Individual variability in the size and organization of the human arcuate nucleus of the medulla

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Abstract
The arcuate nucleus (Arc) of the medulla is found in almost all human brains and in a small percentage of chimpanzee brains. It is absent in the brains of other mammalian species including mice, rats, cats, and macaque monkeys. The Arc is classically considered a precerebellar relay nucleus, receiving input from the cerebral cortex and projecting to the cerebellum via the inferior cerebellar peduncle. However, several studies have found aplasia of the Arc in babies who died of SIDS (Sudden Infant Death Syndrome), and it was suggested that the Arc is the locus of chemosensory neurons critical for brainstem control of respiration. Aplasia of the Arc, however, has also been reported in adults, suggesting that it is not critical for survival. We have examined the Arc in closely spaced Nissl-stained sections in thirteen adult human cases to acquire a better understanding of the degree of variability of its size and location in adults. We have also examined immunostained sections to look for neurochemical compartments in this nucleus. Caudally, neurons of the Arc are ventrolateral to the pyramidal tracts (py); rostrally, they are ventro-medial to the py and extend up along the midline. In some cases, the Arc is discontinuous, with a gap between sections with the ventrolaterally located and the ventromedially located neurons. In all cases, there is some degree of left–right asymmetry in Arc position, size, and shape at all rostro-caudal levels. Somata of neurons in the Arc express calretinin (CR), neuronal nitric oxide synthase (nNOS), and nonphosphorylated neurofilament protein (NPNFP). Calbindin (CB) is expressed in puncta whereas there is no expression of parvalbumin (PV) in somata or puncta. There is also immunostaining for GAD and GABA receptors suggesting inhibitory input to Arc neurons. These properties were consistent among cases. Our data show differences in location of caudal and rostral Arc neurons and considerable variability among cases in the size and shape of the Arc. The variability in size suggests that “hypoplasia” of the Arc is difficult to define. The discontinuity of the Arc in many cases suggests that establishing aplasia of the Arc requires examination of many closely spaced sections through the brainstem.

Keywords Human brainstem · Pontine nuclei · Inferior olive · Sudden Infant Death Syndrome · Cerebellum

Introduction
The arcuate nucleus of the medulla (Arc) is a prominent structure in the human brainstem (Olszewski and Baxter 1982; Mikhail and Ahmed 1975; Essick 1912). We found a similar structure in a minority of chimpanzee brainstems (Baizer et al. 2013a). However, no such structure has been described in the medulla of many other mammalian species including the mouse (Paxinos and Franklin 2004; Sidman et al. 1971), rat (Paxinos 1999; Paxinos and Watson 1997), cat (Berman 1968), and macaque monkey (Paxinos et al. 2000). A brainstem nucleus called the Arc was described in mice, but it is likely not homologous with the human Arc because of neurochemical differences between mouse and the human nuclei (neurons of the human Arc express CR and...
not CB; neurons of the inferior olive express both, discussion in Fu and Watson 2012).

Classically, the Arc is considered a precerebellar relay nucleus, similar to the pontine nuclei, with input from the cerebral cortex and projections to the cerebellum via the inferior cerebellar peduncle (Olszewski and Baxter 1982; Rasmussen and Peyton 1946). A totally different view of the Arc has been proposed on the basis of neuropathological observations showing aplasia of the Arc in the brains of children who died of SIDS (Sudden Infant Death Syndrome). Another term for the same syndrome is Sudden Unexpected Death in Infancy (SUDI). A closely related syndrome is Sudden Intrauterine Death Syndrome (SIUDS). We will use the term SIDS to include all cases of sudden unexpected death, both prenatal and postnatal. Such cases are described in many studies (Filiano and Kinney 1992, 1994, 1995; Kinney et al. 1992; Matturri et al. 2000; Kinney and Filiano 1988; Franciosi and Segura 2004; Biondo et al. 2003; Folgering et al. 1979). Filiano et al. (1990) had suggested that the Arc in humans was the site of chemosensitive neurons similar to those that had been described along the medullary surface in the cat. These neurons are critical for brainstem control of respiration. That interpretation has been widely accepted in many SIDS studies (e.g., Filiano and Kinney 1992; Filiano 1994; Folkerth et al. 2008). Aplasia of the Arc would, by that interpretation, lead to respiratory failure. However, Paradiso et al. (2018) found aplasia and hypoplasia of the Arc in the brains of adults, suggesting that it was not critical for survival.

It is clear that there is significant individual variability in the size and position of the Arc among different human brains. This is well illustrated by its depiction in two atlases of the human brainstem. Olszewski and Baxter (1982) show the Arc as beginning caudal to the obex (plate VIII) and extending rostrally to the pontine nuclei (pn; plate XVIII). Caudally, it is a small cell group ventrolateral to the pyramidal tracts (py). More rostrally it is located ventromedially to the py and extends up the midline. By contrast, the atlas of Paxinos and Huang (1995) shows the Arc as only a small caudal cell group ventrolateral to the py (Figs. 19 and 20, Obex + 2 and + 3 mm) with a rostro-caudal extent of only 1 mm. It does not extend rostrally to the level of the pontine nuclei.

We asked if there were similar variability in Arc size and shape in brainstems from the Witelson Normal Brain Collection (Witelson and McCulloch 1991), and whether we could identify cases in which there was hypoplasia or aplasia. We also examined sections immunostained for a set of antibodies that have been useful in understanding the organization of other brainstem structures. For example, immunoreactivity to calcium-binding proteins revealed subdivisions in the medial vestibular nucleus (Baizer and Broussard 2010; Baizer and Baker 2006) and showed variability among brains in the patterns of immunolabel in the principal nucleus of the inferior olive (IOpr; Baizer et al. 2011b).

Our results confirm the variability in Arc size and shape among cases and additionally show left–right asymmetry. We also show that the Arc is discontinuous in about half the cases. We did not find neurochemically defined compartments within the Arc.

Materials and methods

We studied a subset of brainstems from the Witelson Normal Brain Collection (Witelson and McCulloch 1991). Subjects were recruited from identified metastatic cancer patients who had no history of neurological or psychiatric complications at the time of enrollment. Table 1 shows the critical parameters of the cases we studied including age, sex, and post-mortem interval (PMI).

| Case # | Age | Sex | PMI (h) |
|--------|-----|-----|---------|
| 1      | 57  | M   | 5       |
| 2      | 63  | M   | 3       |
| 3      | 50  | F   | 9       |
| 4      | 51  | M   | 1       |
| 5      | 45  | F   | 3       |
| 6      | 65  | F   | 3       |
| 7      | 55  | F   | 2       |
| 8      | 69  | M   | 3       |
| 9      | 70  | M   | 2       |
| 10     | 63  | F   | 6       |
| 11     | 71  | F   | 3       |
| 12     | 54  | M   | 2       |
| 13     | 69  | M   | 2       |
Initially, sets of sections 2 mm apart from each case were stained with a Nissl stain, Cresyl Violet (CV), following a standard protocol (LaBossiere and Glickstein 1976). For this analysis, we studied Nissl and immunostained sections that had been prepared for studies of other brainstem structures (Baizer et al. 2007, 2011a, b, 2013b, 2018; Baizer 2014; Baizer and Broussard 2010). We here include data from several previously unpublished cases.

**Antibodies and immunohistochemistry (IHC)**

Detailed protocols for immunohistochemistry were described in those earlier publications. Briefly, all immunohistochemistry was done on free-floating sections. Sections were first treated with an antigen retrieval (AR) protocol. Sections were rinsed and then nonspecific label was blocked by incubating sections in a solution of phosphate buffered saline (PBS), 1% Triton–X 100, 1% bovine serum albumin, and 1.5% normal serum. Sections were then incubated in that solution with the primary antibody added overnight at 4 °C on a tissue rocker. Further processing was with the Vector “ABC” method using a Vector Elite kit (Vector Laboratories, Burlingame, CA) and visualization with 3,3′-diaminobenzidine (DAB; Sigma) giving brown staining, or a glucose oxidase modification of that protocol, giving gray–black staining (Shu et al. 1988; Van der Gucht et al. 2006). Sections were mounted on gelled slides, dehydrated in 70%, 95%, and 100% alcohol, cleared in Histosol or Xylene, and coverslipped with Permount (Fisher Scientific). Table 2 shows the primary antibodies and dilutions used.

**Data analysis and photography**

Sections were examined with a Leitz Dialux 20 light microscope, and digital images (1600×1200 pixels) captured with an SPOT Insight Color Mosaic camera. We used the same camera with a Wild Makroscope for lower magnification images. Brightness, contrast and color of the images were adjusted and figures assembled with Adobe Photoshop software (San Jose, CA).

We looked for the presence of the Arc on each section from the most caudal section available to the pontine nuclei. Table 3 shows the total number of sections examined for each case. To determine the total rostro-caudal extent of the Arc, we measured the distance between the sections on which the Arc first could be identified caudally to the appearance of the pontine nuclei rostrally. Table 3 shows those distances. If there were sections on which the Arc was not present, it was considered “discontinuous” and the gap measured (n = 6 cases). It was considered “continuous” in cases (n = 7) in which it could be identified on all sections from its caudal beginning to the pontine nuclei. To measure the area of the caudal Arc (data in Table 3), we identified the section on which the area of the caudal Arc was at its largest. Photomicrographs were taken of the left and right Arc on those sections and the areas measured using ImageJ software.

**Terminology and abbreviations**

With a few exceptions, we follow the abbreviations for anatomical structures used in the atlas of Paxinos and Huang (1995); we have introduced the new terms cArc and rArc for the caudal and rostral cell groups of the Arc. Abbreviations are listed in Table 4.

**Results**

**Size and location of the Arc**

We analyzed Nissl-stained sections from 13 cases (Tables 1, 3). We found an Arc in each case, but variability among cases in its location, size, shape, and degree of left–right asymmetry. We tentatively divide the Arc into caudal and rostral subdivisions on the basis of location of neurons. The

| Table 2 | Antibodies and dilutions |
|---------|-------------------------|
| Antigen                        | Source, Catalogue #      | Host | Dilution |
| Calbindin (CB)                  | Chemicon/Millipore AB1778 | Rb   | 1:2000   |
| Calretinin (CR)                | Chemicon/Millipore AB5054 | Rb   | 1:2000–1:3000 |
| GABA$_A$ Rø1 receptor          | Santa Cruz sc-31403      | Gt   | 1:250    |
| GABA$_B$ R1 receptor           | Chemicon/Millipore AB2256 | GP   | 1:2000    |
| Glutamic acid decarboxylase (GAD$_{65/67}$) | Chemicon/Millipore AB1511 | Rb   | 1:1000   |
| Nitric oxide synthase (nNOS)   | Cayman 160870            | Rb   | 1:200    |
| Nonphosphorylated neurofilament protein (NPNFP) | Covance SMI-32 | Ms   | 1:1000 |
| Parvalbumin (PV)               | Sigma P3088              | Ms   | 1:2000   |

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The Arc in this case has a rostro-caudal extent of about 18 mm. The shape and position of the Arc cell groups were different at caudal and rostral levels. We can distinguish cArc and rArc. There is left–right asymmetry in the Arc at all levels. The cArc began at a level caudal to the appearance of the IOpr as a ventrolateral narrow band of neurons (Fig. 1a; arrows). There was some asymmetry between left and right sides, with the band on the left longer than on the right. Figure 1b shows a section about 2 mm rostral to the one in Fig. 1a; the size and position of the Arc are similar, with the band on the left narrower than the band on the right. As illustrated in Fig. 1c, the neurons of the Arc are apparently randomly scattered and of different soma shapes, typically polygonal but including elongated and oval examples. About 2 mm more rostrally (Fig. 1d), the Arc consists of more compact and more medial groups of neurons (Fig. 1d, arrow shows Arc on the right). This shift in position marks the beginning of the rArc. The development of the midline Arc continues at more rostral levels (Fig. 1e–g). In Fig. 1g, the arrow shows the Arc on the left; it is larger and more complex than on the right. In this case, the neurons of the Arc invade the py (Fig. 1f, g, arrowheads on the right). At the caudal level of the pn, the midline Arc is large (Fig. 1k). The images in Fig. 1h, j show the random scattering of neurons with round, oval, polygonal, and elongated somata. Figure 1l shows that the pn neurons are similar in size, density, and variety of soma shapes. For this case, we did not find any sections on which the Arc was absent.
Variability in cArc and rArc

In Case 158, the cArc consisted of a small cell group ventro-lateral to the py (Fig. 1b, c). However, the cArc had other configurations in other cases. Figure 2 shows examples of the cArc in three additional cases. In Case 171 (Fig. 2a, b), the cArc was much larger, a very broad band of neurons interior to the py (Fig. 2a, arrow). The Arc in Case 125 was also larger than in Case 158, a long band (Fig. 2c) but ventrolateral to the py. In Case 176 (Fig. 2e) the cArc was similar to that in Case 158, a small cell group ventrolateral to the py. Despite the major variations in size, the neurons in the Arc in all three cases (Fig. 2b, d, f) were similar to those in the first case (Fig. 1c) in sizes, shapes, and density. To quantify the variability in size, for each case, we measured the area of the cArc (left and right) on the section on which it was largest (areas in Table 3). The area was similar on left (mean = 0.74 mm², SD = 0.53) and the right (mean = 0.89 mm², SD = 0.58). A paired samples t test did not show a significant difference between left and right sides ($t_{11} = 1.49, P = 0.16$).

There was variability in the configuration of the Arc at more rostral levels. The rArc consists of neurons that are ventro-medial to the py as well as neurons that extend up along the midline, often in a complex shape (as shown in Fig. 1g,h). In Case 125 (Fig. 3a), the midline neurons formed a rather narrow band (arrow) with an irregularly shaped broader band of neurons ventromedially (Fig. 3a, arrowhead). In Case 155 (Fig. 3c), the midline component was broader (arrow) and more complex and the ventro-medial band (arrowhead) narrower, with marked asymmetry. In Case 176, the midline structure was even broader (Fig. 3e, arrowhead).
Fig. 2 Variability of the size and shape of the caudal Arc; three cases. For each pair (a, b; c, d; e, f), the image on the left shows a low magnification image of the Arc. The rectangles show the locations of the higher magnification images on the right. a The caudal Arc in this case is the largest of any of the cases we examined. b The Arc consists of scattered neurons of different soma shapes. c The caudal Arc is again larger than in Case 158, but is restricted to ventral to the py. d The neurons are again randomly distributed. e The section is at about the level of Plate VIII in Olszewski and Baxter (1982). The Arc in this case is similar in size to the Arc shown in Case 158 (Fig. 1b). f Scattered neurons of different soma shapes. Scale bars: a, c, e = 500 µm; b, d, f = 100 µm
Fig. 3  Variability of the rostral Arc in three cases. For each pair, the image on the left shows a low magnification of the Arc. The rectangles show the locations of the higher magnification images on the right. 

(a) In this case, the Arc is well-developed ventral to the py (arrowhead on left), but the midline cell groups are narrow (arrow on the right). 

(b) Randomly scattered neurons with polygonal and elongated somata. 

c In this case, there is a small band of neurons ventro-medial to the py on the right, and the band on the left is bigger (arrowhead). The midline Arc neurons Arc are a broader band than in A (arrow). 

c Somata are similar to those in (b). 

e In this case, there are almost no cells ventro-medial to the py (arrowhead) and the midline group is asymmetrical but large (arrow). 

f The density and shapes of somata are similar to the other cases. Scale bars: a, c, e = 500 µm; b, d, f = 50 µm
arrow), while the ventro-medial band (arrowhead) was narrower. Again, despite the variability in size of the Arc, its component neurons appeared similar in different cases (Fig. 3b, d, f).

Cases also varied in the geographical relationship between caudal and rostral Arc. In some cases (6/13), as in the case illustrated in Fig. 1, they were continuous; the Arc was not missing on any sections examined. It is of course possible that even more closely spaced sections might show a discontinuity. In other cases (7/13), they were discontinuous, with gaps typically of 1–3 mm between the end of the cArc and the beginning of the rArc (Table 3). This discontinuity again supports the idea that the Arc consists of two distinct cell groups, the cArc and the rArc. Table 3 shows the rostro-caudal extent of the Arc, and the size of “gaps” for each case.

Neurochemistry

In examining immunostaining sections, we asked if there were individual differences in protein expression (as was found for the inferior olive, Baizer et al. 2011b) or if immunoreactivity to calcium-binding proteins might define subdivisions in the Arc as in the vestibular nuclei of humans, monkeys, and cats (Baizer and Broussard 2010; Baizer and Baker 2005, 2006). In illustrating these results, we also show more examples of the variability in the configuration of the Arc among cases. We will first describe the expression of proteins in somata of Arc cells, and then the markers for which expression was seen in puncta but not somata, and finally the evidence for GABAergic input to Arc neurons.

CR

Somata of neurons in the Arc express the calcium-binding protein CR. Figure 4 illustrates this finding for the rArc in two cases. Comparison of Nissl sections (Fig. 4a, c) with neighboring immunostained sections (Fig. 4b, d) shows about the same numbers of labeled neurons, suggesting that all cells are CR+. The immunolabeled sections also show more details of Arc organization with strands of immunolabeled processes extending outside the regions of labeled somata (Fig. 4d, arrowheads).

nNOS

We found immunolabel of somata throughout the Arc. As with CR, it appeared that every Arc neuron was immunolabeled. Figure 5a, b compares Nissl and nNOS immunostaining in the Arc of Case 158. At the level illustrated, there are many Arc neurons ventro-medial to the py with a few along the midline. These sections also illustrate the invasion of the py by neurons and processes of the arc (arrows), especially dramatic in the immunolabeled section (Fig. 5b). There is dense immunolabel of somata that are embedded in a region of dense punctate label. The configuration of the midline Arc is shown in Fig. 5c, d. Again, there is label of somata embedded in dense punctate label with strands and islands of immunolabel extending into the py (Fig. 5c, arrows). The higher magnification image shows the dense punctate label and shows densely labeled somata (example at arrow).

NPNFP

The third marker that immunolabeled somata in the Arc was nonphosphorylated neurofilament protein (NPNFP), and the characteristics of the immunostaining were similar across cases. Figure 6a shows the dense immunostaining in patches along the midline in Case 155. The higher magnification image (Fig. 6b) shows immunolabel of somata (example at arrow) embedded in a dense meshwork of immunostained fibers. In addition, the arrowhead indicates immunolabeled fibers of the ventral external arcuate tract. A similar pattern of very dense immunostaining both along the midline and ventro-medial to the py is shown for Case 158 (Fig. 6c). Immunostained somata (Fig. 6d, arrows) are embedded in a dense meshwork of immunostained processes.

CB and PV

While there was immunostaining of somata with the calcium-binding protein CR, the pattern with the two other calcium-binding proteins, CB and PV, was different. With an antibody to CB, immunostaining clearly distinguished the entire Arc, as shown for two cases in Fig. 7. The Arc is shown in Nissl-stained sections (Fig. 7a, d) and immunostaining in adjacent sections (b, e). However, the immunostaining was not of somata but of puncta distributed throughout the Arc (Fig. 7c, f). Furthermore, in both cases, there were regions of lighter and darker (arrows in Fig. 7c, d) immunostaining. For the third calcium-binding protein, PV, there was no immunostaining in the Arc at all. Figure 8 shows this for two cases. The Arc is clearly defined in Nissl-stained sections (Fig. 8a, c) and completely devoid of immunostaining (Fig. 8b, d). There are immunostained fibers in the adjacent py.

GABAergic input to the Arc

We asked if there were evidence for GABAergic input to the Arc. We also used antibodies to GAD65/67, the synthetic enzyme for GABA, as well as to GABA_A and GABA_B receptors to assess possible GABAergic input to the Arc. Figure 9a shows immunostaining for GAD65/67, defines the region of the midline and ventro-medial Arc in Case 155. Figure 9b illustrates the punctate nature of this
immunostaining, consistent with GABAergic terminals. Figure 9c shows that there is immunostaining for GABA_A receptors, again, distributed evenly over the Arc and Fig. 9d shows that both somata and processes are labeled. Figure 9e, f shows immunolabel for GABA_B receptors, with label restricted to somata.

Discussion

We have studied the organization and neurochemical properties of the arcuate nucleus of the medulla in 13 adult human cases. We have found individual variability in its size and shape but similar neurochemical profiles among cases. We will discuss these results first from a neuroanatomical and neurochemical perspective and then revisit the hypothesis that the Arc is a critical structure in SIDS.

Organization of the Arc: cArc and rArc

We found cell groups consistent with the description and location of the Arc in all cases we examined. We describe caudal and rostral cell groups (cArc and rArc) that differ in location of neurons relative to the py. The idea that the Arc consists of rostral and caudal cell groups was also recognized by Fu and Watson (2012). The relative sizes and shapes of cArc and rArc varied among cases. We also saw variability among cases in the extent to which neurons and processes of the Arc invaded the py. Invasion of the py by Arc neurons was described by Mikhail and Ahmed (1975) and by Fu and Watson (2012, Fig. 7b, d).

There was also variability among cases in the continuity of the Arc. In Case 158, (Fig. 1), the Arc was present continuously from a level caudal to the IOpr all the way to the pontine nuclei. In other cases, however, there was a
discontinuity between caudal and rostral subdivisions, of a range typically from 1 to 3 mm \((n = 6, \text{Table 3})\). The Arc in the atlas of Olszewski and Baxter (1982) was discontinuous, with the cArc shown on plates VIII and X, absent on plate XII and the rArc beginning on plate XIV. The atlas of Paxinos and Huang (1995) showed only the cArc. Discontinuity of Arc cell groups was mentioned in the early study of Essick (1912). The overall appearance of constituent neurons in Nissl sections is the same in cArc and rArc and, we have not found neurochemical differences between them. It is unknown if there are different afferents to or efferents from cArc and rArc, and it is also unknown if there might be associated functional differences. The variability in size of the Arc among cases makes it difficult to define “hypoplasia.” The discontinuity of cArc and rArc in some cases suggests the need to examine sections closely spaced throughout the total rostro-caudal extent of the Arc to be sure that its absence on a section represents “aplasia” rather than examination of a section through the “gap.”

**Variability and asymmetry of the Arc and IOpr**

We have shown individual variability in the size, shape, and location of the Arc, consistent with early observations (Essick 1912; Rasmussen and Peyton 1946). Variability, is however, not unique for a human brainstem structure. We have shown variability in two other brainstem nuclei, the principal nucleus of the inferior olive (IOpr) and nucleus paramedianus dorsalis (PMD). For the IOpr, different cases differed in rostro-caudal extent of the nucleus, in folding pattern of the IOpr ribbon, and in neurochemical properties (Baizer et al. 2011b, 2018). We saw no cases in which

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**Fig. 5** nNOS immunoreactivity in the Arc. **a** Nissl-stained section from Case 158; the section is about 1 mm caudal to the section shown in Fig. 1g. The Arc extends into the py (arrow). **b** nNOS-ir section about 1 mm caudal to the section in (a). The Arc is darkly immunostained; both somata and puncta are immunolabeled. **c** nNOS immunolabeled section from the rostral Arc showing extensive immunolabeled strands in the py (arrows). Note also the immunolabel of the IOpr ribbon. The rectangle shows the location of the higher magnification image in (d). **d** The arrow indicates an immunolabeled neuron embedded in labeled puncta. Scale bars: **a**, **b**, **c**=500 µm; **d**= 50 µm.
the IOpr was dramatically bigger or smaller than typical, or missing altogether. We also saw individual variability in the size of another brainstem nucleus, the PMD (Baizer et al. 2007). Older reports have noted variability among cases in other brainstem structures, including the pontobulbar body and circumolivary fascicles (Swank 1934). Individual variability in cortical sulcal and gyral patterns is well established, and has clear functional correlates (Régis et al. 2005; Fedeli et al. 2020; Bonte et al. 2013; Glasel et al. 2011). There is clearly individual variability in brainstem structures in humans as well as in cortex, but the functional correlates for brainstem structures are so far unknown.

Both the IOpr and the Arc show left–right asymmetry in individual cases, both in humans and chimpanzees (Baizer et al. 2011b, 2013a). Asymmetry has been extensively documented in cerebral cortex, and anatomical asymmetry has associated with functional asymmetry, especially for handedness and language (LeMay 1976; Amunts et al. 1999; Toga and Thompson 2003; Volkmann et al. 1998; Geschwind and Levitsky 1968; Gilles and Gomez 2005; Dorsaint-Pierre et al. 2006; Ochiai et al. 2004). We do not know the functional significance of brainstem asymmetries. Our hypothesis for the IOpr was that differences in size or folding pattern might be related to handedness; our data did not support that idea (Baizer et al. 2011b). Asymmetry in brainstem structures could be the result of as yet not understood developmental processes, or a correlate of asymmetries in cortical development (Gilles and Gomez 2005; Naidich et al. 1994). In general, the brains of other species including mice, rats, and cats do not show marked asymmetry of brainstem structures (Berman 1968; Paxinos and Watson 1997; Paxinos and Franklin 2004; Sidman et al. 1971).

**Fig. 6** Nonphosphorylated neurofilament protein (NPNFP) expression in the Arc. **a** NPNFP immunostaining defines rArc. The rectangle shows the location of the image in (b). Immunolabeled neurons (arrows) embedded in a meshwork of immunostained processes. **b** Extensive immunolabel of the rArc showing invasion of the py. The arrowhead marks immunostained ventral external arcuate fibers. **c** Extensive immunolabel of the rArc showing invasion of the py. This section is about 0.5 mm caudal to the Nissl section shown in Fig. 1I. Scale bars **a, c** = 500 µm; **b, d** = 50 µm.
Neurochemical characteristics of the Arc

Somata of Arc neurons expressed CR, nNOS, and NPNFP and not CB, which was expressed in puncta. Expression of CR in Arc neurons was also reported by Stonebridge et al. (2020). These data show that the neurochemical profile of the Arc is different from that of the IO in the absence of immunostaining for CB in Arc somata. CR is expressed in somata of both structures (Baizer et al. 2011b; Stonebridge et al. 2020). Expression of CB and CR in IO neurons has also been seen in other species (Yu et al. 2014; Celio 1990). There was immunolabel for NPNFP in neurons and processes of the Arc. In other areas of the brain, notably the cerebral cortex, NPNFP is expressed in neurons with long axons (Hof et al. 1990, 1997; Hof and Morrison 1990; Bussiere et al. 2003). Its presence in the Arc and the immunolabel of ventral external arcuate fibers is consistent with the idea of the Arc as a precerebellar nucleus, with long projections to the cerebellum (Olszewski and Baxter 1982; Rasmussen and Peyton 1946; Mikhail and Ahmed 1975).

The Arc and the RTN: respiration

It has been widely accepted that Arc is the site of chemosensory neurons necessary for brainstem control of respiration and that Arc aplasia or hypoplasia underlies SIDS (Filiano and Kinney 1992, 1994, 1995, 2003, 2004; Kinney 2009; Kinney and Filiano 1988; Kinney et al. 1992; Franciosi and Segura 2004; Matturri et al. 2002). However, since that initial hypothesis, the localization of the chemosensitive cells critical for respiration has been refined. These cells are found in a nucleus called the retrotrapezoid nucleus (RTN; Akilesh et al. 1997; Cream et al. 2002; Fernandes-Junior et al. 2020; Guyenet et al. 2019; Holloway et al. 2015; Bodineau et al. 2000; Burke et al. 2015; Bourgeois et al. 2019). Neurons in RTN express the transcription factor Phox2b (Stornetta et al. 2006; Kanbar et al. 2010; Onimaru et al. 2009; Kang et al. 2007; Wang et al. 2013, 2014). The discovery of this marker has allowed the localization of the RTN in multiple species including humans (Levy et al. 2019; Lavezzi et al. 2012; Rudzinski and Kapur 2010). The RTN in humans is well rostral and dorsal to the Arc (Rudzinski and Kapur 2010; Lavezzi et al. 2012). These data suggest that the aplasia of the Arc is not the critical cause of SIDS. This interpretation is consistent with the reports that the human Arc is small or absent in a set of adult human cases (Paradiso et al. 2018), and consistent with our data showing individual variability in Arc size. Furthermore, a number of studies have found other CNS abnormalities in SIDS (Ambrose et al. 2018, 2019; Jaster et al. 2008; Kinney et al. 2003; Lavezzi et al. 2012).
The aplasia or hypoplasia of the Arc in neuropathological reports may correlate with other brainstem deficits that are more directly responsible for death. While the reevaluation of the role of the Arc in respiration has been recognized in some studies (Presti et al. 2014), the idea still persists in others (Stonebridge et al. 2020). Understanding the mechanisms of SIDS depends on accurate identification of the critical brain structures, as does understanding the function of the Arc.

The Arc and the pontine nuclei

We would argue in favor of that the older view of the Arc as a precerebellar nucleus. Olszewski and Baxter (1982) and Rasmussen and Peyton (1946) suggested that the Arc is composed of caudally displaced pontine neurons. Our data show with scattered immunostained fibers in the adjacent py. a The Arc in a Nissl-stained section; this section was shown at lower magnification in Fig. 1e. b PV immunoreactivity on a section about 400 µm more caudal. Note the very sparse immunostaining in the Arc.

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The neurons of the Arc are similar in size, shape, and density to the neurons of the pontine nuclei (Fig. 1c, h, j, l). If the Arc is composed of pontine neurons, it would be expected to receive glutamatergic input from cortical pyramidal cells (Conti et al. 1987) and send glutamatergic projections to the cerebellum (Gibson et al. 1977; Glickstein et al. 1985, 1972; Robinson et al. 1984; Bjaalie 1986; Brodal 1968a, b, 1978a; Bjaalie and Brodal 1997; Bjaalie et al. 1997a, b; Brodal and Bjaalie 1992, 1997; Ramnani et al. 2006; Border and Mihailoff 1991). Evidence for corticospinal collaterals to the Arc was discussed by Mikhail and Ahmed (1975); projections to the cerebellum via the inferior cerebellar peduncle were described by Rasmussen and Peyton (1946; they also discussed the older literature supporting projections to cerebellum). However, information processing in the Arc may be more complex than expected in a simple relay nucleus. We found immunolabel with GABA-related antibodies, and

Fig. 8 Parvalbumin is not expressed in neurons or processes in the Arc. a Arc in a Nissl-stained section; this section was shown at lower magnification in Fig. 1e. b PV immunoreactivity on a section about 400 µm more caudal. Note the very sparse immunostaining in the Arc.

2012, 2019; Machaalani and Waters 2014; Paine et al. 2014). The aplasia or hypoplasia of the Arc in neuropathological reports may correlate with other brainstem deficits that are more directly responsible for death. While the reevaluation of the role of the Arc in respiration has been recognized in some studies (Presti et al. 2014), the idea still persists in others (Stonebridge et al. 2020). Understanding the mechanisms of SIDS depends on accurate identification of the critical brain structures, as does understanding the function of the Arc.

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**Fig. 9** GABAergic activity in the Arc. For each pair of images, the rectangle on the left indicates the position of the image on the right. 

a. Immunostaining for GAD in the midline Arc. The section is about 0.5 mm rostral to the section shown in Fig. 3c. Unevenly distributed puncta. b. Immunostaining shows GABA A receptors in the midline Arc. The section is about 1 mm caudal to the section in Fig. 1g. c. Puncta surrounding neurons (arrow) and along processes (arrow-head). d. Immunostaining for GABA B receptors shows labeled neurons in rostral Arc. e. There is punctate label surrounding somata (arrow). Scale bars: a = 500 µm; c = 200 µm; e = 250 µm; b, d, f = 50 µm
that finding suggests that there is also inhibitory input to the Arc. The punctate label for GAD and GABA receptors is not uniform, consistent with the idea that there may be intermingling of neurons with different inputs and outputs, again suggesting more complex circuitry. Complexity is also suggested by several studies that have described cholinergic receptors in Arc, consistent with cholinergic input (Machaalani and Waters 2003a, b; Machaalani et al. 2011). We found the only occasional presence of an Arc in the chimpanzee brain (Baizer et al. 2013a); the idea that these are misrouted pontine neurons is more intuitively appealing than the idea that an entire nucleus is present in some brains and not in others. Examination of the neurochemical characteristics of pontine nuclei neurons might be useful both for testing the hypothesis that the neuronal populations are the same as well as providing further information about information processing in the pontine nuclei.

There are many remaining questions about the connections and functions of the Arc. An especially important question is why it is present in only a restricted set of species. Answering these questions poses a particular challenge, since the Arc is not present in species in which invasive anatomical and electrophysiological experiments designed to understand connections and information processing are possible.

**Author contributions** JSB designed the study, stained sections, analyzed results, and wrote the manuscript. SFW founded the Witelson Normal Brain Collection and made tissue and records available for this study. CJW calculated the cArc areas using ImageJ software and catalogued the sections used for the study.

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**Availability of data and materials** All slides for the photomicrographs shown in the paper and for the analysis of the Arc extent are available in the laboratory of Dr. Baizer.

**Code availability** Not applicable.

**Declarations**

**Conflict of interest** None.

**Ethical approval** None required.

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