The Primary Brain Vesicles Revisited: Are the Three Primary Vesicles (Forebrain/Midbrain/Hindbrain) Universal in Vertebrates?

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Abstract
It is widely held that three primary brain vesicles (forebrain, midbrain, and hindbrain vesicles) develop into five secondary brain vesicles in all vertebrates (von Baer’s scheme). We reviewed previous studies in various vertebrates to see if this currently accepted scheme of brain morphogenesis is a rule applicable to vertebrates in general. Classical morphological studies on lamprey, shark, zebrafish, frog, chick, Chinese hamster, and human embryos provide only partial evidence to support the existence of von Baer’s primary vesicles at early stages. Rather, they suggest that early brain morphogenesis is diverse among vertebrates. Gene expression and fate map studies on medaka, chick, and mouse embryos show that the fates of initial brain vesicles do not accord with von Baer’s scheme, at least in medaka and chick brains. The currently accepted von Baer’s scheme of brain morphogenesis, therefore, is not a universal rule throughout vertebrates. We propose here a developmental hourglass model as an alternative general rule: Brain morphogenesis is highly conserved at the five-brain vesicle stage but diverges more extensively at earlier and later stages. This hypothesis does not preclude the existence of deep similarities in molecular prepatterns at early stages.
Background

According to almost all current textbooks on developmental biology [for example, see fig. 9.9 of Gilbert, 2010], the rostral region of vertebrate neural tubes develops into three distinct swellings or the primary brain vesicles by differential proliferation of neuroepithelial territories: the forebrain, midbrain, and hindbrain (fig. 1a). The brain vesicles are morphologically defined as rostro-caudally arranged dilatations of the primordial brain part of the neural tube, and each vesicle may be composed of several smaller repetitive units known as neuromeres [Nieuwenhuys, 1998]. The three primary vesicles go on to subdivide into a series of five secondary brain vesicles. The forebrain (prosencephalon) and hindbrain (rhombencephalon) are subdivided into the telencephalon/diencephalon and metencephalon/myelencephalon, respectively, whereas the midbrain (mesencephalon) remains undivided (fig. 1b). According to Swanson [2000, 2003], this developmental scheme is based on Malpighi’s classical description and studies by Karl von Baer [1828].

In order to uphold von Baer’s model, it is necessary to show that there exist three initial vesicles in all vertebrates and that the fates of the vesicles follow the scheme. Almost all investigators have agreed on the presence of von Baer’s five brain vesicles at later developmental stages [Nieuwenhuys, 1998]. However, von Baer’s primary vesicles may be an exaggerated view of early brain morphogenesis [Nieuwenhuys, 1998]. Streeter [1933, p. 474] could not confirm the presence of the three primary vesicles in the chick and stated: ‘The subdivision of the embryonic brain into three primary brain vesicles is an arbitrary expedient rather than a natural phenomenon’. Furthermore, our recent studies on the teleost fish medaka (Oryzias latipes) have shown that the molecular prepatterns, which are visible only by gene expressions at early stages, do not correspond to the morphologically defined brain vesicles [Kage et al., 2004]. It is important to survey the early brain morphogenesis in various vertebrates because von Baer’s scheme is currently considered to be a universal rule applicable to all vertebrates. The scheme is a basic tenet of neuroscience. In this review, we survey previous studies based on classical morphology as well as those based on fate maps and gene expression patterns in different vertebrates.
Studies Using Classical Methods of Morphology

Among agnathans, Kuratani et al. [1998] examined early brain development in a lamprey *Lampetra japonica* and reported the presence of two faint constrictions in the initial brain (fig. 2a). The mes/rhombencephalic sulcus first became apparent at stage 21 of Tahara [Tahara, 1988], and later the par/synencephalic (intraprosencephalic) boundary became discernible. Hence, the early brain is divided into three vesicles by these sulci. However, this situation is inconsistent with von Baer’s pros/mes/rhombencephalon model because the brain is divided rostro-caudally into four portions, namely the prosencephalon, mesencephalon plus rostral rhombencephalon, r3, and caudal rhombencephalon [see fig. 1A of Kuratani and Horigome, 2000]. Therefore, the initial morphological subdivisions of the shark brain are also inconsistent with von Baer’s pros/mes/rhombencephalon model. Describing the subsequent stage II of development (22-day or 3.5-mm embryos), Kuratani and Horigome [2000, p. 895] wrote: ‘In *S. torazame* at this stage, rhombomeric boundaries can be seen at the levels of r1/2, r2/3, r3/4, r4/5, and r5/6, but the mid/hindbrain boundary is not detectable’.

In teleost fish, the hollow neural tube is derived from an initially solid neural rod that is homologous to the neural tube in other vertebrates [for a review of teleost neurulation, see Lowery and Sive, 2004]. Kimmel et al. [1995] reported in the zebrafish (*Danio rerio*) that no distinct enlargements were noticed in the early brain; the neural rod developed directly into the neural tube having five brain vesicles [see also Kimmel, 1993]. Our own observations confirmed their results. Although a few shallow constrictions were noticed on the dorsal surface of the mesencephalic region at the 10-somite stage, no constrictions were found ventrally (fig. 3). Hence, the so-called three primary vesicles do not exist morphologically in the initial zebrafish brain.

In the frog (*Xenopus laevis*), Eagleson et al. [1995] reported that two slight constrictions appear separating the prospective three brain vesicles at the late neural plate stage (stage 17 of Nieuwkoop and Faber [1967]). They observed the three distinct brain vesicles in the early neural tube at stage 20 of Nieuwkoop and Faber [1967]. In the same species, Ten Donkelaar [1998] also described that the three primary vesicles can be distinguished by stage 23 of Nieuwkoop and Faber [1967], based on anatomical flexures and constrictions. Thus, von Baer’s model holds true for the anuran neural tube.

There are many detailed studies on chick embryos. According to Vaage [1969], two main brain subdivisions are discernible in living chick embryos at the 7-somite stage.
Vaage [1969] referred to these two divisions as archencephalon (the prospective prosencephalon) and deuteroencephalon (the prospective mesencephalon plus three rhombomeres). At later stages (10-somite stage, HH stage 10), Vaage [1969] reported further transformation of the neural tube into the prosencephalon, mesencephalon, and at least three rhombomeres. At HH stage 10, Hamburger and Hamilton [1951, p. 55] also described that ‘three primary brain vesicles are clearly visible’. In sharp contrast to Vaage [1969] and Hamburger and Hamilton [1951], Streeter [1933] did not find any actual brain subdivisions when he examined inner surfaces of the rostral neural tube in the chick embryos at the 8-somite stage (HH stage 9–10), although three brain vesicles appear to be present in an external dorsal view. The opinion regarding the initial brain subdivisions in chick embryos thus diverges considerably among different researchers [for a review, see Aroca and Puelles, 2005]. The swellings of the subdivisions and constrictions between them may be so faint at the initial stages that they might be overlooked, or they might perhaps disappear or arise as artifacts during preparation. We will further discuss chick brain vesicles in the section Studies Based on Fate Maps and Gene Expression Patterns.

In Chinese hamster (Cricetulus griseus) embryos, Keyser [1972] reported that the first segment-like transverse bulges or neuromeres are present in the open neural plate. In the earliest neural tube at embryonic day 11, Keyser [1972] reported the presence of two or three prosomeres, one or two mesomeres, and several rhombomeres [Keyser, 1972]. He noted: “On a superficial view the external aspect of the brain in the E11 embryo suggests the presence of three vesicles, connected by constriction. In the textbooks these vesicles are called prosencephalon, mesencephalon, and rhombencephalon. On closer scrutiny, however, several segment-like structures are observed within each of these ‘vesicles’, each possessing its own individual outline and configuration” [Keyser, 1972, p.30].

Also in human embryos, the first brain subdivisions do not begin as vesicles but as enlargements of the neural folds at stage 9 [O’Rahilly and Gardner, 1979; O’Rahilly et al., 1989], before any portions of the neural folds have closed (fig. 4). In the initial human neural tube, O’Rahilly and Gardner [1979, p. 129] described that ‘external views of the brain may show at most a swelling of the hindbrain, which is united to that of the forebrain by the angulated and relatively narrow midbrain’. It should be noted that in these mammalian studies the terms prosencephalon, mesencephalon, and rhombencephalon are not used to indicate distinct dilatations of the vesicles (as illustrated in fig. 1a) but only to point out regional locations of brain subdivisions or neuromeres.

The studies surveyed above indicate that the standard von Baer’s model holds true for the frog, but less so for the chicken, and poorly for most other vertebrates. Those previous reports rather suggest diversity among vertebrates in the process of early brain morphogenesis. The swellings that indicate prospective brain subdivisions occur prior to neural tube closure in mammalian embryos but later in many other vertebrates. The rhombencephalic region differentiates much earlier than other brain subdivisions in shark embryos. The numbers and fates of the earliest brain vesicles are also diverse: while frogs have three von Baerian brain swellings, zebrafish have none, and sharks have four. Lampreys exhibit three brain swellings in early development, but they do not follow von Baer’s scheme.

**Studies Based on Fate Maps and Gene Expression Patterns**

As shown in the previous section, distinct swellings of the three primary vesicles may sometimes be difficult to identify based simply on observations of specimens prepared by classical methods. Therefore, it is important to address the issues with more modern methods. To settle the concerns, we surveyed previous reports based on fate map and gene expression patterns, although the interpretation of the latter data requires care since gene expres-
sion may change over time during development. These types of investigations have been performed only in a few vertebrate species. We review in this section the studies on medaka, chick, and mouse embryos.

**Medaka Embryo**

The medaka is one of the fish models used for studies of vertebrate developmental genetics and comparative genomics [Ishikawa, 2000; Kinoshita et al., 2009]. The general development of medaka has been described by Iwamatsu [2004], and the brain morphogenesis has been studied based on a fate map and gene expression patterns [Hirose et al., 2004; Kage et al., 2004; Ishikawa et al., 2008].

In contrast to zebrafish (fig. 3), three enlargements were recognized in the medaka neural rod (Iwamatsu's stage 19) before five brain vesicles were established in the neural tube [Ishikawa, 1997; Kage et al., 2004] (fig. 5). In the latter studies we referred to the large middle enlargement as ‘intermediate brain vesicle (IBV)’ and to the two smaller, adjacent vesicles as ‘rostral brain vesicle (RBV)’ and ‘caudal brain vesicle (CBV)’, respectively (fig. 5b).

Two independent lines of evidence showed that the fate of the intermediate brain vesicle in medaka is quite different from that of the so-called mesencephalic vesicle in von Baer’s scheme. First, the expression patterns of wnt1, which is used as a gene marker for the caudal limit of the mesencephalon in various vertebrates, showed that the intermediate brain vesicle in medaka develops not only into the mesencephalon but also into the caudal diencephalon and metencephalon [Kage et al., 2004; Ishikawa et al., 2008] (fig. 6). Second, single-cell fate mapping has shown that compartments defined by cell migration boundaries are established as early as at stage 16+ [Hirose et al., 2004]. These results are consistent with our interpretation of wnt1 expression (fig. 7). Therefore, von Baer’s scheme does not hold true for medaka.

**Chick Embryo**

As mentioned above, Hamburger and Hamilton [1951] described that the three primary vesicles become visible in the chick embryo at HH stage 10. However, Hidalgo-Sánchez et al. [1999] reported that the so-called ‘mesencephalic vesicle’ at HH stage 10 contains not only the prospective mesencephalon but also a rostral part of the prospective rhombencephalon. This conclusion was based on the spatial expression patterns of developmental genes, Otx2, Gbx2, Pax2, Fgf8, and Wnt1, all of which are implicated in specification of the mes/rhombencephalon boundary domain [see also Martinez and Alvarado-Mallart, 1989; Aroca and Puelles, 2005] (fig. 8).

Hidalgo-Sánchez et al. [1999] noted that the morphological constriction between the so-called ‘mesencephalic’ and ‘rhombencephalic’ vesicles at HH stage 10 is not the true mes/rhombencephalic boundary, which is defined by the Otx2/Gbx2 expression boundary, but an intrametencephalic constriction (fig. 8a). Therefore, they proposed a new term, namely ‘mes/met vesicle’ instead of ‘mesencephalic vesicle’, to refer to the second brain swelling at HH stage 10. Their findings are consistent with the results of fate map analyses using the chick/quail chimeraic system [Martinez and Alvarado-Mallart, 1989; Millet et al., 1996; Hollonet and Alvarado-Mallart, 1997; for a

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**Fig. 5.** Left lateral view of the medaka embryo at Iwamatsu's stage 19 (2-somite stage). Rostral is to the left and dorsal is to the top. A photograph of a living embryo is shown in (a), accompanied by the corresponding line drawing (b). In b, the axial mesendoderm and the notochord (NC), both of which are identified by the expression of shh, are shown as black and striped, respectively. Note that the rostral brain vesicle (RBV), intermediate brain vesicle (IBV), and caudal brain vesicle (CBV) are present. For other abbreviations, see the list. Reproduced and redrawn from Ishikawa [1997] and Kage et al. [2004].

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**Fig. 6.** Line drawing showing the spatial expression patterns of wnt1 in the medaka embryo at different developmental stages: Iwamatsu’s stages 18 (a), 18.5 (b), and 19 (c). In (c), the intermediate brain vesicle (IBV) is the central enlargement, flanked on the left by the rostral brain vesicle (RBV) and on the right by the caudal brain vesicle (CBV). Note the expression of wnt1 in the caudal limit of the mesencephalon (arrow in (c)) and adjacent to the IBV (arrow in (b)). Reproduced and redrawn from Hirose et al. [2004].
Thus, in both chick and medaka embryos, the fates of initial brain vesicles are inconsistent with von Baer’s scheme.

**Mouse Embryo**

Finally, we review developmental studies on the mouse brain. As in cases of the Chinese hamster and human embryos, the first brain subdivisions (prosencephalon, mesencephalon, and two rhombomeres A and B) emerge not as vesicles but as enlargements of the neural folds in the mouse embryo at Theiler’s stage 12 (1- to 7-somite stage, E8–8.5 days), when the neural folds begin to close in the occipital/cervical region [Theiler, 1989; Kaufman, 1994].

Numerous studies of gene expression patterns have been reported in mouse embryos during neurulation [for reviews see Rubenstein et al., 1998; Martinez and Puelles, 2000]. Wnt1, En1, and Pax2 are expressed in the presumptive mes/rhombencephalon boundary domain in the neural plate as early as at the 1-somite stage (Theiler’s stage 12), when the entire dorsal view of the neural plate exhibits a simple spoon shape [Rowitch and McMahon,
Rubenstein et al. [1998] showed in their figures that three rostro-caudally arranged transverse bulges, each of which is marked by shallow lateral notches such as the preotic sulcus (pos), were present in the flattened neural plate at the 7- to 8-somite stage (Theiler’s stage 12–13) [see also Lawson and Pedersen, 1992; Inoue et al., 2000] (fig. 9b). Fate map analyses showed that the rostral, intermediate, and caudal bulges develop into the prosencephalon, mesencephalon plus rostral rhombomeres, and caudal rhombomeres, respectively [Rubenstein et al., 1998; Inoue et al., 2000] (fig. 9b). Moreover, Rubenstein et al. [1998] showed that Wnt1 and Fgf8 are expressed in transverse domains that approximate the prospective mes/rhombencephalon boundary in the intermediate bulge [see also Crossley and Martin, 1995].

Thus, although the three bulges in the mouse neural plate at the 7- to 8-somite stage are not brain vesicles by definition, they seem to be equivalent to the so-called three primary vesicles in the chick neural tube at HH stage 10 in terms of their positions and fates (compare fig. 9b with fig. 8a). That is, both the mouse intermediate bulge and the so-called chick ‘mes/met vesicle’ develop into not only the mesencephalon but also the rostral rhombencephalon.

The neural tube begins to close in the prosencephalic region at Theiler’s stage 13 (8- to 12-somite stage, E8.5–9 days) and is completely closed at the 15- to 18-somite stage (Theiler’s stage 14, E9–9.5 days) [Kaufman, 1994]. According to Kaufman [1994], the three vesicles (prosencephalon, mesencephalon, and rhombencephalon) are formed upon neural tube closure. To our knowledge, however, the anatomical relationship between the three bulges in the neural plate at the 7- to 8-somite stage and the three primary vesicles identified by Kaufman [1994] in the earliest neural tube has not yet fully been documented during mouse neurulation. If neural tube closure is simply tardy in the mouse and the mouse intermediate bulge at the 7- to 8-somite stage develops into the ‘mes/met vesicle’ of Hidalgo-Sánchez et al. [1999] at the 15- to 18-somite stage, the situations of mouse and chick embryos would become much the same. Further detailed studies will be needed to clarify this point in the mouse.
Conclusion

Although von Baer’s three primary vesicles are present in the frog neural tube, there exists rather large morphological divergence in the earliest neural tube in many other vertebrate taxa. Even when three vesicles are present, their fates are different from those of von Baer’s model at least in lamprey, medaka, and chick embryos. Thus, our review of the literature shows that there are many exceptions to von Baer’s rule. We have no choice but to conclude that the existence of three primary vesicles that follow von Baer’s scheme is not a universal rule throughout vertebrates. In short, deep similarities may exist in molecular prepatterns, but little morphological similarity is visible at early stages.

Are there any alternative rules in vertebrate brain morphogenesis other than von Baer’s model? Because five brain vesicles are generally noticed at later developmental stages of vertebrate brain morphogenesis [Nieuwenhuys, 1998], the early variation in brain vesicles may fit the developmental hourglass model [Gilbert, 2010]. According to this model, embryonic development exhibits a conserved developmental stage or period, the so-called phylotypic stage, at which morphological similarity is maximal between the members of each animal phylum [Slack et al., 1993]. The strong similarity at this stage may be due to the phyletic constraints [Hall, 1998]. Importantly, development is much more variable before and after this phylotypic stage. This hourglass model may be applied also to the morphogenesis of vertebrate brains [Kage et al., 2004]. Before and after the middle, five-vesicle stage, vertebrate brain morphogenesis diverges extensively.

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