMEOSTASIS CONTROL AND MANY MOTIVATED BEHAVIOURS

The dominant expression of MCH in the lateral hypothalamus of mammalian brains, a so-called feeding–drinking centre, has generated the proposal that this peptide may regulate appetite and/or energy homeostasis. The role of MCH and MCH-R1 signalling in the control of feeding behaviour and energy balance

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Figure 1. Structure and brain distribution of the rat MCH and MCH-R1. Left panels: structure of the rat MCH (cyclic peptide) and rat MCH-R1 (7TM GPCR); right panels: sagittal sections of the rat brain showing the distribution of MCH neurons and projections and MCH-R1 mRNA expression. Black dots indicate MCH neuron perikarya, and lines correspond to fibres. Brain areas with expression of MCH-R1 mRNA are noted by white dots, redrawn, respectively, from Bittencourt *et al.*⁵ and Hervieu *et al.*¹⁷

is now firmly established in rodents (reviewed in Pissios *et al.*,⁸ Qu *et al.*,¹⁰ Hervieu *et al.*,¹⁷ Nahon,²² Pissios²³).

Acute intracerebroventricular injections of MCH (>5 µg per animal) transiently stimulate food intake in rats, an orexigenic effect that is not mediated by neuropeptide Y, galanin or orexins (also named hypocretins) but could be blocked by α-MSH. Intrahypothalamic injection studies and electrophysiological characterization further identified the paraventricular nucleus, ventromedian nucleus and arcuate nucleus as major targets of MCH action, with differential effects in small-litter and over-nourished rats. Interestingly, MCH also stimulates water consumption independently from its feeding and water osmolality actions, consistent with the strong adiposity observed in LHA-lesioned rats. Chronic intracerebroventricular infusion of MCH in rats or mice induces hyperphagia and body weight gain, especially on a moderate high-fat diet. The development of obesity is accompanied by an increase in liver and adipose tissue weights and an accompanying increase in lipogenic activity in white adipose tissue and concomitant reduction in brown adipose tissue functions. Furthermore, plasma glucose, insulin and leptin levels are also increased in these animals, all these parameters being associated with obesity in men. Furthermore, central administration of MCH-R1 antagonists leads to a sustained reduction in appetite, body weight and fat storage without overt alteration of lean mass. Peripheral MCH-R1 signalling may also be involved in this process through regulation of leptin or insulin secretion. Finally, in agreement with the pharmacological data, genetically modified mouse models also showed an important role of *MCH* and *MCH-R1* genes in appetite and, mainly, energy metabolism. Indeed, transgenic mice overexpressing the *MCH* gene develop hyperphagia, mild obesity and insulin resistance when maintained on a high-fat diet. Conversely, *MCH* gene ablation in mice leads to a leanness phenotype associated with hypophagia and an increase in oxygen consumption, both effects being highly dependent upon the genetic background and diet. In this regard, the reduction in body fat observed in mice combining *MCH* and leptin deficiencies, by crossing *MCH*-null mice and *ob/ob* mice, does not result from feeding behaviour changes (the mice remaining hyperphagic) but from improvements in energy expenditure regulation (increased locomotor activity and resting metabolic rate). It is worth noting that targeted disruption of the *MCH-R1* gene in mice maintained on regular chow also leads to a

lean phenotype associated with both hyperphagia on one hand and increased energy metabolism on the other. In agreement with the hyperactive phenotype, *MCH-R1*-null mice are resistant to high-fat diet-induced obesity.

Overall, pharmacological studies and animal models have shown that *MCH* and *MCH-R1* neuronal networks have a major role in the control of energy homeostasis by increasing food intake and mostly regulating energy expenditure and thermogenesis in mammals. However, the actions of *MCH* appear much more widespread and concern a large spectrum of behaviours. These include sleep–wake regulation, sexual behaviour, grooming, rearing, aggressive behaviour, locomotion, exploratory behaviour, learning and memory formation (these behavioural studies are discussed in exhaustive reviews^{7–9,11,17,24,25}). Recent studies (reviewed in Kemp and Weetman²⁶ and Lakaye²⁷) also addressed the local expression and role of *MCH* and its receptor outside the brain. Indeed, the importance of *MCH*/*MCH-R1* in the digestive tract, adipose and bone tissues and cardiovascular and immune systems, for instance, should be considered in analysing phenotype alterations in *MCH*-null or -overexpressing transgenic mice.

COMPLEX STRUCTURE OF THE PRECURSOR MCH MRNA AND SENSE/ANTISENSE GENES

Two *MCH* genes and/or corresponding mRNAs have been originally cloned and sequenced from salmonids, tilapia, bonito, eel and flounder.²⁸ Both were intronless and displayed the highest level of substitutions when compared within the same species rather than among other species, in agreement with early tetraploidization during teleost fish evolution. The salmonid and tilapia/flounder preproMCHs are 132 and 135 amino acids long, respectively, with the *MCH* peptide located at the C terminus. In contrast with the strong conservation in the *MCH* region between teleost fish species, the remaining part of the precursor displays marked sequence divergences.^{29–30} However, additional putative peptides could be generated from the teleost fish proMCH and named *MCH*-gene-related peptide (*Mgrp*) in tilapia, Neuropeptide-E (glutamic acid)-V (valine; NEV) in chum salmon or Neuropeptide-A (alanine)-L (leucine) in barfin flounder; however, their functional importance, if any, remains elusive. Recently, a second *MCH* gene was identified in the genome databases of zebrafish, fugu,

three-spined stickleback and medaka.³⁰ This gene, named *Pmch2*, shares striking similarities with the mammalian *MCH* gene in terms of structure, genomic synteny and peptide sequence (19–25 amino acids in length). Two different MCH peptides can be expressed in distinct regions of the zebrafish hypothalamus. Although both MCH peptides could have a role in pigmentation,³¹ preliminary functional studies in zebrafish indicate that the *Pmch2*-derived MCH peptide could be selectively involved in food intake regulation, as in mammals. Recently, a single *MCH* gene was found in the scalloped hammerhead shark *Sphyrna lewini*, and shark MCH peptide displayed strong similarities in term of structure and brain distribution to mammalian counterparts as well as under stress responses,³² indicating that the ancestral intronic *MCH* gene may be involved in functions similar to those found in mammals.

The sequence of the single gene encoding the full MCH precursor comprises three exons and two introns and exhibits high conservation in mammalian species. In humans, this gene is located on chromosome 12q24 covering ~1.4 kb of genomic DNA. The first exon encodes the 5'-untranslated region of the mRNA and the N-terminal part of proMCH including the signal peptide that allows targeting to the secretory pathway. The second exon consists of the sequence corresponding to putative neuropeptide(N)-glycine(G)-glutamic acid(E) (NGE), neuropeptide(N)-glutamic acid(E)-isoleucine(I) (NEI) and the first three amino acids of MCH. The last 15 amino acids of MCH and the 3'-untranslated amino acids are localized in exon III. Intron B splits the methionine codon with the nucleotides A on exon II and the TG on exon III. This intronic organization is identical in the rat, mouse and primate *MCH* genes. This unusual intron position for a neuropeptide-encoding gene is of primary importance for generating by alternative splicing a short mRNA encoding a protein named MCH-gene-overprinted-polypeptide (MGOP),³³ whose function remains elusive.

In addition to the *MCH/MGOP* gene, two other *MCH* gene-related transcription units were found in mammalian genomes. First, characterization of high-molecular-weight transcripts recognized by MCH complementary DNA/oligoprobes in the pheochromocytoma PC12 cell line and rat tissues revealed the existence of 'natural' antisense RNAs transcribed from the genomic region complementary to the *MCH* gene. This new gene was named *AROM* (Antisense-RNA-Overlapping-MCH).³⁴ Two major classes of RNAs appear produced from the *AROM* gene: (1) non-coding unspliced RNAs overlapping exon II/exon III and flanking intronic/regulatory sequences of the *MCH* gene; and (2) alternatively spliced mRNAs complementary to the 3'-flanking region of the *MCH* gene and coding for a new family of RNA/DNA-binding proteins. One of these proteins, named PARI, is involved in the control of genomic stability and correct DNA repair.³⁵ Because of the reciprocal regulation of MCH and *AROM* gene expression in PC12 cells, it is tempting to speculate that *AROM* mRNA and/or proteins may control the MCH gene expression, but this remains to be directly tested.

THE PRIMATE-SPECIFIC PMCHL1/PMCHL2 GENES ENCODE NON-CODING RNAs THAT REGULATE MCH GENE EXPRESSION

A decade ago we provided evidence for the emergence of two chimeric genes in higher primates.³⁶ These genes were named *PMCHL1* and *PMCHL2*, based on partial homology with the authentic *MCH* gene, and were found, respectively, on human chromosomes 5p14 and 5q13; that is, they were located on chromosomal regions clearly distinct from this of the genuine *MCH* gene. Detailed structural expression and phylogenetic analyses showed that the *PMCHL1* gene was created about 25 million years ago (mya) by a complex mechanism of exon-shuffling through retrotransposition of an antisense *MCH* messenger RNA coupled to *de novo* creation of splice sites.

PMCHL2 arose 5–10 mya by an event of duplication involving a large chromosomal region encompassing the *PMCHL1* locus. Interestingly, both sense and antisense transcripts from the *PMCHL1* gene are expressed in different areas of the developing human brain, whereas the *PMCHL2* gene is totally silent in the central nervous system. Strikingly, most of the *PMCHL1/PMCHL2* mRNAs expressed in primate testis or brain appeared as long non-coding RNAs (our unpublished data). In particular, we have identified two unconventional RNAs that displayed tissue and developmental-stage specificities in the primate brain, with highest expression in the cerebral cortex. The first one is composed of a *PMCHL1* sense part and an antisense part, and the second one is composed of two *PMCHL1* sense parts but in reverse order. We have established, therefore, cell lines expressing these unconventional transcripts and we investigated further the mechanism underlying the formation of these transcripts and their mode of action. Given the absence of the canonical (GU/AG) splice donor and acceptor sites, our hypothesis was that these transcripts were the result of trans-splicing events that used short complementary sequences. We initiated a mutagenesis approach, in which the different sequence elements were mutated, stably expressed in HEK293 cells and trans-spliced transcripts were analysed. Surprisingly, MCH mRNA expression was found driven by non-mutated trans-spliced transcripts in transfected HEK293 cells as well as in the macaque cortex (our unpublished data). In summary, we have identified unconventional trans-spliced transcripts of the *PMCHL* gene that lead to tissue and developmental-stage expression of the targeted MCH mRNA in primate brain areas outside the hypothalamus. Primate-specific origination of long non-coding RNA-encoding genes that regulate MCH gene expression may contribute to phenotypic traits that would be unique to the human lineage, but this needs further elaboration. The *PMCHL1* gene represents a prototype of the so-called 'primate-specific' genes that are presently under intensive investigation because of their major interest in understanding the basis of human evolution and emergence of novel functions.

MCH-R1 STRUCTURE AND SIGNALLING

A milestone discovery in the MCH receptor field was the quite simultaneous identification by several laboratories in 1999 of an orphan GPCR called SLC-1 (GPR24) as being a *bona fide* MCH receptor, named here MCH-R1.^{13–16} This receptor belongs to the class 1 subfamily of GPCRs and displays strong sequence conservation among mammals (91% protein sequence identity between rats and humans). The *MCH-R1* gene consists of two exons that encode for a 353-amino-acid protein with seven putative transmembrane domains, three N-glycosylation sites in the N-terminal part, which appear necessary for cell surface targeting, and multiple phosphorylation sites in the intracellular loops; some of them, located in its C-terminal tail, are involved in the MCH-dependent internalization of MCH-R1. This receptor is widely and strongly expressed in rat, mouse and primate brains with a distribution that matches with the MCH projection fields, suggesting that this receptor may mediate most MCH actions in mammals (reviewed in Hervieu *et al.*¹⁷).

In cells expressing exogenous receptor, MCH-R1 binds MCH with nanomolar affinity and may couple to Gi, Go and Gq proteins to activate multiple intracellular signalling pathways³⁷ (reviewed in Hervieu *et al.*¹⁷). For example, in CHO or HEK293 cells transfected with MCH-R1, MCH inhibits the forskolin-stimulated production of cAMP and activates mitogen-activated protein kinases (MAPK; Figure 2, left panel). MCH may also stimulate IP3 production and induce an increase in intracellular 'free' Ca²⁺ levels via a Gα_q interaction. Pretreatment with pertussis toxin (PTX), which blocks Gα_i/Gα_o proteins, abolishes the MCH-stimulated inhibition of cAMP production and MAPK activation but only decreases the intracellular Ca²⁺ efflux. Intriguingly,

Figure 2. Representations of the main MCH receptor (MCH-R1 or MCH-R2) signalling pathways in mammalian cells. Left panel: MCH-R1 signalling pathways; right panel: MCH-R2 signalling pathways. The MCH receptors may couple to different G-proteins, including G_i , G_o and $G_{q/11}$. Activation of G_i results in a decrease in adenylate cyclase activity and a reduction in cAMP formation (descending arrows). Activation of G_o (not shown) or $G_{q/11}$ results in MAPK activation via Ras/Raf activation and elevation of Ca^{2+}_i concentrations via IP_3 and PLC/PKC activations (rising arrows). Activation of these cascades would ultimately lead to changes in gene transcription and cell proliferation/differentiation (see text).

combination of MCH with forskolin potentiates MAP kinase phosphorylation in HEK293 cells stably expressing MCH-R1 and in various regions of the rat brain,³⁷ suggesting that a complex interaction between MCH-R1 and receptors coupled to G_{α_s} could be the rule rather than the exception in the mammalian brain.

In contrast to these results, the signalling pathways that mediate MCH action in mammalian cells endogenously expressing MCH-R1 remain poorly explored except in neuroblastoma or melanoma cell models (reviewed in Eberle et al.³⁸). Indeed, in human melanoma SK-MEL-37 cells, MCH inhibited forskolin-stimulated cAMP accumulation and induced MAPK phosphorylation in a PTX-sensitive manner, suggesting the coupling to $G_{\alpha_i}/G_{\alpha_o}$ proteins. The activation of the MAPK pathway led to the production of active Erk1/Erk2-phosphorylated forms. In contrast, MCH stimulates insulin release in insulinoma cells, CR1–G1 and RINm5F cells, through a PTX-insensitive signalling cascade. Expression of MCH-R1 was also reported for human melanocytes and murine 3T3-L1 adipocytes. In these latter cells, MCH affects leptin expression possibly through activation of Erk1/2 MAPK and pp 70 S6 kinase, suggesting a functional MCH signalling pathway.

Interestingly, both p53 and MAP kinase pathways contribute to mediating the MCH effect through genuine MCH-R1 on neurite outgrowth in human neuroblastoma cells,³⁹ suggesting that MCH may have a role in neuronal cell differentiation and plasticity. Very recently, we characterized the expression and functionality of endogenously expressed MCH-R1 in human neuroblastoma SK-N-SH cells and in primary cultured hypothalamic neurons. We found low levels of expression of this receptor in human neuroblastoma cells, close to physiological conditions. We identified a MAPK-dependent signalling cascade that is activated within a few minutes after MCH exposure. This pathway was also found to be involved in stimulation by MCH of a restricted set of COPII complex-encoding genes contributing likely to transdifferentiation of SK-N-SH cells (our unpublished data). Therefore, this study allowed a complete characterization from early receptor activation to late transcriptome remodelling and cellular shape changes in neuronal or neuron-like cells, for a receptor, MCH-R1, particularly important for controlling neuronal activity associated with feeding behaviour and energy balance.

On the basis of neuro-anatomical mapping and electrophysiological data, it was assumed that synaptic transmission represents

the main mode of action of MCH in the brain. However, non-neuronal intercellular communication or 'volume' transmission through the cerebrospinal fluid flow may also be involved. To address the potential contribution of these mechanisms in driving MCH effects, we first mapped non-neuronal cells targeted by MCH fibres. We found numerous MCH fibres in close vicinity to ependymocytes of the ventral part of the third ventricle (3V) in *Pmch-CFP* mice. Then, we demonstrated MCH-R1 mRNA as well as MCH-R1 protein expression in the ventral 3V ependymal cells. Developing new techniques to measure and analyse the ependymal cilia beat frequency (CBF) in acute mouse brain slice preparations, we have shown that the CBF is increased by MCH application on ependymal cells or distantly by LHA stimulation, an effect blocked by a selective MCH-R1 antagonist and absent in MCH-R1-knockout mice. *In vitro* optogenetic activation or inhibition of MCH neurons further demonstrated a tonic control of these neurons on 3V ependymocytes CBF. Finally, using *in vivo* brain MRI and *in vivo* tracking of fluorescent microbeads, we demonstrated that the volume of both the lateral and third ventricles is increased in MCH-R1-knockout mice compared with their wild-type littermates, resulting in faster cerebrospinal fluid flow. Collectively, our data revealed a dynamic control of MCH neurons on spontaneous CBF of MCH-R1-expressing ependymal cells and on cerebrospinal fluid flow in brain ventricles.^{40,41}

MCH-R2 STRUCTURE AND SIGNALLING

The second MCH receptor, named here MCH-R2, was identified in the human genome by use of *in silico* data mining, and its complementary DNA was subsequently isolated from human brain complementary DNA libraries.^{18–19} In contrast to the *MCH-R1* gene, which is intronless in its coding region, the *MCH-R2* gene encompasses five coding and one noncoding exon and may produce receptor variants by alternative splicing that would lead to a truncated version of MCH-R2. It is noteworthy that human MCH-R1 and MCH-R2 display low sequence identity (only 36%), which is rather unusual for a family of GPCRs that bind an identical ligand, suggesting weak evolutionary constraints. As mentioned above, the *MCH-R2* gene is found in teleosts (pufferfish, zebrafish), sharks, dogs, ferrets and primates (including human); however, a functional *MCH-R2* gene is selectively lost in the

rodent/lagomorph lineage. This proved to be useful for elucidating the physiological importance of the single MCH-R1 in rat and mouse but also hampered functional characterization of MCH-R2 in available animal models. Very recently, Maratos-Flier and colleagues⁴² generated a mouse model in which the human *MCH-R2* gene was under the control of the MCH-R1 gene promoter, resulting in coexpression in the MCH-R1R2 mouse brain. Intriguingly, the MCH-R1R2 mice exhibited a resistance to diet-induced obesity due to reduced food intake. Additional investigations with transgenic mice expressing the human *MCH-R2* gene under the genuine human promoter should be performed to validate this interesting study.

In mammals, the overall brain expression pattern of MCH-R2 appears to overlap partially with that of MCH-R1 but with a more restricted expression to the limbic regions, including the medial and central amygdala, the entorhinal and temporal cortex, the CA1 of the hippocampus and to a lesser extent in the ventral hypothalamus and claustrum. Nevertheless, arguing for a coexpression in the same cells seems highly speculative. In monkeys, MCH-R2 is strongly expressed in the arcuate and ventromedial hypothalamic nuclei. The differential distribution of the two MCH receptors, particularly in the human brain, may indicate distinct biological roles in mediating the large spectrum of MCH effects. In the periphery, weak MCH-R2 expression is detected in the pituitary, adipose tissue and pancreas, which also express MCH-R1; however, the precise cellular distribution and functional relevance of MCH-R2 in these organs are presently unknown.

The signalling pathways associated with MCH-R2 activation have been studied only in transfected mammalian cells and proved to be much more restricted than those reported for MCH-R1 (Figure 2, right panel). Indeed, MCH causes an increase in intracellular free Ca^{2+} levels and IP3 production but has no effect on basal or forskolin-induced cAMP synthesis. This action is not blocked by PTX treatment, indicating that MCH-R2 couples mainly to $G_{\alpha q}$. The difference in G-protein coupling between MCH-R1 and MCH-R2 could be ascribed to the marked sequence difference in the second and third intracellular loops, which are essential for specific G-protein interaction in the GPCR family.

CONCLUSION

During the last decade a number of companies have synthesized, tested and patented a variety of non-peptide MCH antagonists acting at MCH-R1 (close to 60 patent applications are presently registered⁴³). As such, the compounds are claimed to be useful in suppressing appetite and in treating or preventing a wide range of diseases, including diabetes, hyperlipidaemia, depression, anxiety and associated somatic symptoms, as well as neurodegenerative and psychiatric disorders. There is no doubt that, among this large number of compounds, suitable candidates will be identified and subsequently entered into clinical trials. However, major questions remain about the role of MCH and MCH-R1 in the control of energy balance (for example, phenotype discrepancy between MCH- and MCH-R1-null mice, role of synaptic versus volume transmission in mediating brain MCH effects) and other behaviours (stress response, sexual activity, sleep and learning). Furthermore, the MCH physiological functions mediated by MCH-R2 in the human brain deserve thorough investigations and the development of suitable animal models.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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