Comment on “Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring”

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Tyzio et al. (Reports, 7 February 2014, p. 675) reported that bumetanide restored the impaired oxytocin-mediated γ-aminobutyric acid (GABA) excitatory-inhibitory shift during delivery in animal models of autism, ameliorating some autistic-like characteristics in the offspring. However, standard practices in the study of these models, such as the use of sex-dimorphic or males-only analyses and implementation of tests measuring social behavior, are lacking to definitely associate their findings to autism.

Autism spectrum disorders (ASD) are a group of neurodevelopmental disabilities characterized by sociability impairments, communication deficits, and stereotyped behavioral patterns (1). The etiology of autism is still not known, and current treatment options provide only a mild relief to some aspects of this condition. Nevertheless, there is mounting evidence that an excitation/inhibition imbalance plays a crucial role in the pathology of ASD (2). Tyzio et al. (3) recently reported that bumetanide maternal pretreatment was able to restore physiological levels of intracellular chloride in CA3 hippocampal neurons in two different animal models of autism: rats prenatally exposed to valproate (VPA) and Fmr1 knock-out mice (FRX). As a consequence, excitatory actions of γ-aminobutyric acid–mediated (GABAergic) signaling were reduced and electroencephalographic patterns were normalized. In addition, bumetanide treatment of pregnant females reversed aberrant maternal-separation–induced ultrasonic vocalization in the offspring. The authors also stressed the importance of oxytocin in the developmental GABA switch from excitatory to inhibitory, because prenatal treatment with the oxytocin receptor antagonist SSR126768A in naïve animals triggered alterations similar to those observed in VPA rats and FRX mice. The use of both genetic and drug-induced autism models strengthen their discovery, which is also supported by their previous finding of bumetanide efficacy in a clinical trial in children with autism (4). However, we strongly feel that some technical issues of their work remained unaddressed and that the final conclusions of this work would be greatly improved by resolving those issues.

One of the most relevant features of autism is the difficulty to establish and maintain reciprocal social interactions with peers. These disabilities in social skills are mimicked in both VPA rats (5) and FRX mice (6) and can be detected by behavioral testing. Tyzio et al. did not evaluate social parameters in the rodents used in their work. We understand that, in principle, impaired ultrasonic vocalizations (USV) can lead to sociability deficits, but this has not been proved yet, and changes in USV in pups may be modulated by several factors not related to social domain—for example, temperature, sensitivity to pain, or general distress (7). Because social behavior requires the integration of a wide variety of neural circuits related to diverse aspects of sensory and cognitive functions (8), they have to be studied in much more elaborate ways—for example, using reciprocal social interaction or social preference tests, possibly in combination with USV recording. We believe that analyses of social behavior and USV emitted during social tasks are necessary to improve the quality and confidence of their data.

It is also important to verify whether the effects of bumetanide and SSR126768A persist over time. To clarify whether those are temporary or long-lasting modifications, the authors should extend the evaluation of the behavioral and electrophysiological phenotypes into different developmental stages, including adulthood and, possibly, the end of the first postnatal week as a rough equivalent of birth-stage development in humans (9).

Another factor that needs to be taken into consideration to fully understand the relevance of Tyzio et al.’s results for autism is the use of both male and female rodents in all experiments. The higher prevalence of ASD in males is very well established, and some animal models of autism also show this male bias. The VPA animal model of autism has demonstrated solid evidence of sex-specific behavioral and morphological outcomes. The VPA rats were extensively analyzed, and most of the autistic-like features, including sociability deficits, were not detected in females (10, 11). In fact, VPA female rats are sometimes used in comparison to males to determine gender-specific alterations, which are likely to be more relevant to autism pathophysiology (12, 13). In addition, there is no complete characterization of autistic-like features in female FRX mice, because the vast majority of researchers use only males in their experiments (13–15). Thus, the authors should consider sex, including in embryos, as a factor in their data analysis, and therefore increase the number of animals per sex per group. Without taking this into account, the results are affected by the possibility of misbalanced numbers of males and females per group.

The concepts analyzed by Tyzio et al. have an enormous potential to help the development of future studies and can represent a turning point in the research of the etiology of ASD. However, giving the topic’s prominence and impact, we believe that our suggestions are important to clarify key aspects discussed by them. It is still premature to think of bumetanide as a prenatal intervention for ASD, but it can be regarded as a meaningful research tool of the molecular underpinnings of this condition.

REFERENCES
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