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 nucleus, presumably motor in function. However, several studies have found aplasia of the Arc in babies who died of SIDS (Sudden Infant Death Syndrome) suggesting a very different role for the Arc in the 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 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 develop a neurochemical profile of this nucleus. Our data suggest that the Arc consists of rostral and caudal subdivisions. There is some degree of left-right asymmetry in Arc position, size and shape at all rostral-caudal levels. Neurons in the Arc express calretinin (CR), neuronal nitric oxide synthase (nNOS) and nonphosphorylated neurofilament protein (NPNFP) but not calbindin (CB) or parvalbumin (PV). There is also immunostaining for GAD and GABA receptors suggesting inhibitory input to Arc neurons. We support the classical idea of the Arc as a precerebellar nucleus; its absence in SIDS cases may not be the cause of death but rather a correlate of other brainstem abnormalities.

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), and cat (Berman 1968) and rhesus monkey (Paxinos et al. 2000). A brainstem nucleus called the Arc was proposed in mice, but its homology with the human Arc questioned on the grounds of neurochemical differences between the mouse and the human nuclei (Fu and Watson 2012).

Classically, the Arc is considered a precerebellar nucleus, with a role in motor function (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; Kinney et al. 1992; Maturri et al. 2000; Filiano and Kinney 1995, 1994; Kinney and Filiano 1988; Franciosi and Segura 2004; Biondo et al. 2003; Folgering et al. 1979). The interpretation of these observations was that the Arc is the site of chemosensitive neurons that are critical for respiration and therefore survival (Filiano et al. 1990). 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.

There is thus uncertainty about the function of the Arc. It is also clear that there is significant individual variability in the size and position of the Arc in 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, atlas of Paxinos and Huang (1995) shows the Arc as a small caudal cell group ventrolateral to the py and well caudal to the pontine nuclei (Figs. 19 and 20, Obex + 2 and + 3 mm).

Very little is known about transmitters, receptors and patterns of expression of calcium-binding proteins in neurons of the Arc. Such information could be useful in understanding the substrate for information processing in the Arc. We have examined Nissl and immunostained sections of 13 brainstems from the Witelson Normal Brain Collection. The results confirm individual variability in size and shape of the Arc, but show similarity across cases in its neurochemical characteristics. The data also suggest that the Arc may consist of distinct caudal and rostral subdivisions.

Material And Methods

We studied Nissl and immunostained sections from brainstems from the Witelson Normal Brain Collection (Witelson and McCulloch 1991). Table 1 shows the critical parameters of the cases including age, sex, and post-mortem interval (PMI). Some sections had been prepared for studies of other brainstem structures (Baizer et al. 2018; Baizer 2014; Baizer et al. 2013b; Baizer et al. 2011a; Baizer et al. 2011b; Baizer and Broussard 2010; Baizer et al. 2007). We here include data from several previously unpublished cases.

Table 1
Cases studied.

Witelson Normal Brain Collection				
	Case #	Age	Sex	PMI (hr)
1	125	57	m	5
2	150	63	m	3
3	155	50	f	9
4	158	51	m	1
5	164	45	f	3
6	166	65	f	3
7	167	55	f	2
8	168	69	m	3
9	169	70	m	2
10	171	63	f	6
11	176	71	f	3
12	180	54	m	2
13	183	69	m	2

Histological procedures. The details of tissue acquisition were given in Witelson and McCulloch (1991). The brains were stored in 10% formalin. The brainstems were dissected away from the cerebrum and cerebellum and then cryoprotected in 15% then 30% sucrose in 10% formalin. Prior to sectioning, we made a small slit was made along one side of the ventral brainstem to allow identification of left and right sides of the brain. This slit can be seen in some of the photomicrographs. Forty μm thick frozen sections were cut on an AO sliding microtome in a plane transverse to the brainstem. All sections were collected and stored in plastic compartment boxes, 5 sections/compartment, at 4°C in 5% formalin. Sets of Sect. 2 mm apart from each case were stained with a Nissl stain, Cresyl Violet (CV), following a standard protocol (LaBossiere and Glickstein 1976). Additional sections were stained as needed to further define the extent of the Arc.

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.

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 Rα1 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

Data Analysis and Photography. Sections were examined with a Leitz Dialux 20 light microscope, and digital images (1600 x 1200 pixels) captured with a 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).

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 subdivisions of the Arc. Abbreviations are listed in Table 3.

Results

Size and location of the Arc.

We analyzed Nissl-stained sections from 13 cases (Table 1). We found an Arc in each case, but variability among cases in the location, size, shape, and degree of left-right asymmetry of the Arc. We tentatively divide the Arc into caudal and rostral subdivisions. The caudal subdivision (cArc) consists of neurons located ventrolaterally to the py whereas the rostral Arc (rArc) is found ventromedially to the py with neurons extending up the midline. In some cases, the neurons and processes of the Arc are not restricted to the surface of the brain but invade the py. We will first illustrate the appearance of the Arc in Nissl-stained sections from a single case, and then show examples from other cases illustrating key aspects of the variability. We will then illustrate the neurochemical characteristics of the Arc.

The Arc in Case 158. The Arc in this case has a rostro-caudal extent of about 8 mm. The shape and position of the Arc cell groups in this case were different at caudal and rostral levels, we 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 (Figs. 1e, f, 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 quite 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.

Variability in cArc and rArc. In Case 158, the cArc consisted of a small cell group ventrolateral 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 3 cases (Fig. 2b,d,f) were similar to those in the first case (Fig. 1c) in sizes, shapes, and density.

There was also variability in the configuration of the Arc at more rostral levels. The rArc consists of neurons that are ventromedial to the py as well as neurons in 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 ventromedial

band (arrowhead) narrower, with marked asymmetry. In Case 176, the midline structure was even broader (Fig. 3e, arrow) while the ventromedial band (arrowhead) 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. This discontinuity again supported the idea that cArc and rArc are independent subdivisions.

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 vestibular nuclei (Baizer and Broussard 2010). 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 of GABAergic input to Arc neurons.

CR. Cells 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 compare Nissl and nNOS immunostaining in the Arc of Case 158. At the level illustrated there are many Arc neurons ventromedial 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, 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. A similar pattern of very dense immunostaining both along the midline and

ventromedial 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 quite 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.

GABA as a transmitter in the Arc. Both the connections and the nature of information processing in the Arc are mysterious. We also used antibodies to GAD_{65/67}, 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 show immunostaining for GAD_{65/67}, defines the region of the midline and ventromedial 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 show 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 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 perspective and then summarize the data concerning a proposed role for the Arc 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, but its relative size and shape varied among cases. In the case that we illustrated in 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. 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. In the atlas of Paxinos and Huang (1995) only the cArc was shown. This discontinuity leads to the suggestion that the Arc should be considered as two subdivisions, the caudal subdivision in which neurons are ventrolateral and the rostral subdivision in which neurons are found ventromedially and also continue dorsally along the midline. The overall appearance of constituent neurons is the same in cArc and rArc. 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. 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).

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; Baizer et al. 2018). We saw no cases in which 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). 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). Our data clearly show that there is variability in brainstem structures in humans as well, but the functional correlates 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. 2013a; Baizer et al. 2011b). 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; Volkman 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).

Neurochemical characteristics of the Arc. Somata of Arc neurons expressed CR, nNOS, and NPNFP and not CB. Expression of CR in Arc neurons was also reported by Stonebridge et al. (2020). These data show again that the neurochemical profile of the Arc is different from that in 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; Hof and Morrison 1990; Bussiere et al. 2003; Hof et al. 1997). Its presence in the Arc is consistent with long-distance projections to cerebellum.

Information processing in the Arc. The paper of Mikhail and Ahmed (1975) stated that “it is believed that the nucleus receives “different impulses” by way of collaterals of the corticospinal tract. These observations suggest that the Arc is a simple relay nucleus, receiving signals from cortex and sending efferents to the cerebellum. This would be a similar pattern of connections as seen for pontine nuclei (Gibson et al. 1977; Glickstein et al. 1985; Glickstein et al. 1972; Robinson et al. 1984; Bjaalie 1986; Brodal 1978b, a; Bjaalie and Brodal 1997; Bjaalie et al. 1997; Brodal 1968a, b, c; Brodal and Bjaalie 1997, 1992; Ramnani et al. 2006).

However, the data from immunolabel with GABA-related antibodies suggest that there is also inhibitory input to the Arc, suggesting more complex information processing. The data are also consistent with the idea that there may be intermingling of neurons with different inputs and outputs since the punctate label for GAD and GABA receptors is not uniform. The source of the GABAergic input is not known.

The Arc and the RTN: Respiration. There are many studies reporting aplasia or hypoplasia of the Arc in children who died of SIDS (Filiano and Kinney 1994, 1992, 1995; Kinney 2009; Kinney and Filiano 1988; Kinney et al. 1992; Franciosi and Segura 2004; Matturri et al. 2003; Matturri et al. 2002; Matturri et al. 2004). The Arc has also been implicated in apnea with Multiple System Atrophy (Benarroch et al. 2007). These data have been interpreted as showing that the Arc is the site of chemosensory neurons and hence critical for survival. One caution about these studies concerns how many sections were evaluated. Since the Arc is variable among cases and can be discontinuous, examination of closely spaced sections is needed to confirm/deny its presence. It is possible it was small rather than absent in some of the neuropathological cases. A much more important point, however is the reevaluation of the role of the Arc in respiration. The initial view of the Arc as the locus of chemosensitive neurons critical for respiration was proposed on the general similarity in microscopic appearance of Arc neurons to putative chemosensitive neurons in the ventral medulla of the cat (Filiano et al. 1990). However, since that hypothesis, the localization of the chemosensitive cells critical for respiration has been refined. Such 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; Wang et al. 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 factor in 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). Further, a number of studies have found other CNS abnormalities in SIDS, (Ambrose et al. 2019; Jaster et al. 2008; Kinney et al. 2003; Lavezzi et al. 2012; Lavezzi et al. 2019; Ambrose et al. 2018; 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 brainstem structures.

We would argue that the Arc should be considered a precerebellar nucleus. Olszewski and Baxter (1982) proposed that the Arc neurons were caudally displaced pontine neurons. The neurons of the Arc are similar in size, shape and density to the neurons of the pontine nuclei (Fig. 1C,H,J, L), consistent with that suggestion. However, embryological analysis found that the Arc neurons arise earlier in development than do the pontine neurons Essick (1912), suggesting that they are separate anatomical and functional entities. Presumably, projections from the Arc to the cerebellum are another source of mossy fibers. However, there are many remaining questions about the details of the connections and information processing in the Arc, and 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 anatomical and electrophysiological experiments designed to understand connections and information processing are possible.

Declarations

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Availability of data and material. All slides for the photomicrographs shown in the paper are available in the laboratory of Dr. Baizer.

Code Availability. Not applicable.

Authors' 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.

Ethics approval. None required.

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Figures

Figure 1

The Arc in Case 158 shown on 8 sections through its rostro-caudal extent (16 mm). a is the most caudal. The rectangles in panels b, g, i, f show the location of the higher magnification images in the panels immediately to the right (c, h, j, l). a. The arrow indicates the Arc on the right, a small cell group ventral to the py. It is present on the left as well but note the left-right asymmetry in size. The section is caudal to the appearance of the IOpr. b. The Arc on a section about 2 mm more rostral, again the Arc is present bilaterally but asymmetrical in size. The section is about at the level shown in Plate VIII of Olszewski and Baxter (1982). The rectangle shows the location of the higher magnification image in c. c. Neuronal size and spacing in the caudal Arc. d. The Arc on a section 4 mm more rostral. On both sides the neuronal group is closer to the midline than in c. e. This section is 1 mm more rostral than the section in d with the Arc neurons present ventral to the py and extending up the midline (arrow on the left). f. Section 2 mm more rostral showing the Arc along the midline (arrow on the left). g. Section 3 mm more rostral showing extensive development of the Arc along the midline on the left (arrow). The rectangle shows the location of the higher magnification image of neurons in h. h. Neurons appear randomly scattered and examples with round, oval, elongated and polygonal somata are all seen. i. 2 mm further rostral the Arc is at its largest; it is present ventromedial to the py and extends up the midline. The rectangle shows the location of the image in j. j. Again, neurons appear randomly scattered with a number of different soma shapes. k. Section at the level of the appearance of the pontine nuclei (pn) about 2 mm more rostral than i. The Arc is still present along the midline. Jj Neurons in the pn are similar in size, shape and density to those in the Arc. Scale bars: a, b, d, e, f, g, i, k = 1 mm; c, h, j, l = 100 μ m.

Figure 2

Variability of the size and shape of the caudal Arc; 3 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 .

Figure 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 ventromedial to the py on the right, the band on the left is bigger (arrowhead). The midline Arc neurons 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 .

Figure 4

Neurons in the Arc express calretinin (CR). a. Nissl-stained section showing neurons in the rostral midline Arc. b. An adjacent section showing CR-ir neurons and processes in the Arc. The arrow indicates CR-ir neurons in the IOpr. c. The rostral midline Arc in a Nissl-stained section of another case. This section is mm rostral to the section in Fig. 2e. d. Again, the neurons and processes of the Arc express CR. Scale bars: a-d = 500 μm .

Figure 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 Figure 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 μ m; d= 50 μ m.

Figure 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. c. Extensive immunolabel of the rArc showing invasion of the py. This section is about .5 mm caudal to the Nissl section shown in Fig. 1I. Scale bars a, c = 500 μm ; b, d= 50 μm .

Figure 7

Calbindin (CB) expression in the Arc is found in puncta not somata. a. Nissl-stained section in rostral Arc. b. Adjacent section immunostained for CB. The Arc is immunostained but somata are not apparent. The rectangle shows the location of the higher magnification image in c. c. Uneven immunostaining of puncta with darker (arrow) and lighter regions. Somata are not immunostained. d. Nissl-stained section of rArc from another case. e. Adjacent section immunostained for CB; the entire Arc shows immunolabel. The rectangle shows the location of the image in f. f. Uneven immunostaining of puncta with darker (arrow) and lighter regions. Somata are not immunostained. Scale bars: a, b, d, e = 500 μm ; c, f = 50 μm .

Figure 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 with scattered immunostained fibers in the adjacent py. c. The Arc in a Nissl stained section from another case. d. Lack of PV-immunostaining in the Arc on a section about 200 μm more rostral. Again, there are PV-ir fibers in the py. Scale bars: a, b, c, d = 250 μm .

Figure 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 .5 mm rostral to the section shown in Fig. 3c. b. Unevenly distributed puncta. c. Immunostaining shows GABA A receptors in the midline Arc. The section is about 1 mm caudal to the section in Fig. 1g. d. Puncta surrounding neurons (arrow) and along processes (arrowhead). e. Immunostaining for GABA B receptors

shows labeled neurons in rostral Arc. f. There is punctate label surrounding somata (arrow). Scale bars: a = 500 μm , c = 200 μm ; e = 250 μm ; b, d, f = 50 μm .