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*Research article*

# Autism-modifying therapy based on the promotion of a brain enzyme: An introductory case-report

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**Abstract:** An interdisciplinary study of autism led to implicate the relatively poor degradation of synaptic *serotonin*, a molecule involved in brain development. Consequent metabolic imbalance of *monoamine* neurotransmitters is assumed to impede memory encoding across sleep stages, hence the building of aberrant neural structures linked with autistic symptoms. A medication can be derived from this theoretical approach, with the aim of regulating neuromodulation whereby proper neural networks may start growing over impaired ones. *The Valproate* anticonvulsant has been prescribed here for its contribution to the promotion of a relevant brain enzyme known as *Monoamine oxidase A* (MAOA). Whereas case-studies usually focus on a subset of symptoms for less than three months in mild to moderate autism, the evolution of every autistic symptom has been witnessed across one year in an 11-year boy with severe autism. Rapid improvement of sleep, followed by the rising of visual exploration, preceded positive shifts of the core symptoms noticeable nine months later, however still impeded by bursts of hyperactivity. The adjunctive medication of *Methylphenidate psychostimulant* permitted afterwards to increase the attention span without interfering with Valproate. Such combination of MAOA-inducer and psychostimulant eventually favored the gradual acquisition of social conditioning, without fully erasing poor habits issued from ten years of autism. Because restricted to a disease-modifying action, this dual therapy relies on accompanying educational assistance, as notably learnt from its exploratory monitoring. Other insights focus on specific biomarkers as well as *functional polymorphisms* of relevant genes promoters, with the aim of guiding future clinical trials.

**Keywords:** case study; autism; neuromodulation; epigenetics; monoamine enzymes; neurotransmitters metabolism; valproate; biomarkers; sex ratio

**Abbreviations:** 5-HT: serotonin; ASD: Autism Spectrum Disorders; COMT: Catechol-O-MethylTransferase; DA: Dopamine; EEG: *ElectroEncephaloGram*; HDAC: *Histone DeACetylase*; GABA: gamma-aminobutyric acid; GP: Guided Propagation; IME: Educational and Medical Institute; MAOA: MonoAmine Oxidase A; MAOB: MonoAmine Oxidase B; MPH: Methylphenidate; NE: NorEpinephrine; TPH: Tryptophan hydroxylase; VPA: Valproic Acid; SV: Sodium Valproate

## 1. Introduction

Autism Spectrum Disorders (ASD) cover a large set of conditions encompassing sensory impairments, lack of social conditioning, and self-standing repetitive behaviors, which together impede communication. Besides the social withdrawal that results from these disabilities, underlying biological factors can be considered at both molecular and neural levels. Following advances in human genetic sequencing, pharmacological treatments have recently been tested in rodent models of ASD, thus providing guidelines for clinical investigations [1]. So far, drugs officially approved for the treatment of ASD in children *(risperidone* and *aripiprazole*) target peripheral symptoms such as irritability and associated temper tantrum, aggressive trend and self-injury. Global neurobiological models remain scarce, except an influential theory assuming brain excitatory/inhibitory imbalance [2], notably consistent with epileptic signs often associated with ASD. Despite years marked by "pervasive developmental deficits", it seems implicitly believed that drugs can rapidly rescue core ASD symptoms, as if behavioral knowledge had anyway been stored in the neural tissue, and only needed chemical regulations to be accessed and replayed at the right time. Without neglecting pharmacology to reinstate proper learning conditions, the present approach rather relies on both lifelong *neurogenesis* and long-term reeducation for an autistic person to start over. An interdisciplinary study brought together factual and hypothetical pieces of the autism puzzle, which emphasized a disrupted metabolism of monoamines neurotransmitters, itself possibly caused by a prenatal excess of the *monoamine oxidase A* enzyme (MAOA) [3]. The main assumption is that epigenetic regulations compensate for this transient enzymatic excess, but inappropriately survive the latter, even across human generations. Epigenetic traits conveying the resulting metabolic imbalance (weak promotion of MAOA) could be masked by either "safe-X" protection in women or "Low-activity" genetic variant of another enzyme (*Catechol O-Methyl Transferase*: COMT) in everybody. Epigenetic alterations would remain hidden and silently inherited until a different COMT polymorphism is met on the occasion of a new combination of genes. Through a calculation, this coincidence between an epigenetic regulation and genetic variants is found to affect four times more men than women, reflecting the actual autism sex-ratio. In other words, MAOA would temporarily be overexpressed during gestation, giving rise to its enduring epigenetic downregulation. This would explain a significant reduction of MAOA in the plasma [4], cerebellum and frontal cortex [5] of autistic children: a deficit possibly offset by COMT which however does not affect serotonin (5-HT) [6], thus eliciting a metabolic mismatch between monoamines. This disparity would be enhanced by the

known gradual action of the *monoamine oxidase B* (MAOB) enzyme towards dopamine (DA) in the early development; in fact, MAOB reaches a mature level about two years after birth, which can be linked to the delayed onset of autistic symptoms. In computer simulations of the related *Guided Propagation* (GP) model, such imbalanced metabolism of monoamines is represented by a learning parameter which, across "offline" cycles, comes back too slowly to the baseline value that allows encoding. Within processing channels, consequent aberrations include supplementary convergent connections inducing local over-activity, as well as divergent structures coding for repeated meaningless sequences. Cross-connections which convey rapid anticipations from emotional/'global perception' modules towards 'sensory-motor'/'central perception' modules are either impaired or lacking [7].

This theoretical approach has guided a preliminary medical trial conducted for one year, without notice towards involved clinicians and caregivers. This case study is reported here.

## 2. Materials and methods

The procedure implemented here followed the usual medical care required for an epileptic child. The process of gathering data in the course of this case-study did not interfere with the child life. With regard to the "Privacy and confidentiality" point 24 of the WMA Declaration of Helsinki, the names of involved individuals do not appear in the article, including the Acknowledgements. Consent for the publication of the case report was obtained from the parents of the minor patient.

An eleven-year-old Caucasian boy was diagnosed with ASD at the age of four. He is the youngest child into a family of three boys and one girl. Among siblings, the second youngest boy has a mild development disorder regarded as *Asperger syndrome* (now referred to as High Functioning ASD). At the age of 35, their mother experienced bouts of exogenous depression across the gestations of the two boys who later showed developmental problems. Both parents are qualified workers in the tertiary sector, without contact with chemicals, whereas the maternal grandmother worked in a laboratory of chemical products.

Before the rising of atypical behaviors, the boy is described by his parents as an "easy baby", sleeping full-night after 3 weeks. By 7 months, he was able to sit down, and could walk on his own at 12 months of age. He looked and smiled at his parents, played with his siblings. His first words ("maman", "papa") were uttered around one year, followed by a stagnation of language. By 2 years of age, at the beginning of child's crib, irrelevant behaviors began, including repeated swinging, sudden bursts of hand-flapping, and unexpected laughs. The following year, the boy became silent and taciturn, with difficult afternoon naps, and a trend to bring everything to his mouth. When he almost choked on swallowed sand at the day nursery, his parents decided to consult a Medico-Psychological Center. There, they met a psychologist trained to the *post-Freudian psychoanalytic* approach. Because of the time spent focusing on the mother-baby relationship, this is only two years later that the development level of the boy could be assessed during a 3-week observation at the Child Psychiatry Department of the Necker Hospital (Paris). At the age of 4 years and 2 months, the Psycho Educational Profile-Revised (PEP-R) tool [8] showed an average development level slightly above 2-years, with pronounced deficits in language, and best scores in motor skill. This case was scored 34 on the CARS scale, corresponding at that time to an average level of autism. After 3 years of hard time at school "*between misunderstanding and open hostility*" (mother's words), an Institute of Medical Education (IME) opened nearby, which proposed methods of *Applied Behavior*

*Analysis* (ABA) into its program [9]. Eventually not admitted in this public structure for unknown reasons, the 6 year-old boy then stayed either at home or in a local association where he was followed by private practitioners. This period marked a turning point in the child medical care, since it started involving drugs. A series of 8 *chelation* sessions were planned by an alternative practitioner across the next 4 years, in association with food supplements, as well as antibiotics against intestinal upsets and mood/sleep-oriented drugs (*Prozac, Melatonin, Polaramin*); a cocktail of vitamins, minerals, herbs and homeopathic intakes were supposed to counteract the toxic side-effects of chelation. Intended to remove heavy metals from the body, chelation did not prove efficient, whereas cures of antibiotics seemed to improve digestion. Chelation was discontinued, and a neurologist took over the boy case.

The author of this report came into play by first questioning the chelation procedure, noticing that blood tests showed increased amounts of toxins after four years of treatment. Furthermore, the coincident discovery that autistic brains are denser than typical ones contradicted the assumption of a neuronal depletion induced by heavy metals. The serious sleep problems undergone by the boy were revealed to the author in parallel with his design of a computational model of sleep. In this model, aberrant memory structures may be generated, owing to incorrect settings of 'offline' periods representing sleep cycles. At that time, a publication had uncovered a metabolic pathway through which *Valproic Acid* (VPA) stimulates the MAOA gene promoter, thereby increasing enzymatic activity by 60% [10]. Interestingly, this feature is noticeable in former experiments showing elevated level of the 5-HT main metabolite after VPA injection in rodents [11]. This molecule could thus be considered as capable of preventing an assumed overnight imbalance of synaptic neuromodulators. At the same period, the potential value for autism of VPA was to be studied in a clinical trial planned at the Boston hospital for Children [12]. Although withdrawn because no eligible patients could be enrolled, this project increased the relevance of using this molecule (in the form of *Sodium valproate*: SV, also termed "Valproate" in the following) in the boy case. There was however one condition to be satisfied for a SV prescription, namely epileptic signs in the sleep electroencephalogram (EEG). Once this diagnosis established, the boy Neurologist agreed to prescribe SV rather than another antiepileptic drug, up to the usual daily dosage of 30 mg/kg in two takes. His prescription also involved a gluten-free diet, as well as food supplements against intestinal disorders. For their judgement not to be influenced, none of the other intervening parties knew that SV was not only prescribed against epilepsy. Among "side effects" of the antiepileptic molecule, rapid improvements of sleep had been predicted by the model. The main outcomes of the following months are summarized in Table 1, while Table 2 provides a timeline of the autistic symptoms variations.

## 3. Results

For its MAOA-inducing effect, an antiepileptic drug based on VPA was given daily from May 2015 to a boy with severe autism. Visible effects of this medication have been monitored until May 2016. Among variations of the autistic traits noticed during that period, and listed in Table 1, significant sleep improvements happened in a few days. Eight months later, an EEG aimed at assessing the antiepileptic effect of the treatment showed restoration of the first sleep cycle. Thereafter, gradual shifts of behavior concerned visual exploration, social interaction and decreased sensitivity to noisy environments (after 2 months). Seeds of voluntary natural language have then grown up to a small size (i.e., "I want x", where x stands for either food or an action). Although less and less repetitive

and more linked with the ongoing context, poor habits continued during the first six months of treatment, namely: hand-flapping, murmuring, teeth-grinding, shouting, biting, and spitting. Stimulated by the drug, the boy activity fed at that time into the limited range of habits acquired across ten years of autism. Whereas nighttime had become peaceful, daytime nervousness was rather difficult to bear for family and educators, although the situation slightly improved with the rising of more relevant behaviors. However, peaks of activity, as well as attention deficits occurred early and all along the treatment.

Apart from the initial setting of a sleep-EEG which permitted the anticonvulsant medication, no extra medical examination has been added to the boy ongoing follow-up. Incidentally, this non-intrusive approach bore witness to an instance of educational and medical care towards an autistic child in a developed country. Of note, the lack of coordination between actors may explain why the boy did not meet a *speech pathologist*, as recommended by a neurologist who measured progress in object recognition, which opened the way to object naming. An EEG allowed verifying the regression of epileptic signs, but no quantitative autistic test — such as ADOS [13] and ADI-R [14] — has been organized by the medico-educational environment unaware of the one-year study. However, a qualitative assessment of visual skills has been conducted by the aforementioned neurologist three months after the beginning of the SV treatment; results were compared with those of similar tests performed the year before. In her neuro-visual follow-up, this hospital practitioner reported that the boy stayed focused 45 minutes on four (among five) tasks respectively focused on: visual pursuit, fixation time, visuo-motor coordination, and pattern recognition. Despite weak dissociation between head and eye movements, a target could be followed in every spatial dimension, and eye motion often preceded gesture. Fixation time remained short (3 to 4 seconds), but the child intentionally searched the clinician's eyes, and stared at her for about 2–3 seconds. A good manual dexterity was observed, both quick and precise despite a trend not to keep sight on gesture. Couples of drawings of the same object could quickly be matched, and non-figurative patterns be retrieved among 5 to 6 distractors. The "visual scanning" last test was refused, likely because of both its complexity and the child tiredness. In conclusion, "*behavioral improvements cannot be denied. The boy is now capable of staying seated and focused during 45 min. He generally displays proactivity, except when defeated by a given task. Of note, his attention was attracted by the experimental equipment.*" [15].

After the case study, the boy evolution continued being recorded at IME where he is still welcome during school hours. Besides good night sleeps, he displayed awareness and sociability as a sound basis for reeducation, however still impeded by hyperactivity and bursts of aggressiveness. Two years after the beginning of the SV treatment, no toxic effect had been detected across periodic blood analyses. Despite the potential antagonism of SV with androgen/progesterone receptors, pubertal changes have arisen. At the behavioral level, no sign of regression occurred, and the boy made further progress in stages regarding autonomy, attention span and generalized ability to obey rules. Among poor habits, murmuring, teeth-grinding and occasional biting remained, although less frequently.

**Table 1.** Long-term case-study based on every criterion of autism. Shifts of behaviors were recorded over one year of daily treatment, including educational care for the last three months. DSM-5 criteria of autism are displayed in the left-hand column, supplemented here by: 'Architecture of Sleep', 'Attention', and 'Restlessness' (marked by *).

| Criteria of autism | Pre-treatment | Post-treatment | First shift of behavior |
|---|---|---|---|
| *Architecture of sleep | Disturbed and short sleep, less than 6 hours.<br>No REM observed during the first sleep cycle (sleep-EEG).<br>Frequent awakenings at night. | At least 9-hour sleep<br>Normal sleep architecture (only assessed on the first sleep-cycle)[1] | $t_0$+ 3 days : "good night sleep: 10pm-9am" (family)<br>$t_0$+ 8 months (m.): correct 1st sleep cycle, including REM (EEG): "Well-defined architecture of sleep" (clinician) [1] |
| | | **Social/Communication** | |
| *Attention, awareness | Very limited | More alertness.<br><br>Some ability to concentrate, however disturbed by more nervousness. | $t_0$+ 3 m.: 50 min of close attention at visual tasks (Neurologist)[2]<br>$t_0$+ 8 m. : quiet at the doctors (family)<br>$t_0$+ 9 m. Poor attention at sport (IME)<br>$t_0$+ 11 m. 90 min of full attention at the movie-theatre, watching a 3-D movie (but refusing 3-D glasses). |
| Reduced sharing of interest | Water-related plays (alone) | Plays with relatives.<br>Trend to join in other children activity. | $t_0$+ 7 m. Plays with dad before sleep<br>$t_0$+ 11 m. Spontaneously sings with peers (IME: Medical & Educational Institute) |
| Response to social request | Limited.<br>Moving around can be stopped; a word can be repeated on request, with a toneless voice. | Smiles, answers to easy questions with some prosody.<br>Can refuse to perform a task.<br>Takes account of reprimands. | $t_0$+ 9 m. Can reply to "Qu'est-ce que tu veux? "[What do you want?] (IME)[3]<br>$t_0$+ 9 m : Tooth brushing is more efficient; instructions are better followed when apparently understood (family). |
| Initiation of social interaction | No. | Beside new 'shyness', some trials to attract interest of relatives.<br>Voluntary easy request.<br>Shows that he identifies his relatives. | $t_0$ + 9 m. Trend to stay apart from a group of peers, or even hide himself (IME)[3]<br>$t_0$+ 10 m. "Bonjour Grand'père! "<br>$t_0$+ 12 m. Initiates a splashing play with kids at the swimming-pool (IME) |
| Facial expression | hardly | Sometimes: in-context expressions, usually overplayed. | $t_0$+ 2 m. Trials to draw attention through mimics (family)<br>$t_0$+ 11m. Burst of sadness: silent tears (IME)<br>$t_0$+ 11 m. imitates funny-faces (family) |

*Continued on next page*

| Criteria of autism | Pre-treatment | Post-treatment | First shift of behavior |
|---|---|---|---|
| Visual tracking | Sideways glance.<br><br>"deficit of the voluntary control of sight which may impact visual exploration and its coordination with movements" (Neurologist) | Significantly improved scanning of his surroundings and relatives.<br>Still a trend to follow moving objects with the peripheral vision.<br>The peripheral vision appears more functional than the foveal one. | $t_0$+ 1 m.: looking around as if discovering his environment and people (family)<br>$t_0$+ 3 m.: Eye movements anticipate gesture. 3-4 seconds of focus on a clinician (Neurologist)[2]<br>$t_0$+ 9 m.: capable of identifying and naming most fruits (IME)[3]<br>$t_0$+ 10 m. : capable of naming relatives from a set of pictures (family) |
| Adaptation to context | No autonomy.<br>Necessary guidance by parents. | Trend to adapt behavior to types of person (stranger, adult, child).<br>Adapt movements to obstacles.<br>Autonomy in some specific contexts (prior to sleep, at the bathroom). | $t_0$+ 6 m. : Shyness in front of strangers (family)<br>$t_0$+ 8 m. : Quieter at the Doctor (family)<br>$t_0$+ 9 m. : No apprehension in psychomotor tasks (IME)[3]<br>$t_0$+ 11 m.: came back home by himself during a walk. |
| **Restricted, repetitive behavior** | | $t_0$+ 3 m.: "behavioral improvements cannot be denied" (Neurologist)[2] | |
| *Restlessness | High level | Even higher hyperactivity by periods (fluctuating).<br>Appears to tire easily.<br><br>Less nervousness after an acupuncture session. | $t_0$+ 1 week: Drift of activity (family)<br>$t_0$+ 7 m. Restlessness that can be repressed through language (family)<br>$t_0$+ 9 m. Trend to escape and to ignore calls (IME)[3]<br>$t_0$+ 11 m. Calm after acupuncture session (family) |
| Stereotypies | Echolalia, hands-flapping. | Almost no echolalia. | $t_0$+ 10 m. Hands-flapping remains (family) |
| Impaired speech | Almost speechless: toneless voice, no syntax, no social use of speech. | Efforts with pronunciation and prosody.<br><br>A few spontaneous, constructed and 'on purpose' phrases. | $t_0$+ 4 m. "shh'iiiil te plait" [Ppp'leaaase]<br>$t_0$+ 10 m. Coming to his mother, tapping on her arm: "Je veux gateau." ["I want cake"] (family)<br>$t_0$+ 11 m. Spontaneous production of a non-rehearsed word ("Biscotte" [rusk])<br>$t_0$+ 12 m. "Let me think! " : reaction to an educator request. |
| Distress at changes | Tantrums | nd | No more tantrums |
| Perseverative interest | Playing with water, mud, can spend hours in the bathtub. | Less likely to act repetitively. | $t_0$+ 10 m. Still interested in water plays, extending to kids' books, videos and music (family) |
| Indifference to pain and temperature | Significant. | More sensitivity to cold. | $t_0$+ 11 m. Cannot stay in cold water (sea shore) as long as before (family) |
| Adverse response to noise | Adverse reaction to musical moods (screaming)… Knows how to switch-off the music player.<br>Escapes from family meetings. | More noise-resistant.<br>Interest for classical music. | $t_0$+ 2 m. Walks across a noisy family-meeting.<br>$t_0$+ 8 m. Expressive face when listening to music (Mozart, Jazz) with close intention up to 15 min (family) |
| Unusual sensory interest | Smelling<br>"Hyposensitivity" which impedes cognitive acquisitions (Neurologist) | Still smelling. Still a trend to put things in his mouth.<br>Calls for hugs. | $t_0$+ 9 m. Interest for all sensory input, especially touch (IME)<br>Attracted by pets (IME)[3] |

*Continued on next page*

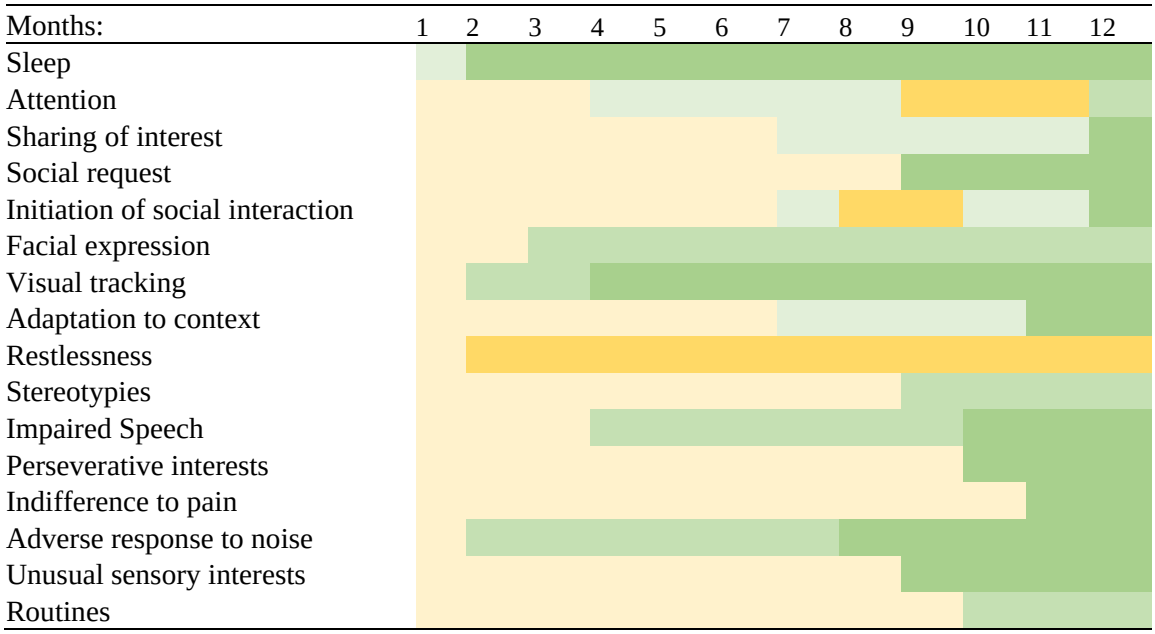
| Criteria of autism | Pre-treatment | Post-treatment | First shift of behavior |
|---|---|---|---|
| Routines | Recurrent habits (see the right-hand column), including water plays.<br><br>Self-injury. | Some routines have stopped but several poor habits remain. | $t_0$+ 10 m: (family)<br>Have become rare or have disappeared:<br>- Tantrums, hitting head<br>- Opening, leaning out of windows<br>- Throwing himself backward<br>- Objects dropping<br>Still remain occasionally:<br>- Murmuring, teeth-grinding<br>- irrelevant laughs<br>- biting, spitting<br>$t_0$+ 12 m: No more calloused skin at the wrist (previously caused by self-injury) |

[1] Vespignani H. (2015) Compte-Rendu d'EEG, 30/10/2015, *boy name*, Neurophy, 1 page in French.

[2] Renault D. (2015) Compte-rendu de suivi Neuro-visuel du 24/07/2015 de *boy name*, Unité Fonctionnelle Vision et Cognition, Fondation Ophtalmologique Adolphe de Rothschild (Hôpital Rothschild, Paris), 3 pages in French

[3] Hubert A., Auger S., Wirrmann S., Do Ceu L., Auberval C. (2016) Bilan de stage d'observation -18/02/2016 - *boy name*, Institut Médico-Educatif Le castel, Gazeran, 8 pages in French.

**Table 2.** Timeline of the evolution of autistics symptoms (left column) across 12 months (other columns), compiled by the author from parents' oral reports, formal reports written by physicians, as well as caregivers the last 3 months. The greener the box, the more positive the change. Worsening symptoms are displayed in yellow.




More than two years after SV medication, the pharmacological effects of the *Methylphenidate* (MPH) psychostimulant were found to possibly contribute to bring the pattern of monoamines closer to its balanced distribution by increasing both DA and Norepinephrine (NE) synaptic concentrations. In fact, whereas hyperactivity can actually be linked with depressed DA levels [16], the involvement of the NE monoamine is still questioned [17]. In any event, a long history of MPH prescription against children hyperactivity at school contributed to validate this complementary

solution, conditionally upon the compatibility with the basic SV drug, a requirement satisfied if the two molecules follow different metabolic pathways. So far, one knows that they work on different grounds: MPH inhibits NE and DA reuptake, while SV contributes to their breakdown. SV and MPH can therefore be prescribed together [18], provided that attention is paid to possible rapid adverse effects that have once been reported, namely *dyskinesia* and *bruxism* [19]. The MPH dosage was the lowest possible, namely 5 mg in the morning, resulting in a significant increase of the attention span (up to twenty minutes), and a quieter attitude. Because the usual higher dosage (5 mg three times a day) induced significant loss of appetite and weight, an identified side-effect of this molecule, the boy's parents decided to keep on a single uptake of 5 mg in the morning. After six months of this bi-therapy associating MAOA promotion and psycho-stimulation, the boy notably developed the non-autistic capacity to be sad in relevant situations (e.g., when missing a parent), as well as some ability to express his sadness on request ("Je suis triste": I am sad). He also showed autonomy in conducting sensorimotor tasks involving learnt rules (e.g., to come back home on his own, with streets to cross). Four years after the beginning of the SV prescription, his on-going spontaneous language remains rare, however. Surprisingly, he once took initiative of asking his father: "Je peux t'aider ?" (I can help you?). According to the report of a recent visit to a "vision and cognition" hospital service, the visual recognition of objects was confirmed accurate enough to consider pairing objects with elements of language under the supervision of a speech pathologist: An upcoming component of reeducation.

## 4. Discussion

### *4.1. The wide-spectrum effects of valproate*

High dosage of Valproate (up to 600 mg/kg) given to pregnant rodents elicits autistic traits in their offspring, and the latter are today considered as the standard "animal model" of ASD for experimental purpose [20]. Owing to this fact, that Valproate be presented as a condition-modifying treatment of severe ASD may sound paradoxical. It is undeniable, however, that a given foreign molecule is not passively assimilated by its host organisms, irrespective of their ongoing chemical signaling. Depending on both dosage and context of its intake, a given drug with known immediate benefits can induce various side effects, possibly opposite of the targeted one. To illustrate this point, while coffee is known to prevent headaches, it can also cause them, if either overconsumed or drunk by someone already dehydrated. With regard to the Valproate dosage against epilepsy, 30mg/kg is twenty times lower than what induces deficits in animal models. But this dosage-based explanation of SV paradoxical effects appears insufficient, since it happened that pregnant women treated with the usual low-dose of SV gave birth to children with autism. Indeed, exposure to valproate during pregnancy has actually been shown to increase the risk of ASD in the offspring [21]. Context-dependency seems a more relevant concept for addressing this question. Although a given drug elicits immediate changes in a living organism, these changes may be annulated so as to maintain the balance of critical functions. At the earliest gestational period of stem cells differentiation, the fetus epigenetic system is busy fixing the identity of each cell, to be kept life-long (e.g., a neuron should preferably never turn into a bone cell). Foreign molecules similar to inner epigenetic modulators are not expected to interfere then, and the MAOA expression is orchestrated once for all in order to generate the proper baseline level of synaptic monoamines. If a previously initiated MAOA

overstimulation stops once the "neuron score" is written, the MAOA baseline level is reduced accordingly. Only remains this permanent enzymatic deficit. But the situation is different outside of the timeframe within which a specific pattern of genes expression is assigned to the neuronal cell. If an autistic patient receives Valproate after the completion of this prenatal critical period, the genetic identity of their neurons cannot be edited anymore. Immediate effects of the drug prevail, including the stimulation of MAOA, assumed here to reinstate the correct level of monoamines that occurred in the early gestation, namely as long as both environmental MAOA overstimulation and consequent epigenetic regulations co-existed. However, apart from the favorable drug effect, other properties of Valproate are worth considering.

Following its approval as an anti-epileptic drug in 1967, Valproate has been used in a variety of pathologies, including *bipolar disorder* [22] and migraine headache; it is now investigated against carcinomas, and has recently been proposed as an agent capable of reopening developmental critical periods for learning purpose [23]. These various medical applications remain to be precisely set against a series of microbiological features. Among the latter, the promotion of MAOA is central in the study reported here, although barely mentioned in the scientific literature. One cannot rule out other biological properties, especially those concerning the brain excitability and plasticity, for their potential link to autism. With regard to excitability, Valproate is known to both raise brain levels of the inhibitory synaptic neurotransmitter GABA and impair neural conductivity (as a *sodium channel blocker*). The resulting shift of brain Excitatory-Inhibitory (E-I) balance has been proposed as a promising medical approach to autism [24], as well as in other pathologies showing over-activity. Rather than questioning the balance of two main brain circuits with opposite global effects, the approach followed here enhances the part of synaptic enzymes in regulating the brain development. It is also emphasized that, during sleep cycles, when some monoamines are switched off, abnormal brain over-excitability may only be counteracted by efficient enzymes. If not, development problems are assumed to occur. More than a global on-line excitability, these are the inner "spontaneous/contextual/predictive flows" of the computational model that undergo, offline, a lack of precision, hence faulty memory encoding [3]. While the "E-I imbalance" theory provides a systemic account of ASD core symptoms, the proposed "enzymatic imbalance" appears to bind molecular events to other characteristics which remained elusive so far, such as the sex ratio and the delayed onset of symptoms.

With respect to brain plasticity, an epigenetic action of Valproate, the inhibition of *Histone Deacetylase* (HDAC), has been observed to facilitate learning and memory in mice [25]. Being the first recorded HDAC inhibitor, VPA can thus be thought to reinstate adaptive processes in a brain undergoing developmental deficits. However, HDAC inhibition is a non-specific mechanism through which a large set of genes (*chromosome*) becomes prone to epigenetic modifications, owing to *chromatin remodeling*. A loosely packaged DNA induced by Valproate allows more specific effects, including the one targeted here towards the MAOA promoter [10].

The present theory relies on neurogenesis for new proper neural structures to result from reeducation, itself supported by enzymatic rebalancing. Recent studies however raise concern about the selective nature of neurogenesis under valproate modulation, favoring inhibitory (GABAergic) neurons [26] while causing the death (*apoptosis*) of stem cells that are fated to become excitatory neurons [27]. Unlike high phases of bipolar disorders which benefit from this shift towards global inhibition, the long-term reeducation of autistic individuals would rather require a well-balanced neurogenesis. Interestingly, the active genomic regions of a given cell type is supervised by different

HDAC families which participate in stem cells differentiation during the embryonic development, as well as adaptive responses in mature cells [28]. As a consequence, among HDAC inhibitor molecules capable of eliciting MAOA, some may favor a more balanced neurogenesis than Valproate, a perspective which deserves further investigation.

### *4.2. Relevant biomarkers*

Given pharmacological hypotheses supported here, the baseline level of brain MAOA should in principle have been assessed before starting a treatment assumed to promote this enzyme. An enzymatic deficiency could be expected, as recorded in other children with ASD [4,5]. A test kit was acquired for carrying out relevant measures, but left unused because "health and safety" conditions could not be met outside of an official framework. Measurement of the MAOA baseline level had also been planned to contribute to the Valproate dosage, except that the latter had first to comply with the antiepileptic prescription. In any case, this priority meant limited freedom to guide the medical protocol according to other factors, at that time. Hopefully, this limitation might be overcome in future investigations in which Valproate would officially be assessed for its autism-modifying potential. Furthermore, tools of genetic engineering would then be involved, notably for testing predictions of the underlying model, including the following one.

The MAOA gene is located on the X chromosome, which is one of the two sex chromosomes. In women (who have two X chromosomes), MAOA alterations, including epigenetic marks, must occur in both copies of the gene to be effective, which may explain why autism is less frequent in women than in men (who only have one X chromosome)… Less frequent, but not as unlikely as in diseases actually caused by a defective X chromosome (i.e., *hemophilia*, *Brunner syndrome*), which means that other masking mechanisms than the "double X" protection must be uncovered. Of note, the respective targets of two enzymes (MAOA and COMT) which degrade synaptic monoamines only partly overlap, which can be highlighted as a potential cause of discrepancy between the impact time of serotonin on the one hand, and that of dopamine and norepinephrine on the other hand. This is when both enzymes are brought into play that an imbalanced metabolism of monoamines may be revealed. Under the genetic polymorphism with lowest promotion of COMT, all monoamines likely stay about equally longer in the *synaptic cleft*, since catabolized at about the same rate. The MAOA-epigenetic mark would thus remain masked across human generations as long as the Low-COMT variant participates in genetic combinations. Related predictions concern the COMT variants of the parents, as a function of the gender of their autistic child (Table 3). When considering the distribution of COMT polymorphism in a western population [29], this hypothesis is consistent with the ASD sex-ratio currently recorded: four times more men than women. Accordingly, this rate increases for eastern people [30,31] who less likely own the Low-COMT variant, and gets slightly closer to the prevalence of diseases caused by a gene mutation located on the X chromosome. Among biomarkers to be tested in a complete clinical study, genetic polymorphisms influencing the metabolism of monoamines would contribute to confirm, modulate or refute the above assumption.

**Table 3.** Inheritance of epigenetic marks (under-promotion of the MAOA enzyme: "**MAOA-**") combined with variants of the COMT enzyme distributed over a given population [29]: Low (L) 28%; Medium (M) 50%; High (H) 22%. Whereas the mother's "**MAOA-**" carried by a X chromosome is masked by her second and safe X (and possibly by her **Low-COMT** variant), an autistic boy would inherit of his father's phenotype that permits a significant production of COMT, hence eliciting imbalanced metabolism between serotonin and other monoamines. More rarely, an autistic girl would inherit two chromosomes (X', X") which both convey the "**MAOA-**" epigenetic mark, combined with her mother's **COMT**, not low enough to protect her. According to this theory, the girl would tend to be a "healthy carrier" if inheriting the protective **Low-COMT** of her father.

| ***Mother*** | | ***Father*** | | |
|---|---|---|---|---|
| *Healthy carrier* *L+M* (78%) | **MAOA-** (**Low-**) **COMT** | *Non-carrier* *H+M* (72%) | (High-) **COMT** MAOA | **X** ***Boy*** |
| *Healthy carrier* *M* (50%) | **MAOA-** **COMT** Double-X protection | *Healthy carrier* *L* (28%) | **MAOA-** **Low-COMT** | **X'** **X"** ***Girl*** |

Note: Sex ratio = (L+M) * (H+M) / (M * L) = 78*72 / 50*28 = 4

From a broader perspective, the following biomarkers are deduced from this case-study and its background theory.

(1) Phenotyping of genes promoters responsible for the management of monoamines

Although linked here with persistent epigenetic regulations, the severity of autistic symptoms is evidenced to depend on allelic variants of specific genes promoters [32,33], with emphasis on the genes that monitor the production, transport and degradation of monoamines. Phenotyping is worth carrying out in parents, notably for assessing the Low-COMT masking hypothesis presented above. Contrary to other points of this tentative protocol, phenotyping is done once for all.

(2) Sleep EEG

ASD patients can start a SV treatment provided epileptic signs, visible in their sleep EEG. Given the key role of impaired sleep in the underlying GP model of autism, EEGs should provide patterns of the sleep architecture before the treatment, to be compared to those recorded at the beginning of the medication, and less frequently afterwards.

(3) Analysis of the eyes movements

If, as assumed by the GP model of autism, the completion of cross-connections between brain areas is impaired, interaction between parallel systems of perceptions is impacted, including between peripheral and central vision. This is why visual testing is regarded here as central in assessing the patient evolution.

(4) Blood test of Valproate

Following-up the SV blood rate is required to avoid potential harmful effects towards detoxification organs. Across the treatment, this biomarker must be kept below 95% of the top end of the recommended range in case of epilepsy (50–100 mg/kg), and far from the 150 mg/kg limit at which toxicity is known to occur.

(5) Levels of neuromodulators (5-HT, DA, NE), metabolizers (MAOA-B and COMT) and their metabolites

The treatment can be initiated and monitored with biomarkers of the neurotransmitters respective metabolisms, notably assessed in their urine metabolites (i.e., 5-HIAA, issued from serotonin degradation). Brain MAOA deficiency can for instance be reliably diagnosed by measuring the ratio of two specific amine metabolites in urine samples [34]. Considering the theoretical joint variation of '5-HT'-noise and impairment level [3], it would be relevant to try adapting the dosage of SV to the ongoing MAOA level of activity.

### *4.3. Situation among related case-studies*

To use the informatics terminology, medical approaches to ASD tend to focus on the chemical 'software' running on a neural 'hardware'. Implicitly, the neural matrix is assumed to incorporate well located and connected cells, either undergoing reversible damage (e.g., in *dendritic spines*), or tentatively unable to be properly activated. Hopefully, then, drug therapies are supposed to quickly improve these dysfunctions. Although this view may apply to mental diseases which start affecting the young adult, the picture is different for disorders accompanying the child development, since involving the growth of neural circuits that account for a unique range of life experiences. Context-dependent social conditionings develop across childhood and later on, allowing quick decisions to be made in situations previously encountered (being at the restaurant, taking turns, dealing with conflict…). This fully integrated skill is impaired in ASD. It would therefore be surprising if social deficits could be fully rescued by a brief pharmacological treatment, unless assuming that they have just been rendered either inaccessible or intrinsically ineffective for biochemical reasons. While the global brain architecture and peripheral perception are both likely to develop according to an inherited genetic program, the precise spatial targets of cells issued from neurogenesis rely instead on individual experiences, themselves mediated by both accurate perception and emotion assignment. Even mild forms of autism suffer from perceptual disturbance (i.e., noise management, face recognition) and difficulties with social cues. The more severe the case, the more difficult the acquisition of context-dependent rules supporting complex social skills. Thus, it is perhaps not by chance that molecules of potential interest are mostly tested on High-Functioning ASD, furthermore by implementing contrived social situations. In recent trials, *oxytocin* and *propranolol* have enhanced abilities that contribute to proper social behavior: single dose propranolol improved language at the behavioral level, while modulating the functional brain activity. Brain imaging permitted to display a changed "functional connectivity" between the resting-state network and cortical areas. Besides reducing stereotypies, the allocation of visual attention was improved. Oxytocin has been shown to

increase activity in brain regions involved in social skills, including *amygdala*, *medial prefrontal cortex* and *right anterior insula* [35]. Regarding clinical trials focused on Valproate, only its short-term impact have been explored in human, furthermore on peripheral symptoms of ASD, namely aggression [36] and irritability [37]. Not surprisingly, a meta-analysis of anti-epileptic drugs, including SV, led to the conclusion that such medications do not show a large effect size to treat behavioral symptoms [38].

Now leaving clinical investigations in human beings, the experimental approach in rodent models of autism applies to a shorter life (about 2% of the human longevity). A given experimental duration thereby corresponds to a much longer trial in human. Among 40 drugs tested on rodent models of autism since 2011 [1], Valproate improved the long-term recognition of novel objects, presumably by rescuing the *dendritic spine* density of *hippocampus* neurons [39]. In line with a question discussed above, Valproate was found to exhibit opposite long-term effects at both cellular and behavioral levels, whether absorbed before or after birth (shortly before the usual appearance of ASD-like visual recognition deficits, at 9 weeks of age). The drug dosage was similar to the anti-epileptic one in human (30 mg/kg/day), during 5 weeks and starting the 4$^{th}$ week. An equivalent test in human would have thus lasted about 5 years and be initiated at almost 4 years of age. This long-term chronic administration of valproate appears in line with the present case-study, consistently with a pathology that involves long-term modifications of the neural tissue. Nevertheless, the respective underlying hypotheses differ: the SV property assumed to be responsible for the mice behavioral improvement is there "HDAC inhibition", rather than also involving the stimulation of MAOA, here. Of note however, both interpretations are linked to epigenetic regulations. As a final example of the current research trend involving animal models of autism, a recent experiment concluded that social deficits were rescued for about 3 weeks after a brief treatment with a highly potent HDAC inhibitor [40]. Significant results have been obtained with a test of social preference in which the mice were considered as sociable when choosing to get closer to a chamber hosting another mouse rather than the alternative chamber containing a non-social item (piece of wood).

An interpretation of other experimental outcomes requires focusing on the 'offline' sessions during which input representations are internally replayed. This feature is salient for the GP implementation of learning, because the 'online' anticipation of environmental stimuli performed by any memory module is not compatible with the encoding, at the same time, of new events in this very module. Simulated 'offline' periods are therefore necessary for the system to periodically become free of vital ongoing anticipations. Offline, the growth and consolidation of memory paths may occur as soon as the "serotonin" parameter has decreased enough [3]. In the brain, clearing synapses after the shutdown of monoamines at every sleep phase relies on the effective action of relevant enzymes. If an enzymatic deficit can partly be compensated for by 'online' mechanisms (i.e., lower release of monoamines), such process cannot run 'offline', when the release of both 5-HT and NE is temporarily suspended. Sleep can thus be regarded as a fragile condition during which a low metabolism of 5-HT cannot be prevented. Whatever the inflow rate of 5-HT when its release is stopped across a sleep cycle, an enzymatic deficit may cause a "5-HT noise" that remains in the synapse. The following experimental data, seemingly inconsistent with the present model, may be addressed with this feature in mind: even a lowered 5-HT baseline does not prevent from 'offline' 5-HT noise. *Tryptophan* is a precursor of serotonin, and must be obtained from the diet. Trials of tryptophan-free diet causing acute 5-HT depletion have unexpectedly worsened autistic traits, including mood-related ones in a case study [19,41]. Serotonin depletion also led to a significant

increase of irrelevant repetitive behaviors in eleven among 17 patients, with higher tryptophan plasma level correlating with the autism severity and significant exacerbation of symptoms [20,42]. High level of tryptophan in the plasma can be elicited by its low transformation into serotonin, mediated by the isoform 2 of an enzyme (*Tryptophan hydroxylase*: TPH2). Indeed, TPH2 is the rate-limiting enzyme in the synthesis of serotonin and its gene showed significantly reduced expression levels in autism [43]. The release of 5-HT would thus be decreased in the autistic brain, as an 'online' reaction to its defective catabolism. The acute depletion of 5-HT income reported in [41,42] would exacerbate the underproduction of serotonin, and therefore generate symptoms usually associated with low 5-HT levels, such as irritability and depression. Regarding the worsening of core symptoms in a cohort of persons with ASD, they can also occur in computer simulations of the GP model with parameters set into the 'stimulus-driven' dysfunctional area coding for the lowest "serotonin" parameter [3]. If, as in GP networks, stereotypy originated in the activation of memory paths that mistakenly implement repetitions [7], this irrelevant behavior could not permanently be erased by any drug intake. Again, one may emphasize that the growth of well-formed paths is here supported by a disease-modifying molecule providing rebalanced neuromodulation, along with re-education aimed at lessening the reactivation of aberrant neural structures.

By contrast with the aforementioned couple of articles on 5-HT depletion, a recent case report appears consistent with the current study, a psychostimulant being there shown to supplement an enzyme-inducer. Indeed, a comparable combination of drugs is involved, except that several years of MPH intake precede there (rather than follow, here) the co-administration of an antiepileptic and enzyme-inducer (*Phenytoin*) [44]. The two treatments would be considered equivalent if, besides its effects on liver enzymes, Phenytoin were found to stimulate the production of MAOA, such as SV. If evidenced, this property would make these two case-studies fall into the same theoretical frame, suggesting at least two complementary types of drugs for dealing with core and peripheral symptoms of ASD.

### *4.4. Limitations and future prospects*

This case-study lacked access to relevant tools for monitoring biomarkers, especially beforehand, as well as over the first weeks during which eyes movements and sleep improved the most. However, this is only after having analyzed preliminary data over a long period, and having reported on every dimension of the condition that the relevance of specific biomarkers turned out to be essential. Shortly after the beginning of the treatment, onsets of restored sleep and visual exploration did not happen by chance. A placebo effect was unlikely in a level-3 autistic boy, already used to take food supplements and pills. The first months of treatment have also revealed daytime drops of hyperactivity oriented toward ongoing poor habits (e.g., murmuring, teeth-grinding), at the time new context-dependent behaviors had not yet been acquired. As a matter of fact, most changes regarding high-level skills such as seeds of spoken language and social relationship became noticeable after the ninth month of daily medication, shortly before educational care handled at IME. Thirty-two months after the treatment initiation, *Methylphenidate* at low dose was able to compensate for the over-metabolism of both DA and NE, improving attention and quietness without interfering with the basic drug.

Concerning future prospects, other molecules than SV should be investigated, not only as alternative treatments, but also for the protection of gestation. This concerns molecules that are

capable of stimulating the genetic promotion of MAOA, including *forskolin* [45], notably used as weight loss supplement. Small *fatty acids* share molecular features with VPA [46], namely *nonanoic* (*pelargonic*) and *decanoic* (*capric*) acids. Described as "natural" given their organic origin (pelargonium, coconut, palm oil), these molecules have been widely introduced in our environment through the food market, cosmetics, cleaning products and agrochemicals. Although likely able to replace SV as life-long autism modifiers, the risk these products represent for pregnancy should be put forward, mainly due to their capacity to cross the fetal *Blood Brain Barrier*. One may still believe that our environment contains negligible quantities of such MAOA inducers, with a dose-dependent effect. The amount of small fatty acids in food could thus be considered ineffective if maintained far from the usual valproate dosage. The following easy calculation questions this belief. Food concentrations of nonanoic acid, notably used as antifungal and antimicrobial in fruit juices and dairy products, range from 10 to 10,000 ppm [47]. Given that one ppm = 1 mg/kg, this rather wide range corresponds to up to 10 g of additive for 1 kg of food. For this strongest dosage, only 100 g of food contains 1 g of additive. For a woman weighting 60 kg, the consumption of food containing this additive corresponds to 1000 mg/60 kg, that is 16 mg/kg, a value to be compared to the SV posology which starts at 15mg/kg/day.

If an acute excess of MAOA in the early gestation were confirmed as a cause of postponed development troubles in the toddler, recommendations would be worth expressed towards women who plan to bear a child. Fortunately, the prescription of Valproate is now under medical control regarding pregnancy. Given their chemical similarities to Valproate, the aforementioned marketed molecules would deserve supplementary assessment and consequent regulation. The connection between MAOA and nicotine brings another reason to either fully avoid smoking or to stop long enough before gestation, since withdrawal from heavy smoking likely generates depressed mood associated with increased enzymatic level [48]. More generally, acute depression has been linked with excess of MAOA [49]. The latter is also associated with the resolution of inflammation in macrophage cells, those involved in host defense and immunity [50], which may be why about 10% of autistic cases are attributed to severe infections occurring early across gestation, namely rubella, mumps and measles. With respect to vaccination of the young child, sometimes noticed to precede the emergence of symptoms in regressive forms of autism, its implication in ASD has clearly been refuted, notably through the recent analysis of data from more than 650 thousands Danish children [51]. According to the present underlying theory, gradual maturation of MAOB in fetal brain might account for the late onset of ASD symptoms, instead [3]. Last environmental factor but not least, exposure of pregnant women and infant to ambient pesticides has experimentally been shown to cause neurodevelopmental impairment, an outcome which also resulted from a recent epidemiological study focused on a large population of individuals with ASD [52]. In connection with the "enzymatic disruption" hypothesis developed here, further research could focus on how monoamines metabolic pathways can be influenced by each type of pesticide.

## 5. Conclusions

This article has presented an exploratory monitoring of disease-modifying treatment based on a new theory of autism. Given the heritability of epigenetic traits with an environmental origin, the classical distinction between genetic and environmental factors of autism may rather apply to "built-in genetic" and "reversible epigenetic" features, both being heritable. The possibility that no

significant genetic mutation be involved is a reassuring hypothesis, since it means that cessation of environmental causes would break the disease progression, owing to the gradual extinction of epigenetic alterations after several generations. However, the same approach suggests a hidden part in current assessments of autism rate. In addition to recorded cases of overt-autism, several healthy carriers of epigenetic alteration (i.e., "**MAOA–**") are likely to increase the ASD prevalence in future generations, conditionally upon mating with someone conveying specific genetic variants (i.e., Medium to High-COMT).

Given the variety of cases that build upon autism disorders, promising results obtained in a single case-study must be put into perspective. The background theory even suggests that persons with high-functioning autism would get more disadvantage than benefit from the proposed bi-therapy, since the latter cannot only target affected brain structures. More precisely, the fewer the structures hosting the imbalanced neuromodulation metabolism in question, the wider the depressing trend predicted on unaffected circuits [3].

Despite the present focus on pharmacological rather than educational aspects, it would be useless to improve learning capacities thanks to drugs without properly feeding memory and favoring social interactions through a reeducation program. Even worse in absence of educational drive: Irrelevant and repetitive behaviors are likely more consolidated than without the treatment. Because the actual target of this study was ignored by medico-social participants, dialogue and consultations could not be conducted among all parties concerned. Future official investigations will require close coordination between the family and medical practitioners, educators, teachers and researchers, not to mention the autistic patients themselves, as soon as their treatment hopefully provides them with enough self-sufficiency.

The last words of this article bring the focus back to the boy who was the main actor of this study. For someone now interested in his pairs, attending school with a teaching assistant would be more than relevant, provided an inclusive education trend. But considering the uncertainties linked to the evolution of educational care, as well as the continuation of the current medical treatment—or availability of alternative drugs—, how much the boy development delays may still be remedied can hardly be anticipated today.

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## Conflict of interest

The author declares no conflicts of interest in this paper.

## References

1. Kuo HY, Liu FC (2018) Molecular pathology and pharmacological treatment of autism spectrum disorder-like phenotypes using rodent models. *Front Cell Neurosci* 12: 422.

2. Uzunova1 G, Stefano PS, Hollander E (2016) Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics. *World J Biol Psychiatry* 17: 174–186.
3. Béroule DG (2018) Offline encoding impaired by epigenetic regulations of monoamines in the guided propagation model of autism. *BMC Neurosci* 19: 80.
4. Essa MM, Al-Sharbati MM, Al-Farsi YM, et al. (2011) Altered activities of monoamine oxidase A in Omani Autistic children—a brief report. *Int J Biolog Med Res* 2: 811–813.
5. Chauhan V, Gu F, Chauhan A (2016) Impaired activity of monoamine oxidase A in the brain of children with autism. Conference Abstract: 14th Meeting of the Asian-Pacific Society for Neurochemistry.
6. Hensler JG, Artigas F, Bortolozzi A, et al. (2013) Catecholamine/serotonin interactions: Systems thinking for brain function and disease. *Adv Pharmacol* 68: 167–197.
7. Béroule DG, Encoding of memory across online/offline alternations, screencast of running computer simulation, 2016. Available from: https://perso.limsi.fr/domi/Movie-S1_DGB_nov16.mov.
8. Alwinesh MTJ, Joseph RBJ, Daniel A, et al. (2012) Psychometrics and utility of psycho educational profile-revised as a developmental quotient measure among children with the dual disability of intellectual disability and autism. *J Intellect Disab* 16: 193–203.
9. Baer DM, Wolf MM, Risley TR (1968) Some current dimensions of applied behavior analysis. *J Appl Behav Anal* 1: 91–97.
10. Wu JB, Shih JC (2011) Valproic acid induces monoamine oxidase A via Akt/Forkhead Box O1 activation. *Mol Pharmacol* 80: 714–723.
11. Whitton PS, Oreskovic D, Jernej B, et al. (1985) Effect of valproic acid on 5-hydroxytryptamine turnover in mouse brain. *J Pharm Pharmacol* 37: 199–200.
12. Treatment of Children With Autism Spectrum Disorders and Epileptiform EEG With Divalproex Sodium. Available from: https://clinicaltrials.gov/ct2/show/NCT02094651.
13. Lord C, Rutter M, Goode S, et al. (1989) Autism diagnostic observation schedule: A standardized observation of communicative and social behaviour. *J Autism Dev Disord* 19: 185–212.
14. Lord C, Rutter M, Le Couteur A (1994) Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 24: 659–685.
15. Renault D (2015) Compte-rendu de suivi Neuro-visuel du 24/07/2015, Unité Fonctionnelle Vision et Cognition, Fondation Ophtalmologique Adolphe de Rothschild (Hôpital Rothschild, Paris), 3 pages in French.
16. del Campo N, Chamberlain SR, Sahakian BJ, et al. (2011) The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biol Psychatry* 69: 145–157.
17. Vanicek T, Spies M, Rami-Mark C, et al. (2014) The norepinephrine transporter in attention-deficit/hyperactivity disorder investigated with positron emission tomography. *JAMA Psychiatry* 71: 1340–1349.
18. Santos K, Palmini A, Radziuk AL, et al. (2013) The impact of methylphenidate on seizure frequency and severity in children with attention-deficit-hyperactivity disorder and difficult-to-treat epilepsies. *Dev Med Child Neurol* 55: 654–660.

19. Gara L, Roberts W (2000) Adverse response to methylphenidate in combination with valproic acid. *J Child Adol Psychop* 10: 39–43.
20. Nicolini C, Fahnestock M (2018) The valproic acid-induced rodent model of autism. *Exp Neurol* 299: 217–227.
21. Christensen J, Grønborg TK, Sørensen MJ, et al. (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA* 309: 1696–1703.
22. Chateauvieux S, Morceau F, Dicato M, et al. (2010) Molecular and therapeutic potential and toxicity of valproic acid. *J Biomed Biotechnol* 2010: 479364.
23. Gervain J, Vines BW, Chen LM, et al. (2013) Valproate reopens critical-period learning of absolute pitch. *Front Syst Neurosci* 7: 102.
24. Ajram LA, Horder J, Mendez MA, et al. (2017) Shifting brain inhibitory balance and connectivity of the prefrontal cortex of adults with autism spectrum disorder. *Transl Psychiatry* 7: e1137.
25. Ganai SA, Ramadoss M, Mahadevan V (2016) Histone Deacetylase (HDAC) Inhibitors-emerging roles in neuronal memory, learning, synaptic plasticity and neural regeneration. *Curr Neuropharmacol* 14: 55–71.
26. Fujiki R, Sato A, Fujitani M, et al. (2013) A proapoptotic effect of valproic acid on progenitors of embryonic stem cell-derived glutamatergic neurons. *Cell Death Dis* 4: e677.
27. Laeng P, Pitts RL, Lemire AL, et al. (2004) The mood stabilizer valproic acid stimulates GABA neurogenesis from rat forebrain stem cells. *J Neurochem* 91: 238–251.
28. Giorgio ED, Brancolini C (2016) Regulation of class IIa HDAC activities: It is not only matter of subcellular localization. *Epigenomics* 8: 251–269.
29. Al-Asmary S, Kadasah S, Arfin M, et al. (2014) Genetic association of catechol-O-methyltransferase val (158) met polymorphism in Saudi schizophrenia patients. *Genet Mol Res* 13: 3079–3088.
30. Le TV, Chu TTQ, Le BN, et al. (2019) Prevalence of autism spectrum disorders and their relation to selected socio-demographic factors among children aged 18–30 months in northern Vietnam, 2017. *Int J Ment Health Syst* 13: 29.
31. Lai DC, Tseng YC, Hou YM, et al. (2012) Gender and geographic differences in the prevalence of autism spectrum disorders in children: analysis of data from the national disability registry of Taiwan. *Res Dev Disabil* 33: 909–915.
32. Cohen IL, Liu X, Schutz C, et al. (2003) Association of autism severity with a monoamine oxidase: A functional polymorphism. *Clin Genet* 64: 190–197.
33. Hranilović D, Novak R, Babić M, et al. (2008) Hyperserotonemia in autism: The potential role of 5HT-related gene variants. *Coll Antropol* 32: 75–80.
34. Abeling NG, van Gennip AH, van Cruchten AG, et al. (1998) Monoamine oxidase A deficiency: Biogenic amine metabolites in random urine samples. *J Neural Transm* 52: 9–15.
35. Frye RE (2018) Social skills deficits in autism spectrum disorder: Potential biological origins and progress in developing therapeutic agents. *CNS Drugs* 32: 713–734.
36. Hellings JA, Weckbaugh M, Nickel EJ, et al. (2005) A double-blind, placebo-controlled study of valproate for aggression in youth with pervasive developmental disorders. *J Child Adol Psychop* 15: 682–692.

37. Hollander E, Chaplin W, Soorya L, et al. (2010) Divalproex sodium vs placebo for the treatment of irritability in children and adolescents with autism spectrum disorders. Neuropsychopharmacology 35: 990–998.
38. Hirota T, Veenstra-Vanderweele J, Hollander E, et al. (2014) Antiepileptic medications in autism spectrum disorder: A systematic review and meta-analysis. *J Autism Dev Disord* 44: 948–957.
39. Takuma K, Hara Y, Kataoka S, et al. (2014) Chronic treatment with valproic acid or sodium butyrate attenuates novel object recognition deficits and hippocampal dentritic spine loss in a mouse model of autism. *Parmacol Biochem Behav* 126: 43–49.
40. Qin L, Ma K, Wang ZJ, et al. (2018) Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition. *Nat Neurosci* 21: 564–575.
41. McDougle CJ, Naylor ST, Goodman WK et al. (1993) Acute tryptophan depletion in autistic disorder: A controlled case study. *Biol Psychatry* 33: 547–550.
42. McDougle C, Naylor ST, Cohen DJ, et al. (1996) Effects of tryptophan depletion in drug-free adults with autistic disorder. *Arch Gen Psychiatry* 53: 993–1000.
43. Boccuto L, Chen CF, Pittman AR, et al. (2013) Decreased tryptophan metabolism in patients with autism spectrum disorders. *Mol Autism* 4: 16.
44. Bird PD (2015) The treatment of autism with low-dose phenytoin: A case report. *J Med Case Rep* 9: 8.
45. Gupta V, Khan AA, Sasi BK, et al. (2015) Molecular Mechanism of monoamine oxidase A gene regulation under inflammation and ischemia-like conditions: Key roles of the transcriptions Factors GATA2, Sp1 and TBP. *J Neurochem* 134: 21–38.
46. Minkiewicz P, Darewicz M, Iwaniak A, et al. (2016) Internet databases of the properties, enzymatic reactions, and metabolism of small molecules—search options and applications in food science. *Int J Mol Sci Dec* 17: 2039.
47. Koenraad PM, Braber AF (2016) Use of nonanoic acid as an antimicrobial agent, in particular an antifungal agent, patent A61Q17/005 (Antimicrobial preparations), 1999. Available from: https://patents.google.com/patent/WO2001032020A2/en.
48. Meyer JH, Ginovart N, Boovariwala A, et al. (2006) Elevated monoamine oxidase A levels in the brain: An explanation for the monoamine imbalance of major depression. *Arch Gen Psychiatry* 63: 1209–1216.
49. Bacher I, Houle S, Xu X, et al. (2011) Monoamine oxidase A binding in the prefrontal and anterior cingulate cortices during acute withdrawal from heavy cigarette smoking. *Arch Gen Psychiatry* 68: 817–826.
50. Cathcart MC, Bhattacharjee A (2014) Monoamine oxidase A (MAO-A): A signature marker of alternatively activated monocytes/macrophages. *Inflamm Cell Signal* 1: e161.
51. Hviid A, Hansen JV, Frisch M, et al. (2019) Measles, mumps, rubella vaccination and autism: A nationwide cohort study. *Ann Intern Med* 170: 513–520.
52. von Ehrenstein OS, Ling C, Cui X, et al. (2019) Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: population based case-control study. *BMJ* 364: l962.

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