Review

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Review

The Domestic Chick as an Animal Model of Autism Spectrum Disorder (ASD); Developmental Homology Gives Validity

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Abstract: Equipped with an early social predisposition immediately post-birth, humans typically form associations with mothers and other family members through exposure learning, canalized by a spontaneous predisposition to biological motion, face configuration, and other cues of animacy. If impaired, reduced social preferences can lead to social interaction impairments such as autism spectrum disorder (ASD) via misguided canalization. Despite being taxonomically distant, domestic chicks also follow a homologous developmental trajectory toward adaptive socialization through imprinting, which is guided via predisposed preferences that are homologous to those of humans, thereby suggesting that chicks are valid animal models of ASD. In addition to the convergent similarities in predisposition with human newborns, accumulating evidence suggests the construct validity of the chick model. Considering the recent progress in the evolutionary neurobiology of vertebrates, we reviewed the advantages and limitations of the chick model of developmental mental diseases in humans.

Keywords: autism spectrum disorder; chick; valproic acid; nicotinic acetylcholine receptors; biological motion; face; animacy; thyroid hormone; bumetanide

1. In search of valid animal models of developmental psychiatric disorders

Biological psychiatry and comparative psychology share the common scientific issue of neurocognitive homology among animals under study. Since the Cambrian period, the nervous system of vertebrates has held a highly conserved "Bauplan" [1–3]. Accordingly, homologies are widely found from the level of composite molecules (transmitters, hormones, receptors, and second messengers) to neural pathways and cytoarchitectonic organizations at the macroscopic level [4,5] (specifically for the mammal-bird homologies [5]). Despite its conservative nature, huge phenotypic diversification occurs in the behavioral and cognitive aspects of vertebrates through differentiated development [6], complicating the untangling of homology issues.

In the present review, encouraged by recent progresses of avian model studies [7,8], we argue that chicks and humans exhibit an evolutionary convergence in neurobehavioral traits and examine the validity of domestic chicks as autism spectrum disorder (ASD) animal models on the surface and construct validity (as Willner [9] proposed for the depression model). The former (surface validity) includes an examination of the similarity of behaviors and developmental processes, particularly the perceptual predisposition to faces and biological motion. The latter (construct validity) includes shared neural substrates and molecular cascades, particularly the potential roles of nicotinic acetylcholine (nAChR) transmission in the fetal brain in controlling excitation-inhibition balance in neonates.

2. Diverse environmental risk factors of ASD remain to be specified

ASD is the most prevalent developmental disorder primarily characterized by underdeveloped social interactions and communication [10,11]. It is speculated that the heterogeneous diagnostic

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phenotypes, as well as the dimensional nature of this disorder, could be associated with a wide range of underlying genetic and environmental factors [12]. In addition to apparent genetic risks [13] (for an exhaustive hereditability study in Sweden); also see [14] for de novo mutations and [15] for common variations), exposure to environmental toxicants during pregnancy and the neonatal period remains a major social and scientific concern [16]. For example, a large-scale twin study [17] revealed high concordance rates among siblings, indicative of the role of genetic factors; however, the authors also reported that common environmental factors shared by these twins could substantially contribute to autism/ASD liability. Complex interactions are thought to occur between the genetic background of ASD susceptibility and the chemical agents acting during pregnancy and the early post-natal period.

However, this hypothesis does not imply that these chemicals need to be eliminated. For example, valproic acid (VPA) has been identified as a risk factor for ASD (see below for details); however, it remains an indispensable antiepileptic medication [18]. The associated risks must be precisely evaluated in close consideration with known benefits. But, a lack of reliable measures often obscures environmental risk management. Studies using appropriate animal models are critical [19]. Rodents (mice and rats) are the most popular model animals [20,21] but cannot be deemed identical to humans. Rodents and primates have undergone distinct evolution since their separation during the late Cretaceous period over 65 million years ago.

As an alternative animal model, we have focused on newly hatched domesticated chicks (*Gallus gallus domesticus*) [22–25]. Birds are descendants of theropod dinosaurs, the major group of sauropsids, whereas primitive mammals diversified as a minor group of synapsids during the Carboniferous Period, ~ 300 million years ago. Considering such a taxonomically distinct animal as a valid model for human psychiatric disorders may sound unrealistic. If the adult phenotypes are solely compared, humans can never be chickens. However, as the hourglass bottleneck theory suggests [26], humans and chickens achieve developmental convergence during the prenatal/early neonatal periods in terms of specific neurocognitive aspects (Figure 1). Determining whether certain animals are human-like demands the elucidation of how phenotypic similarities arise through their respective ontogenies.

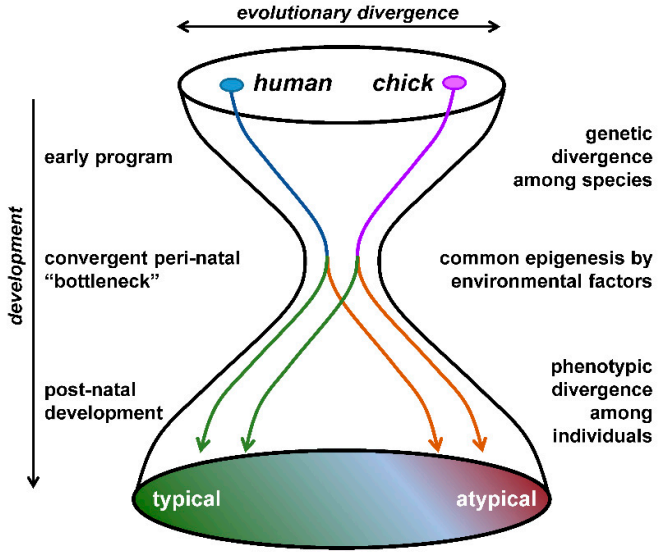

Figure 1. Hourglass "bottleneck" model of the convergent evolution of peri-natal epigenetic control of social behaviors.

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3. Visual predispositions canalize the development of social behaviors: common developmental features for the surface validity.

It is speculated that the development of socialized behaviors during the early neonatal period depends on a predisposed visual preference. Human babies exhibit biased visual attention, which subsequently forms social attachments with siblings and parents. The predisposed "Conspec" [27] or "core knowledge" [28] could include a variety of visual features (for reviews see [29–34]), however, most studies have focused on face configuration, biological motion, and other cues of animacy.

Face Differential tuning to face configuration has been demonstrated in adolescents with ASD when compared with typically developing controls (for one of the earliest reviews, see [35,36]; also see [37] for a recent study using pictures resembling the food-face paintings by *Giuseppe Arcimboldo*). Based on the finding that infants prefer faces at 2–4 months old [38,39], a predisposed face preference has been suggested as a reliable diagnostic indicator for ASD. Typically, a definitive ASD diagnosis can be achieved in toddlers aged ~3 years; reliable biomarkers at earlier ages, if available, could facilitate treatment at the early stages [40]. In addition to behavioral measures, physiological measures, such as electroencephalography (EEG), can be employed in newborn babies [41].

Based on data from brain studies, it can be suggested that the subcortical visual pathway (superior colliculus/pulvinar to the amygdala) is responsible for the proto-face configuration (three blobs configured at a low spatial frequency) in human neonates [36]; see [42] for the most reliable account of this pathway in rhesus monkeys. Visually naïve monkeys deprived of social stimuli (including human faces) preferred faces [43], indicating robust development of face perception. Even human fetuses in the third trimester of pregnancy reportedly exhibit head-turn responses to upright face configurations [44]. Consistently, damage to the amygdala has been shown to cause some ASD-like deficiencies according to the "theory of minds" (ToM [45]), although the causal link between the ToM and ASD remains controversial [46].

Considering that the subcortical face pathway is functional in the early prenatal period and critical for the typical development of social behavior [47], we would expect to detect differentiated visual attention to the face in infants with familial risk of ASD and more specifically, in those who are later diagnosed with ASD. However, the use of the "face pop-up" task in these infants revealed clear attention to face similar to that observed in control individuals (or even higher) at 7 and 14 months of age [48]. Conversely, another longitudinal developmental study has reported a steady decline in selective eye-fixating behavior in infants who were subsequently diagnosed with ASD; the finding was observed in typically developed individuals [49]. Therefore, developmental changes need to be carefully examined [50]. More recently, it has been reported that newborns with a high familial risk of ASD (6–10 days of age) show a reduced face preference when compared with low-risk controls [51]; also see [52] for the follow-up longitudinal study on 4-month-old infants).

In addition, visually naïve, newly hatched chicks exhibit an evident inborn preference for faces [53–55]. Fetal exposure to VPA substantially reduced the face preference score [56], possibly paralleling that observed in humans. The subcortical visual pathway of birds (optic tectum and arcopallium/nucleus taeniae amygdala) is known to functionally mature early, and newly hatched chicks start actively pecking small conspicuous objects at 1–2 days old. Furthermore, immediate early gene imaging studies have revealed that telencephalic limbic nuclei are involved in the predisposition preference of chicks [57–59]. Moreover, traditional rodent models fail to spontaneously exhibit a comparable visual predisposition.

Biological motion Biological motion (BM) preference comprises another aspect of the "Conspec" process; it is easily tested using highly reduced animations composed of relatively few light points (~ a dozen). Both human and chick neonates exhibit BM preference without visual experience ([60,61] in human infants; [62] in chicks; also see [63] for the association of the BM preference with brain asymmetry). In addition to the commonality in their appearance at the early neonatal stage, both human infants and chicks show a clear inversion effect, that is, a preference for the upright walking motion over the inverted upside-down display ([64] in human infants; [65] in chicks).

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The motion characteristics of both local movements (such as the movements of the lower limbs) and global features (shape of the body) are critical in humans [66,67]. However, it remains unclear which of these visual components is associated with the development of social cognition. [68] reported that infants depend more on local cues, whereas [69] highlighted the importance of translational displacement of the body. [70] have proposed an integrative hypothesis of the two-process theory, wherein the "step detector" responsible for the local motions of feet below the body precedes the "bodily action evaluator" that processes the global processing of action types and styles. Several studies in both human adults and children have suggested the association between reduced sensitivity to BM and ASD [71–74] (also see the recent meta-analysis [75]). Importantly, it should be noted that, in our current context, visual preferences for social stimuli (face inversion, averted eye gaze, and BM) markedly differ between the two groups of infants with high and low familial risk of autism [51]. Further longitudinal studies are needed to determine the association between visual preference and the subsequent development of social interactions.

Animacy In addition to the face and BM, both chicks and humans have a visual predisposition to other cues of animacy ([25,76,77] for reviews). For example, living organisms are characterized by self-propelling animacy, a well-established preference in both human babies [78] and visually naïve chicks [79,80]. Unpredictable speed changes in motion are a critical feature of animacy in newborn human babies [81], as observed in newly hatched chicks with distinct inherited variability [82,83] (for involvement of septal and hypothalamic nuclei, see [84]). Both variability in body orientation and the unpredictable temporal contingency of motion are critical in chicks [85,86]. It should also be noted that avoidance of looking at (threatening) objects is also considered to be innately predisposed [87].

4. Imprinting and the early process of attachment formation

In chicks, the BM preference is functionally linked to filial imprinting. Imprinting is a complex process that involves predisposition and experience-based learning; thus, it may be homologous to the processes of attachment formation in human babies. Newly hatched domestic chicks and ducklings form lasting attachments, even when the first object seen is a non-biological artifact rather than a conspecific animal [88,89]. Artifacts such as rotating blue boxes were actually effective as imprinting objects [90]. However, contrary to this popularly accepted idea, memorized preference for artifacts is short-lived and is gradually replaced by more naturalistic stimuli such as stuffed hens [91–93]. Accordingly, an innate predisposition gradually emerges after learned attachment fades. In contrast, BM preference emerges first and subsequently guides learning.

Perfectly naïve chicks show an apparent preference for BM, although with a relatively small effect size [62]. When imprinted by motion pictures, the BM preference is enhanced or "permissively induced" [94]. Induction is nonspecific to the exposed stimulus, and any motion (even randomized point-light animation) is a similarly effective inducer. Furthermore, the BM preference facilitates imprinting [95]. BM animations were more effective than non-BM animations, and chicks with a higher BM preference exhibited higher imprinting scores.

Imprinting memory is coupled with BM induction through enhanced thyroid hormone activity. Exposure to motion increases the expression of *Dio2*, which is responsible for the conversion of circulating thyroid hormone (T_4) to its active form (T_3) in the epithelial cells of telencephalic capillaries [96]. The enhanced T_3 influx into the dorsal pallium (intermediate medial mesopallium, IMM, an avian homolog of the mammalian neocortex, including the association areas) reopens the sensitive period and acutely strengthens learning and BM scores [97,98]. In aged chicks, T_3 can reactivate the preference for animate objects [99]. Accordingly, imprinting allows chicks to remain imprintable for a prolonged period, guiding subsequent learning during the extended sensitive period to objects bearing BM features. Notably, these two aspects of imprinting (i.e., memory formation and induced predisposition) appear tightly coupled and not dissociable [100].

Newly hatched domestic chicks could serve as a valid animal model for studying environmental risk factors for ASD, at least at the surface phenomena level. The following section examines how ASD-like deficiencies could arise in the chick model and whether the underlying mechanisms are shared.

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5. VPA, an anticonvulsant drug, mediates ASD-like impairment of social behavior development and acute suppression of spontaneous fetal movements

Given that VPA was identified as an environmental risk factor for ASD during pregnancy [101–103], studies have attempted to clarify the underlying mechanisms using rodent models (see reviews [20,21,104]). Fetal exposure to VPA impairs social behavior also in chicks [105–109]. These studies have consistently reported impaired social behavior in newborns and hatchlings after fetal VPA exposure, although drug-induced phenotype disorders were not necessarily identical, probably due to task-dependent variation among individuals. For example, [109] reported low imprinting scores exclusively in individuals with a low BM preference,.

VPA has a wide spectrum of pharmacological effects, including actions on N-methyl-D-aspartate type glutamate receptors (NMDA-R [110]) and inhibitory GABA transmission [111]. Moreover, VPA is well-accepted as a potent inhibitor of histone deacetylases (HDACs [112]). Acute anticonvulsant action on the fetus may induce ASD-like phenotypes, as VPA effectively suppresses fetal motion [109]. Spontaneous motion is ubiquitous among fetuses of vertebrates [113] (see also [114,115] for more recent reviews), although its functional roles remain unclear. Nevertheless, suppression of fetal movements fails to account for ASD-like deficiencies, since similarly effective suppressers (e.g., selective blockers of NMDA-R MK-801) failed to cause ASD-like symptoms.

The brain regions and molecular events that are responsible for the VPA-mediated ASD phenotypes remain elusive. In a rat model, VPA enhanced NMDA-R expression and synaptic potentiation in the hippocampus [116], thereby causing an imbalance between excitatory and inhibitory transmission (E-I imbalance; see [117,118] for comprehensive reviews). In support of the hyperexcitation hypothesis, post-natal blockade of NMDA-R by memantine (a drug prescribed for Alzheimer's disease) rescued social interaction impairment [119]. Consistent with studies performed in rodent models, administering bumetanide (a selective blocker of NKCC1 co-transporter) immediately before training could rescue chicks with VPA-induced impaired imprinting [109]; the impact of bumetanide will be discussed subsequently.

6. Selective impairment of BM predisposition via fetal interference with nAChR receptors, including neonicotinoid insecticides

Pesticide chemicals, particularly considering the rapidly increasing consumption of neonicotinoid insecticides (NNs [120]), are another serious concern in the etiology of ASD. NNs were designed to selectively block cholinergic neurotransmission in insects with low toxicity in vertebrates. Early ecological reports have highlighted the population decline of insectivorous birds [121]. Following concerns regarding the high persistence of NNs in plants and soil, NNs were found to impair the migratory ability of granivorous birds [122,123]. Several recent epidemiological studies have reported the risk of maternal exposure to environmental NNs [124–127]. An early study [124] estimated the association between the indoor usage of imidacloprid (IMI; one of the most heavily used NNs for flea and tick treatment for pet animals) and ASD, detecting an alarming odds ratio of ~2.0. Prenatal exposure to agricultural pesticides was found to be associated with low intelligence quotient and verbal comprehension [125]. A large-scale study on the association between ambient pesticide usage (NNs included) and ASD in California's agricultural region [126] detected considerable odd ratios for various pesticide chemicals; the effects of prenatal exposure were boosted by additonal exposure in neonatal infants. A rodent model study assessing acetamiprid (ACE; another NNs) has reported the abnormal development of social and anxiety-related behaviors in males after prenatal and lactational exposure [128] (see [127] for a recent systemic review).

Our study using a chick model [109] revealed high concordance with these reports in humans and rodents. Selective and non-selective blockade of nAChR (using tubocurarine and selective $\alpha 7$ subtype inhibitor), as well as impaired nAChR transmission mediated by IMI, could suppress fetal movements and impair the BM preference of hatchlings. Notably, nAChR blockade did not impair imprinting memory formation, thus revealing distinct dimensions of social behavior malformation from those induced by VPA.

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7. Thyroid hormone, E-I imbalance in humans and chicks

Maternal hypothyroidism (gestational hypothyroxinemia) is another risk factor for ASD [129–131]. Circulating levels of thyroid hormones (THs, T_4 in particular) could be a potential early biological marker for ASD. To date, no consensus has been reached regarding the role of THs in the development of ASD, given that both positive [132] and negative [133] results have been reported. In addition to being a critical determinant of imprinting in chicks [96], TH plays critical roles in diverse neurodevelopmental processes [134], particularly in the maturation of GABAergic transmission via the rapamycin (mTOR; mechanistic target of rapamycin) cascade [135]. The mTOR-GABA cascade may mediate the acute facilitatory effects of THs in chicks [96,136,137]. T_3 acutely enhances GABAergic transmission in slice preparations of the chick pallium [138], although its functional link to the behavioral effects remains elusive.

Interestingly, in humans, symptomatic autism comorbid with fragile X syndrome and tuberous sclerosis complex (TSC) is accompanied by mutations in the mTOR signaling pathway. Rapamycin was shown to rescue social impairment in a mouse model of TSC [139]. A more recent study addressing macrocephaly in infants with ASD has suggested that synaptic pathology related to the mTOR pathway is responsible for hyperconnectivity [140].

In the central nervous system, the metabolic control responsible for the balanced management of energy income and growth may underlie appropriate socialization during the early stages of life in humans and chicks. In addition, premature E-I balance (or delayed GABA switch from depolarizing to hyperpolarizing response) could be a key event in neural maturation, affording a potential target for developing effective pharmacotherapies for ASD.

8. GABA switch, nicotinic transmission, and treatment using bumetanide and oxytocin

GABA exerts depolarizing transmission via GABA-A receptors in the embryonic stage, and excitatory GABA exerts trophic functions for the functional maturation of the brain. During the perinatal period, excitation is converted to adult-type inhibitory neurotransmission ([141–143] for comprehensive reviews; [144] for the hippocampal development; also see [145] specifically for the schizophrenia etiology); this conversion (referred to as the GABA switch) is mediated by a reduction in the intracellular chloride ion ($[Cl^-]_i$), which, in turn, can be attributed to the enhanced expression of cation-linked co-transporters responsible for the efflux of Cl^- (KCC2 over NKCC1). To identify regulatory mechanisms underlying the GABA switch, nicotinic cholinergic transmission was found to be critical in the chick ciliary ganglion [146]. Further analyzing the phosphorylation of KCC2 molecules revealed protein kinase C-mediated modulation by glutamatergic and serotonergic actions, as well as the activation of muscarinic acetylcholine receptors [147] (for more recent comprehensive reviews see [148,149]).

The association between GABA switch retardation and ASD was based on the finding that diazepam (an anxiolytic drug, a positive modulator of GABA-mediated inhibition used to relieve anxiety) could paradoxically increase aggressive behaviors in autistic children [150]. Subsequent studies have shown that dysfunctional GABA inhibition could play a pivotal role in ASD etiology [151,152]. Bumetanide has attracted attention as a potent candidate for ameliorating ASD symptoms, given that this agent selectively blocks the NKCC1 co-transporter responsible for Cl^- influx [153]. Although initial open-label small-sized trials appeared positive and promising [154,155], recent phase-2 trials failed to afford positive outcomes [156]. A detailed follow-up analysis has identified heterogeneous phenotypes of neurocognitive impairment in patients with ASD, some of which were unaffected by bumetanide [157]. In a chick model, bumetanide treatment immediately before training rescued impairments in both imprinting (by VPA) and BM preference (by nAChR blockade) [109]. Further studies are required to identify underlying targets and pharmacology.

Oxytocin and related nonapeptides comprise another group of candidate drugs for ASD. In humans, intranasal application of oxytocin can acutely ameliorate social deficiencies such as BM perception and social communication in cases of relatively low severity [158,159]. In typically developing individuals, oxytocin receptors peak during early childhood, whereas this peak is absent in those with ASD [160]. Similar facilitatory effects of oxytocin on prosocial behaviors have been

observed in dogs [161,162], birds [163–165], and fish [166]; also see [167] for the zebrafish model of developmental disorders), suggesting its ubiquitous role in vertebrates. Considering the underlying mechanisms, a study using oxytocin-receptor knock-out mice has suggested that KCC2 is regulated by oxytocin [168]. A more recent study in mice identified a link between the genetic risk of ASD and the oxytocinergic signaling pathway [169]. In chicks, intracranially administered mesotocin (an avian counterpart of oxytocin) enhanced the preference of naïve chicks [165].

Although disadvantageous as a genetic model of ASD owing to the limited availability of powerful tools for gene manipulation, the chick model could substitute rodent models in several technical aspects. Firstly, maternal complications are disregarded in chicks as direct fetal administration is possible in eggshells. Secondly, the time course and dosage of chemicals can be precisely controlled. The short incubation time and quick testing (usually 8 days after drug application) enable the rapid screening of harmful chemicals. Third, the use of chicks satisfies the 3Rs of animal experiments (https://nc3rs.org.uk/who-we-are/3rs): replacement with mammals, reduction in number due to controlled chemical treatments, and refinement by sophisticated visual predispositions like humans.

Chicks are unique models for studying neurocognitive disorders in humans. The fundamental question is, *why?* Why did chicks converge to human neonates despite distinct evolutionary separations over 300 million years? This remains an unresolved puzzle, which could be addressed by analyzing behavioral phenotypes and disorders (surface validity) and the underlying mechanisms (construct validity). Biological psychiatry and comparative psychology ask the same question: *What are we?*

Conflicts of Interest: Both authors decleare no conflict of interests.

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