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Comparative Study of the Distribution and Localization of Neuroglobin Expression in the Mammalian Brain: A Literature Review

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ABSTRACT

Neuroglobin (Ngb) was recently identified as a member of the vertebrate hemoglobin family. Several studies have been conducted on Ngb in mammals, but none have compared its expression and localization among different mammals. This review compared the distribution and localization of Ngb expression and explained the different functions of Ngb in the brains of mammals. Intel's integrated performance primitive (IPP) analysis was employed to obtain the expression levels in each region of the mammalian brain. Ngb is widely expressed in the adult yak brain and distributed in different areas, similar to its expression in cattle. The relative expression of the Ngb gene in the cerebral cortex (262.69±9.19) was significantly higher than that in the cerebellar cortex (137.00±7.29), hippocampus (1.00±0.22), medulla oblongata (3.43±0.76), striatum (7.65±0.61) and olfactory bulb (2.14±1.22). Findings in the rat brain showed low Ngb protein expression. The mouse brain showed Ngb over expression in a transgenic variant (Ngb-Tg), while in the human brain, the level of Ngb was higher in the hypothalamus, amygdala and pontine tegmental nuclei than in other parts of the brain. The expression levels, distribution and localization of Ngb differ across the brains of different mammals, so it is appropriate to explore the precise distribution and localization of Ngb before comparison or analysis in these mammals.

Key words: Brain, Distribution, Expression, Localization, Mammals, Neuroglobin.

Neuroglobin (Ngb) was identified as a novel endogenous neuroprotectant by scientists but the regulatory role of this protein still remains under debate (Adelman *et al*., 2000). Although it is a hypoxia-inducible protein, Ngb has a cytoprotective effect in Alzheimer's disease and related disorders in animal model of stroke (Adelman *et al*., 2000). Ngb binds reversibly to oxygen and has varying levels of expression in different areas of the brain (Burmester *et al*., 2000; Dewilde, 2001). Ngb in mammals protects neuronsin the brain from hypoxic-ischemic insults and experimentally induced stroke *in vivo* (Sun *et al*., 2001; Sun, 2003). Additionally, *Ngb* is 151 amino-acids long with a molecular mass of 17 kDa which has shown to promote neuron survival under hypoxia and could potentially limit brain damage (Burmester *et al*., 2000; Dewilde, 2001). It is an intracellular hemoprotein expressed in the central and peripheral nervous system, cerebrospinal fluid, retina and endocrine tissues (Pesce *et al*., 2002). It has hexa-coordinated heme-Fe atoms that display O_2 affinities comparable to those of myoglobin (Pesce *et al*., 2002; Reuss *et al*., 2002). It is hypothesized that this protein enhances the O_2 supply to the mitochondria of the metabolically active neurons and resides in metabolically active cells and subcellular compartments (Pesce *et al*., 2002). Also, the concentration of neuroglobin is closely correlated to the distribution of mitochondria; however, it is not entirely localized in specific organelle (Reuss *et al*., 2002). The exact localization of Ngb is still unclear. Several studies have demonstrated the expression of Ngb in mammalian brains, but no study has compared its expression level and localization in different mammals. Ngb has been found in the cerebral cortex, cerebellar cortex, hippocampus, medulla oblongata, striatum and olfactory bulb of the adult yak (Tong-fang *et al*., 2015). This research also showed the Ngb distribution in brain tissues (Tong-fang *et al*., 2015). In every area of the brain, Ngb decreases with increasing age, stabilizing at approximately 24 months (Ni *et al*., 1992). Research conducted on the rat brain showed that Ngb expression changed minimally in the control group than the subarachnoid hemorrhage (SAH) (Wei-De *et al*., 2013).

Ngb was shown to be highly expressed in the brains of mice with traumatic brain injury (TBI); specifically, TBI resulted in significantly increased expression of Ngb, with the post injury protein levels significantly higher than the pre injury levels (Zhao *et al*., 2012). According to this report

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(Zhao *et al*., 2012), higher expression of Ngb limits the volume of traumatic lesions, by reducing oxidative stress. Research has shown that human Ngb was previously present in uncharacterized cDNA databases (expressed sequence tags, ESTs), as shown in both the mouse and human brains (Burmester *et al*., 2000). Following this study, several other investigations were conducted involving Ngb in humans. The results mentioned here regarding the human brain showed that Ngb is highly expressed in various regions (the hypothalamus, amygdala and pontine tegmental nuclei), but not in the hippocampus (Hundahl *et al*., 2013). The expression of Ngb in the cerebral cortex is limited but it is enhanced and controls stroke in the human brain (Hundahl *et al*., 2013). In addition, scientists have shown that Ngb might be a target for pharmaceutical intervention because it is widely expressed in the human brain and plays a regulatory role in the peri-infarct zone of stroke patients (Jin *et al*., 2010). Ngb is also widely expressed in other animals, but this review focuses on the above mentioned mammals. Given the reported results, it is appropriate to explore the Ngb distribution and localization in detail before conducting a more thorough investigation.

Survey methodology

Several studies have been conducted on the expression of Ngb in mammals. However, only a few of these studies have presented explanations for the distribution and localization of Ngb expression and none of them have compared the distribution and localization patterns across different mammals. Therefore, this study collected, documented and compared detailed results on Ngb in different mammals. The literature search was conducted by using the following search term: "The distribution and localization of neuroglobin expression in mammals". As there are few studies on the distribution and localization of Ngb expression, the authors included available information found in scientific databases, from reading available books and reports and from searching scholarly journals for research articles on the mammals involved in this study. In addition, Intel's integrated performance primitives (IPP) analysis was employed to obtain the level of Ngb expression in each region of the mammalian brain. In this literature review, the authors respected the original authors' definitions, descriptions, methodology and the reported results. During the literature review search, various information and results were obtained on the expression of Ngb, but the review's objectives were prioritized.

The authors are aware that there might be doubts with regard to the analytical results presented in some of the figures or tables. It should be understood, however, that each laboratory uses a specific analytical tool and that it is practically impossible to streamline the results in such a way that they could be compared using the same standards. The readers are referred to the original articles for further details. Below are the methods used by the original authors to obtain specific results.

A study conducted on the adult yak showed the distribution of Ngb in the yak brain (Tong-fang *et al*., 2015) (Fig 1). The results showed that Ngb could be expressed in the following areas: A. Neurons of yak cerebral cortex, I and IV showed the laminae I, VI of cerebral cortex, M. indicates medulla, bar= 200 μm; B. Neurons of yak cerebellar cortex, ML. Indicates molecular, PCL, indicates Purkinje cell, GL. indicates granular layer, M. indicates medulla, bar= 100 μm; C. Neurons of yak hippocampus, bar= 20 μm; D. Striatum, bar= 200 μm; E. Olfactory bulb, bar= 100 μm; F. Medulla oblongata, bar= 100 μm; Arrow shows Ngb-positive cells.

According to a study done by Liang *et al*. (2013) on the distribution of Ngb in different neuronal regions of the adult yak (Fig 2). Ngb was mainly expressed in the following areas of the brain as compared to the study done by Tong *et al*. (2015). The quantities of expression and distribution pattern differ. The localization of distribution almost similar (Plate 1). The expression of NGB in neurons of yak cerebral cortex. I and VI represent the I and VI layers of the cerebral cortex, M. the medulla of the brain, Immunohistochemistry bar= 200 μm (Plate 2). The expression of NGB in Neurons of yak hippocampal shows the NGB positive-cells. Immunohistochemical staining bar= 20 μm (Plate 3). The expression of Ngb in neurons of the yak medulla oblongata. N-neurons show the NGB positive-cells. Immunohistochemical staining bar= 100 μm (Plate 4). The expression of Ngb in neurons of yak cerebellar cortex. ML-Molecular; PCL-Purkinje cell; GL-Granular layer; M-Medulla; shows the NGB positive-cells. Immunohistochemical staining bar= 100 μm (Plate 5). The expression of Ngb in neurons of yak spinal cord. VH-Ventral; WM-White matter; N-Neurons shows the NGB positive-cells. Immunohistochemical staining bar= 100 μm (Plate 6). The expression of Ngb in yak adrenal cortex. Co-Cortex; M-Medulla shows the NGB positive-cells. Immunohistochemical staining bar= 100 μm (Plate 7). The expression of NGB in neurons of the yak cerebral cortex. I and VI represent the I and VI layers of the cerebral cortex, M-the medulla of the brain, Immunohistochemistry bar= 200 μm (Plate 8). The expression of NGB in neurons of yak hippocampal shows the NGB positive-cells. Immunohistochemical staining bar= 20 μm (Plate 9). The expression of Ngb in neurons of the yak medulla oblongata. N neurons show the NGB positive-cells. Immunohisto- chemical staining bar= 100 μm (Plate 10). The expression of Ngb in neurons of the yak cerebellar cortex. ML-Molecular; PCL-Purkinje cell; GL-Granular layer; M-Medulla; shows the NGB positive-cells. Immunohisto-chemical staining bar= 100 μm (Plate 11). The expression of Ngb in neurons of yak spinal cord. VH-Ventral; WM-White matter; N-Neurons shows the NGB positive-cells. Immunohistochemical staining bar= 100 μm (Plate 12). The expression of Ngb in the yak adrenal cortex. Co-cortex; M-Medulla shows the NGB positive-cells. Immunohisto-chemical staining bar= 100 μm (Table 1a) comparison of the studies from Liang *et al*. (2013) and Tong-fang *et al*. (2015) showed the Ngb distribution in different regions of the adult yak brain. Expression was found mainly in the following areas:

A. Yak cerebral cortex neurons: laminae; M indicates medulla (200 μm). B. Yak cerebellar cortex neurons: molecular, Purkinje cell and granular layers; medulla (100 μm). C. Yak hippocampus neurons (20 μm). D. Striatum (200 μm). E. Olfactory bulb (100 μm). F. Medulla oblongata (100 μm) (Tong-fang *et al*., 2015).

The results from Liang *et al*. (2013) showed that Ngb proteins are expressed in the following areas: the neurons of the cerebral cortex (200 μm), cerebral cortex layers I and IV (200 μm), medulla (200 μm), neurons of the hippocampus (20 μm), spinal cord (100 μm), ventral region (100 μm), white matter (100 μm), neurons (100 μm), adrenal cortex (100), cortex (100) and medulla (100 μm) (Liang *et al*., 2013).

Table 2 which was replicated from one of the included studies (Liang *et al*., 2013) on the adult yak, different lowercase letters in each column indicate a normal level of significance ($P<0.05$), while capital letters indicate a high level of significance ($P<0.01$). The Ngb expression levels in different areas of the adult yak brain were significantly different. The relative expression of the Ngb gene in the cerebral cortex was significantly higher than that in the cerebellar cortex, medulla oblongata, striatum and olfactory bulb. The expression level in the hippocampus was different from that in the other regions, with a high level of significance.

Table 3 shows positive (+) = expression of Ngb, negative (-) = no expression. This table shows that Ngb is widely expressed in different regions in mammals.

Table 4 shows the results of double immunofluorescence staining to determine Ngb expression and distribution. The study reported that Ngb was expressed at a lower level in the control group and significantly more highly expressed in the SAH group after 24 h (Wei-De *et al*., 2013). Ngb was expressed in regions similar to those found in the yak study.

Expression levels after subarachnoid hemorrhage (SAH)

Study conducted on the rat brain reported that WB analysis was conducted to show the levels of Ngb protein found in the post-SAH temporal cortex (Wei-De *et al*., 2013). The level of Ngb expression was reported lower in the control group (Wei-De *et al*., 2013). Following induction of SAH, the levels of Ngb protein increased after 24 h and then decreased between 48 and 72 h after SAH (Wei-De *et al*., 2013). There was a significant difference in the expression level between the control group and the SAH group after 24 h. In addition, the quantitative real-time PCR analysis was performed to show the level of Ngb protein expression in the brain of rat (Wei-De *et al*., 2013). The level of Ngb in the control was low, while in the SAH groups (Wei-De *et al*., 2013),

Table 1: Quantitation of Ngb distribution in the adult yak brain.

References	Brain regions	Quantity of expression
Tong-fang *et al*., 2015	Neurons of the cerebral cortex	0.05*
	Laminae (I, VI)	0.44
	Cerebral cortex (VI)	0.33
	Medulla (200 μm)	0.38
	Neurons of the cerebellar cortex	0.03*
	Molecular	0.81
	Purkinje cells	0.14
	Medulla (100 μm)	0.55
	Granular layer	0.13
	Hippocampus (20 μm)	0.10
	Striatum (200 μm)	0.22
	olfactory bulb (100 μm)	0.98
Liang *et al*., 2013	Neurons of cerebral cortex (200 μm)	0.05*
	Cerebral cortex I and IV (200 μm)	0.11
	Medulla (200 μm)	0.06
	Neurons of hippocampal (20 μm)	0.09
	Medulla (100 μm)	0.11
	Spinal cord (100 μm)	0.10
	Ventral (100 μm)	0.14
	White matter (100 μm)	0.12
	Neurons (100 μm)	0.12
	Adrenal cortex (100 μm)	0.06
	Cortex (100 μm)	0.12
	Medulla (100 μm)	0.04

**Significant level.

Source: Authors constructed table.

Table 2: Mean Ct values and relative content of neuroglobin (Ngb) in the brain of yak brain.

Samples	Mean Ct value of the Ngb gene	Mean Ct value of the β-actin gene	ΔCt value	ΔΔCt value	$2^{-\Delta\Delta Ct}$ value
Cerebral cortex	27.57±0.24	27.24±0.14	0.33±0.24	-8.04±0.05	262.69±9.19
Cerebellar cortex	21.99±0.17	20.72±0.11	1.27±0.17	-7.10±0.08	137.00±7.29
Hippocampus	26.75±0.22	18.38±0.04	8.37±0.22	0.00±0.22	1.00±0.22
Medulla oblongata	26.69±0.49	20.08±0.07	6.61±0.49	-1.76±0.31	3.43±0.76
Striatum	26.76±0.19	20.62±0.10	6.14±0.19	-2.91±0.36	7.65±0.61
Olfactory bulb	28.75±0.66	21.33±0.72	7.42±0.66	-0.95±0.80	2.14±1.22

Source: Reproduced from Liang *et al*., 2013.

Table 3: Distribution and expression of neuroglobin (Ngb) in the brain of mammals.

Samples	Yak	Cattle	Rat	Mice	Human
Hypothalamus	+	+	-	-	+
Cerebral cortex	+	+	+	+	+
Cerebellum	-	+	-	-	-
Medulla	+	+	-	-	-
Olfactory bulb	+	+	-	-	-
Striatum	+	+	-	-	+
Spinal cord	+	-	-	-	-
Adrenal cortex	+	-	-	-	-
Temporal cortex	-	-	+	-	-
Laminae	+	-	-	-	-
Cerebellar cortex	+	-	-	-	-
Periaqueductal gray	-	-	-	-	+
Hindbrain	-	-	-	-	+
Lateral tegmental area (LTA)	-	-	-	-	+
Purkinje cells	-	+	-	-	-

Positive (+) = Expression of Ngb, (-) = No expression. in this table, Ngb is widely expressed in the brain regions of mammals.
Source: Authors constructed the table.

Table 4: Ngb expression in the control group/level of Ngb expression in the control group.

Factors	Group I		Group II		Group III		References
	Control	24h	Control	24h	Control	24h	
Ngb	0.50	0.014	0.13	0.18	0.10	0.44	Wei-De *et al*., 2013
Cell type	0.52	0.35	0.50	0.05*	0.22	0.45	Wei-De *et al*., 2013
DAPI	0.12	0.41	0.60	0.27	0.47	0.01**	Wei-De *et al*., 2013
Merge	0.36	0.92	0.23	0.55	0.23	0.005**	Wei-De *et al*., 2013

*Significant; **Highly significant. Source: Authors constructed the table.

the Ngb levels increased early after 3 h after SAH and reached a maximum at 6 h, which was three times than the control group. The Ngb rate gradually decreased after this time.

Immunohistochemical study of Ngb after subarachnoid hemorrhage (SAH)

In a study by Wei *et al*. (2013). immunohistochemical analysis was performed to locate Ngb in the control group and the SAH group after 24 h. Table 4 shows Ngb-positive cells in the temporal cortexes of the control group and the SAH group after 24 h. Less Ngb was observed in the control group. The SAH group showed significantly more Ngb-positive cells in the cerebral cortex after 24 h (Wei-De *et al*., 2013). Ngb expression was also observed in neurons (Wei-De *et al*., 2013). The semi-quantitative results demonstrated that the level of Ngb was significantly different in the temporal cortexes of the SAH group (80.5%) and the control group (55.3%) after 24 h ($p<0.01$).

Ngb expression among the mammals

According to the study performed by Hundahl *et al*. (2013). Ngb has a smaller distribution, weaker expression and fewer effects on neuronal morphology in the human brain than in the rodent brain. Low levels of Ngb expression were found in small neurons of the cerebral cortex and the protein was less widely distributed in the medium-sized neurons but had clear expression (Hundahl *et al*., 2013).

As in yaks and mice, Ngb was also found in the cerebral cortex, but the relative expression level differed. Sections of the hypothalamus showed that Ngb-IR was scattered and observed in the processes of large neurons. As in yaks, Ngb was also found in the hypothalamus (Tong-fang *et al*., 2015). No Ngb-IR was observed in the striatum of humans, unlike yaks (Tong-fang *et al*., 2015). The greatest distribution was observed in the nuclei of the hindbrain pontine, while the strongest Ngb expression in the yak was in the cerebral cortex (Hundahl *et al*., 2013). Overall, Ngb was highly co-expressed in the laterodorsal tegmental area and weakly expressed in other brain areas (Hundahl *et al*., 2013).

Yak

The distribution and expression of Ngb in various regions of the adult yak brain were demonstrated by the immunohistochemical staining ISPs method and real-time fluorescence quantitative PCR as shown in Fig 1 (Tong-fang *et al*., 2015). The results indicated that Ngb was widely distributed in different regions of the adult yak brain (Tong-fang *et al*., 2015), while in the human brain (Hundahl *et al*., 2013), Ngb had a more limited levels of distribution, weaker expression and fewer effects on neuronal morphology (Tong-fang *et al*., 2015). These differences could be the result of the varied methods and laboratory procedures used in each study. Ngb participation in the uptake and storage of oxygen by nerve cells can improve the rate of oxygen usage by nerve cells (Sun *et al*., 2001). Ngb upregulation can protect nerve cells, improving the tolerance of brain tissue to ischemia and hypoxia and reducing damage to the brain under these conditions (Greenberg *et al*., 2001). As yaks live in a high-altitude hypoxic environment for a long period, the levels of Ngb in different regions of the brain perform key functions in enhancing the oxygen utilization rate (Zhang *et al*., 2008). The nervous system maintains the normal physiological function of the brain (Zhang *et al*., 2008). Due to the high expression of Ngb in functional nuclei, the function of oxygen storage may be closely related (Dewilde, 2001); however, this expression may also reflect the difference in activity and oxygen consumption in different areas of the brain. In addition, the distribution of Ngb in other regions of the brain and in the cells of the yak may also be related to the oxygen-consuming activities of these regions and cells (Zivin, 2008). First, the expression of the Ngb gene in different regions of the yak brain was found by fluorescence quantitative PCR and the results showed significant differences in the expression of Ngb in various areas of the yak brain. The level of Ngb quantity of expression in the cerebral cortexwas the most significant (Tong-fang *et al*., 2015). Compared to its expression in humans, a large amount of Ngb was observed in the hypothalamus, but the difference was not significant (Hundahl *et al*., 2013). Both yaks and mice showed Ngb in the cerebral cortex, but the levels of expression differed (Tong-fang *et al*., 2015; Wei-De *et al*., 2013). The rat brain also showed higher expression in the cerebral cortex, but the difference was not significantly different compared to the other brain regions (Guo *et al*., 2011). The positive expression of Ngb in the cerebral cortex of yaks was significantly higher than that in the cerebellar cortex, hippocampus, medulla oblongata, striatum and olfactory bulb (Wang *et al*., 2004).

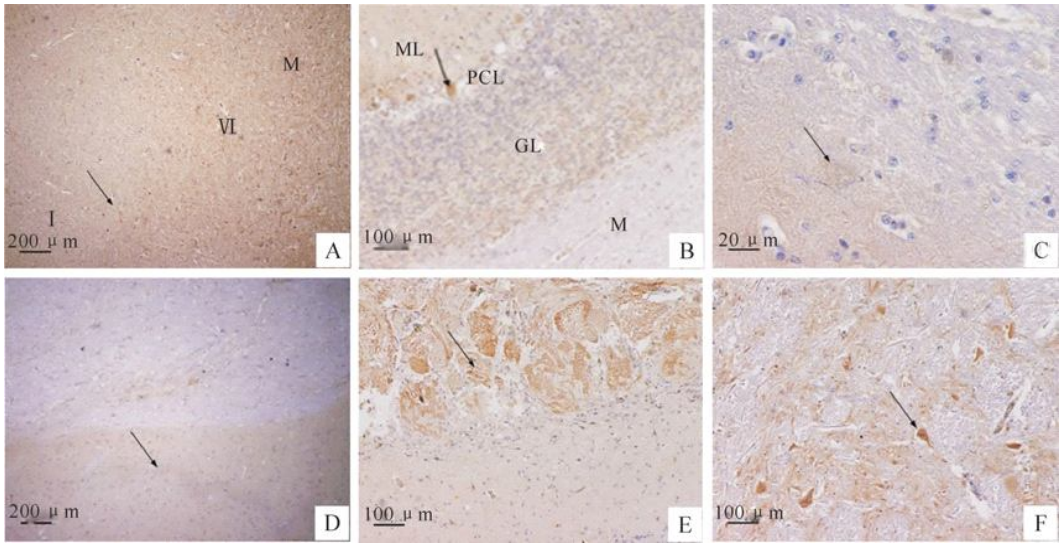

Fig 1: Distribution of Ngb in different areas of the adult yak brain (Tong-fang *et al*., 2015).

A study conducted on the adult yak showed the distribution of Ngb in the yak brain (Tong-fang *et al*., 2015). The results showed that Ngb could be expressed in the following areas: A). Neurons of yak cerebral cortex, I and IV showed the laminae I, VI of the cerebral cortex, M-Indicates medulla, bar= 200 μm; B). Neurons of yak cerebellar cortex, ML-Indicates molecular, PCL-Indicates purkinje cell, GL-Indicates granular layer, M-Indicates medulla, bar= 100 μm; C). Neurons of yak hippocampus, bar= 20 μm; D). Striatum, bar= 200 μm; E). Olfactory bulb, bar= 100 μm; F). Medulla oblongata, bar= 100 μm; Arrow shows Ngb-positive cells.

Yaks and cattle

The study of Liang *et al.,* used the immunohistochemistry to show the distribution of Ngb in the brain of the adult yak (Liang *et al*., 2013) as shown in Fig 2. All twelve (12) layers of the cerebral cortex contained Ngb-positive cells that were distributed throughout the layers and the level of expression was significantly higher than that in the cerebellar cortex, hippocampus and striatum (Liang *et al*., 2013). Ngb-positive cells were also found in the medulla (Liang *et al*., 2013). The Ngb distribution and localization were similar in the cerebral cortexes of the cattle and yaks (Tong-fang *et al*., 2015). The overall levels of Ngb expression in the brains of cattle were lower than those in the brain of yaks (Liang *et al*., 2013). In the cerebellar cortex of the yak, Ngb-positive cells showed high levels of expression in purkinje cell layers and lower levels in the granular layers (Liang *et al*., 2013). The distribution and localization of Ngb-positive cells in the cerebellar cortex of cattle was similar to that in the yak, but the intensity of the reaction was weaker overall (Liang *et al*., 2013). In various regions of the hippocampus in the yak, Ngb-positive cells were mostly found in pyramidal cells, with positive reaction sites but weak Ngb expression found in nerve processes (Liang *et al*., 2013). The similarities of these results might be due to the identical methods and laboratory procedures used. In separate areas of the cattle hippocampus, the distribution and localization of Ngb-positive cells were similar to those of the yak, but the intensity of the reaction was weaker in the yak brain (Tong-fang *et al*., 2015). Additionally, in the medulla oblongata of cattle and yaks, the distribution and localization of Ngb-positive cells were weakly expressed and the overall intensity was weaker in the cattle than in the yak (Tong-fang *et al*., 2015). Ngb-positive cells in the striatum of the yak were widely distributed in the caudate nucleus and the Ngb-positive reactions were stronger than those in the hippocampus, medulla oblongata and olfactory bulb, but Ngb was more weakly expressed than in the cerebral cortex and cerebellar cortex (Liang *et al*., 2013). In the medulla oblongata of the yak, Ngb-positive cells were mainly distributed in the gray matter (Liang *et al*., 2013) and Ngb-positive cells were also scattered in the white matter (Liang *et al*., 2013). Ngb was also expressed in the mitral cell layer of the yak olfactory bulb, with notable staining and large cells (Liang *et al*., 2013); however, the staining intensity of the Ngb-positive cells was weaker than that of medulla oblongata and stronger than that of the hippocampus (Liang *et al*., 2013). The distribution and localization of Ngb-positive cells in the mitral cell layer of the olfactory bulb of the cattle was similar to that of the yak (Tong-fang *et al*., 2015); the staining intensity was higher than that of the hippocampus, weaker than that of the medulla oblongata and significantly weaker than that of yak (Tong-fang *et al*., 2015). Ngb-positive cells were distributed primarily in the peripheral nerve plexus and ganglia and mostly scattered in some of the nerve

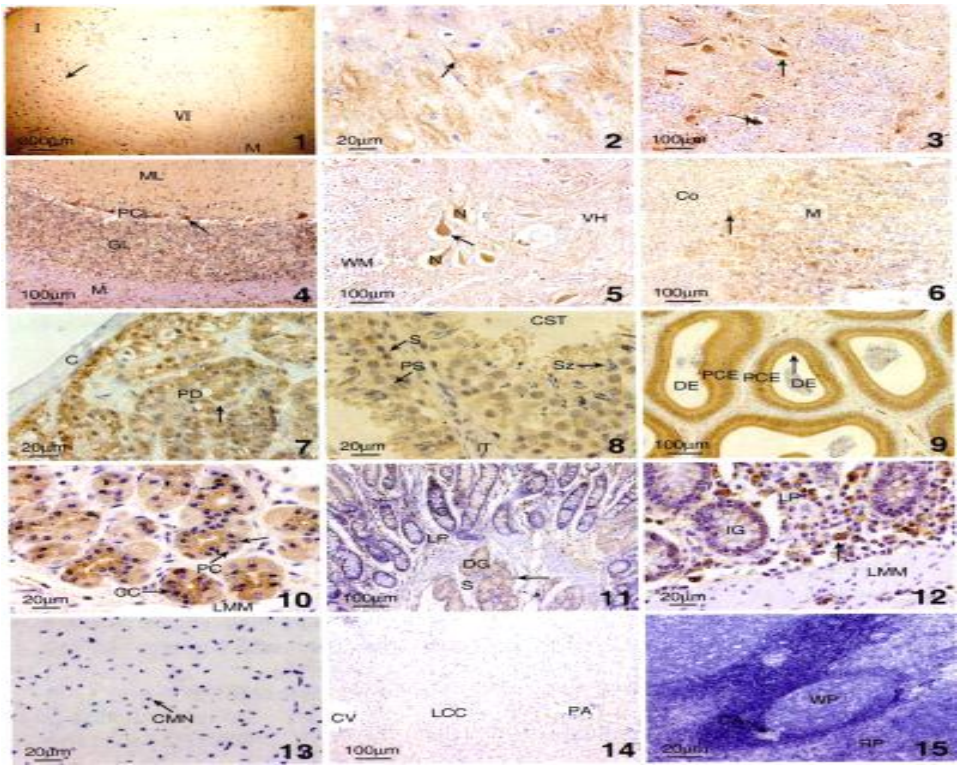

Fig 2: Expression of Ngb in different regions of the yak brain Liang *et al.* (2013).

According to a study done by Liang *et al.* (2013) on the distribution of Ngb in different neuronal regions of the adult yak. Ngb was mainly expressed in the following areas of the brain as compared to the study done by Tong *et al.* (2015). The quantities of expression and distribution patterns differ. The localization of distribution almost similar:

cells in the peripheral nervous system but at low quantities (Liang *et al*., 2013). In the peripheral nervous system of the cattle, the distribution and localization of the Ngb-positive cells were similar to those of the yak, but the intensity of the reactions was on average weaker than that of the yak (Tong-fang *et al*., 2015). Ngb is distributed in the cortex and medulla, similar to that observed in rats and human (Wei-De *et al*., 2013; Hundahl *et al*., 2013).

The $2^{-\Delta\Delta Ct}$ values of the Ngb gene in the adult yak cerebral cortex, cerebellar cortex, medulla oblongata, striatum, olfactory bulb, adrenal gland, abomasum and duodenum tissue were 262.69, 137.00, 3.43, 7.65, 2.14, 7.82, 16.26 and 30.23, respectively (Tong-fang *et al*., 2015). The Ngb gene expression levels in the above brain regions were 262.69 times, 137.00 times, 3.43 times, 7.65 times, 2.14 times, 7.82 times, 16.26 times and 30.23 times higher than the expression levels, respectively, of the hippocampus (Tong-fang *et al*., 2015).

The above reported results are similar to the results obtained with immunohistochemistry (Tong-fang *et al*., 2015). Ngb gene expression was significantly higher in the cerebral cortex and cerebellar cortex than in other areas. In contrast, the lowest expression levels were in the medulla oblongata, olfactory bulb and hippocampus (Tong-fang *et al*., 2015). The $2^{-\Delta\Delta Ct}$ values of the Ngb gene in the cerebral cortex, cerebellar cortex, medulla oblongata, striatum, olfactory bulb, adrenal gland, abomasum and duodenum tissue of the cattle were 165.53, 136.29, 1.53, 1.98, 2.19, 4.18, 12.91 and 20.29, respectively (Tong-fang *et al*., 2015). The Ngb gene expression in the cerebral cortex and the cerebellar cortex was the highest among and significantly higher than that in the other tissues (Tong-fang *et al*., 2015). The lowest expression levels were found in the striatum, medulla oblongata, olfactory bulb and hippocampus (Tong-fang *et al*., 2015).

Expression of Ngb in the nerve cells of the adult yak

Some researchers observed that positive expression of Ngb in the adult yak nervous system was mainly in the brain, which was basically consistent with the results of RNA dot hybridization in humans and mice (Burmester *et al*., 2000). Various parts of the brain showed significant Ngb expression (Tong-fang *et al*., 2015). In the cerebral cortex, positive expression was significantly higher than that in the hippocampus, cerebellum and thalamus (Tong-fang *et al*., 2015). This observation was consistent with the sensitivity of different regions of the brain (Zivin, 2008). Ngb participation in the uptake and storage of oxygen by nerve cells can improve the rate of oxygen usage by nerve cells (Greenberg *et al*., 2001). Ischemia and hypoxia (Greenberg *et al*., 2001) can also enable the upregulation of Ngb expression, protect nerve cells, improve the tolerance of brain tissue to ischemia and hypoxia and reduce damage to the brain under these conditions (Greenberg *et al*., 2001). Higher Ngb expression in the brain is important for enhancing the oxygen utilization rate of the yak nervous system and maintaining the normal physiological function of the brain (Tong-fang *et al*., 2015). Shang *et al*. (2006) used immunofluorescence double-labeling staining to identify the syndromes. Endogenous Ngb is specifically present in rat neurons, which was also observed in the study by Laufs *et al*. (2004). Positive Ngb expression was observed innerve cells, similar to what was reported in the studies by Shang *et al*. (2006) and (Shang *et al*., 2006; Laufs *et al*., 2004) and in astrocytes cultured *in vitro*, as reported by in the studies by Chen *et al*., 2012 and Aviv *et al*. (2010); Khan *et al*., 2008). The expression of Ngb in glial cells could be related to the oxygen-consuming physiological activity of these cells (Avivi *et al*., 2010; Khan *et al*., 2008). The specific location of Ngb needs further confirmation. The distribution and localization of Ngb in the adult yak spinal cord were mainly in poliomyelitis neurons, especially in anterior horn somatic motor neurons, similar to the results of the study by Shang *et al*. (2006) study. The distribution of Ngb in the spinal cord adapts to the function of the corresponding region. In addition, these results indicate that Ngb is widely involved in the physiological activities of the spinal cord. There was no positive expression of Ngb, which was similar to the results of the study by Reuss *et al*. (2002); (Hundahl *et al*., 2011).

Expression of Ngb in the rat brain

A pilot study (Wei-De *et al*., 2013) conducted on the rat brain investigated the expression level and distribution pattern of Ngb in the temporal cortex of an SAH model. The key findings were as follows: (1) The expression of the Ngb protein in the control rat brain was low and it increased gradually after SAH until reaching a maximum 24 h later (Wei-De *et al*., 2013). (2) After SAH, the mRNA of Ngb increased at 3 h and reached a maximum at 6 h (Wei-De *et al*., 2013). (3) In the cerebral cortex, Ngb was highly expressed and the proportion of Ngb-positive cells in the temporal cortex was 80.5% (24 h after SAH group) which was greater than that of the control group (55.3%) (Wei-De *et al*., 2013). (4) In addition to astrocytes, Ngb was observed in neuronal and microglial cells (Wei-De *et al*., 2013). The results showed that Ngb plays an important role in protecting the brain (Wei-De *et al*., 2013). The results from different studies have shown that the Ngb protein increases immediately after injury caused by hypoxic ischemia (Sun *et al*., 2013). In addition, the proportion of Ngb increased dramatically, as indicated by WB, immunohistochemistry and double immunofluorescence staining (Sun *et al*., 2013). As suggested by neuronal expression, Ngb plays an important role following SAH (Sun *et al*., 2013). In 2000, a study conducted by Brumester *et al*. (2000) first identified Ngb in neurons, which was further confirmed in the studies reviewed here. Since its discovery, several studies have been conducted on the structure, molecular mechanisms and neuroprotective effect of Ngb. Some studies have confirmed that Ngb, as a heme proteins contains monomeric. The sequence identity of Ngb with vetebrate myoglobin and hemoglobin has been shown to be 21 and 25% respectively (Brumester *et al*., 2000; Pesce *et al*., 2003).

In contrast to myoglobin and hemoglobin, the proximal and distal histidines located in the pocket of the heme in the Ngb protein can be bind directly to the heme iron (the Fe_2 or Fe_3 oxidation states) because of the his-histidine six-coordinate heme geometry (Dewilde, 2001). A study reported by Kriegl *et al*. (2002) rationalized the six-coordinate bond displacement with the distal histidine 64 residue. The binding of several ligands to heme iron can be enabled by Ngb, not excluding gaseous ligands of diatomic compounds such as nitric oxide (NO) and carbon monoxide (CO) (Kriegl *et al*., 2002; Capece *et al*., 2009). The P_{50} value reported for O_2 binding to Ngb ranges from 1-2 mm Hg at 20°C (Kriegl *et al*., 2002; Capece *et al*., 2009). Because of its similar structure to hemoglobin and myoglobin and its ability to bind oxygen, Ngb functions in O_2 storage and transportation (Kriegl *et al*., 2002). However, the concentration of Ngb is relatively low (11 M) (Brumester *et al*., 2000) and comparatively weak under O_2-binding physiological conditions (Kriegl *et al*., 2002). The key functions of Ngb are O_2 storage and transportation; however, Ngb may function as an O_2 sensor, as reported by Krigel *et al*. (2002). Other results showed that Ngb performs other functions, such as reactive oxygen species (ROS) scavenging in the brain, due to NO binding enabled by Ngb (Dewilde, 2001; Fago *et al*. (2004). The idea that Ngb overexpression enables a decrease in NO-induced cell death was supported by Jin *et al*. (2008). In addition to its ability to function as an O_2 sensor and a ROS scavenger, it is hypothesized that Ngb might also act as a signal transducer (Wakasugi *et al*., 2003). Ngb enables guanine nucleotide dissociation inhibitor (GDI) activity (Wakasugi *et al*., 2003), as reported by other studies, performing important functions for its protective role against hypoxia by interacting with the polarization of cytoskeletal and lipid raft-dependent death signaling through the Rho GTPase pathway (Khan *et al*., 2008), functioning as a redox-regulated nitrite reductase (Chen *et al*., 2012) and activating the Akt signaling pathway (Yu *et al*., 2013). In addition to themolecular mechanisms described above, the mitochondrial mechanisms of Ngb were recently discussed. As indicated by experimental studies, Ngb could interact with voltage-dependent anion channels (VDACs) and inhibit the depletion of hypoxia/oxygen-glucose (OGD), which induces the mitochondrial permeability transition pore (mPTP) to open and release cytochrome c from the mitochondria (Li *et al*., 2008) and the level of Ngb overexpression can attenuate beta-amyloid-induced mitochondrial dysfunction (Duong *et al*., 2009). Despite the controversy concerning the molecular mechanisms of Ngb and its role in neuroprotection has been supported by many *in vivo* and *in vitro* studies (Duong *et al*., 2009). The overexpression of Ngb in mice was reported to result in smaller infract volumes and fewer markers of oxidative stress in the brain after transient focal (Khan *et al*., 2008) or global (Duong *et al*., 2009) ischemia. In a similar manner, the intracerebral administration of an Ngb-expression-increasing adeno-associated virus vector reduced the infarct size in rats after focal cerebral ischemia, while Ngb downregulation worsened ischemic outcomes (Sun *et al*., 2003). The most important factor was the *in vitro* overexpression of Ngb, which reduced the sensitivity to hypoxic reoxygenation injury in a neuronal cell culture (Liu *et al*., 2009). Additionally, increasing evidence has demonstrated the important role of Ngb in protecting neurons against other related neurological disorders beyond hypoxic/ischemic brain injury. For instance, Ngb overexpression has shown protective effects against beta-amyloid and NMDA toxicity in both cultured neurons and an Alzheimer's disease model in mice (Khan *et al*., 2008). All these results confirmed the neuroprotective functions of Ngb.

Expression of Ngb in the mouse brain

Studies have shown that Ngb plays an important role in neuroprotection against hypoxic/ischemic brain injury, stroke and other related neurological disorders (Lin *et al*., 2010; Chuang *et al*., 2010; Shang *et al*., 2012). Other studies have demonstrated that a reduction in tissue infarction volume and markers of oxidative stress is induced by Ngb overexpression in a mouse model of focal stroke (Lin *et al*., 2010). Recently, researchers reported that after TBI, the gene expression of Ngb was increased, but the results were not investigated in detail (Wei *et al*., 2011; Jain, 2008). In this brief study, the hypothesis was tested and the outcomes indicated that Ngb overexpression might play a protective role against traumatic injury in the mouse brain. In addition, three sets of experiments were conducted to examine the following: (1) Ngb protein expression in the brain following TBI; (2) the effects of Ngb overexpression on mechanistic endpoint-oxidative tissue damage and (3) the neurological outcomes of neurobehavioral deficits and traumatic lesion size three weeks after TBI (Wei *et al*., 2011; Jain, 2008).

During the first experiments, the researchers found that the Ngb protein level significantly increased in the brains of Ngb-Tg and WTmice, similar to the sham controls, 6 h after TBI. The results of this immunohistochemistry and WB examination were similar to the transient increase in Ngb brain expression found in rat, which showed a peak 6 h after TBI (Jain, 2008). In the mouse study, WB showed that the baseline levels of both Ngb protein expression (149% of WT sham control mice) and CCI-induced Ngb protein levels at 6 h after TBI (196% of WT sham control mice) were increased, which was highly significant than the Ngb-Tg mouse brains and the WT TBI controls (147% of WT sham control mice) (Jain, 2008). Despite the different levels of expression demonstrated after TBI, Ngb protein expression was induced. Both baseline and TBI conditions also induced Ngb protein levels, which are still reported to be significant in the Ngb-Tg mouse brains, validating the association between the various levels of brain Ngb protein expression and the numerous neurological results after TBI in Ngb-Tg and WT control mice (Jain, 2008). Other studies have demonstrated that the overexpression of Ngb enables a reduction in hypoxia/ischemia that induces oxidative damage

in cultured neurons and focal cerebral ischemia in mice (Lin *et al*., 2010; Jin *et al*., 2011). The second experiment recorded a common oxidative tissue damage biomarker, 3NT, in TBI-injured brains of Ngb-Tg and WT control mice (Lin *et al*., 2010; Jin *et al*., 2011). The study reported a significant reduction in the level of 3 NT 6 h after TBI in the Ngb-Tg mouse brains compared to the WT controls, indicating that TBI-induced oxidative tissue damage could be diminished by Ngb overexpression (Lin *et al*., 2010; Jin *et al*., 2011). During the third experiment, the researchers focused on the neurological outcomes of neurobehavioral deficits and the sizes of brain lesions for a period of three weeks after TBI (Lin *et al*., 2010; Jin *et al*., 2011). At 0, 1, 3, 5, 7, 10, 14 and 21 days after TBI, the functions of Ngb were assessed by neurological scores and the hanging wire tests. Body weight loss was recorded on each day (Lin *et al*., 2010; Jin *et al*., 2011). Data from the experiment were reported to be significantly different across the 7-day deficits after TBI (Lin *et al*., 2010; Jin *et al*., 2011). By day 21 after CCI, all deficits recovered close to pre-injury baselines (Lin *et al*., 2010; Jin *et al*., 2011). However, no significant differences between the Ngb-Tg and WT mice were observed in any of the assessments during the 3-week TBI recovery period (Lin *et al*., 2010; Jin *et al*., 2011). The morris water maze was used to assess spatial acquisition memory between 15 and 21 days after TBI, but no significant difference was observed between Ngb-Tg and WT mice in the latency on the hidden and visible platform trials, or for the probe trials (Lin *et al*., 2010; Jin *et al*., 2011). Last, the researchers quantitatively examined traumatic brain lesion volumes and found that they were reduced significantly in Ngb-Tg mice compared to WT mice 21 days after TBI (Lin *et al*., 2010; Jin *et al*., 2011). Over the past two decades, neuroprotectants designed to block or inhibit a specific step in the TBI cascade have not been medically successful (Xin *et al*., 2012). TBI triggering of endogenous protective mechanism scan prevent or limit damage to the brain. New methodologies that seek to augment the endogenous protection of the brain and its repair signals can lead to new therapeutic strategies for stroke and related disorders (Hundahl *et al*., 2008a). Ngb is among the few unique molecules that could be utilized for endogenous neuroprotection according to the above experiments, functioning to stabilize neuronal function and prosurvival genes under both normal resting and hypoxic/ischemic conditions, protect against oxidative stress and preserve mitochondrial function (Hundahl *et al*., 2008b; Hundahl *et al*., 2010a; Hundahl *et al*., 2010b). Investigators have further attempted to explain the gene regulation mechanisms of Ngb, detecting small molecules that can specifically upregulate endogenous Ngb protein expression for the development of a group of strategies called novel endogenous neuroprotection, for treating neurological disorders (Jin *et al*., 2011; Bederson *et al*., 1995). The researchers first examined whether Ngb overexpression affects neuroprotection in the TBI model of mice (Jin *et al*., 2011; Bederson *et al*., 1995) and the results showed the following: (1) there was a significant increase in Ngb in the peri lesion areas of the ipsilateral cortex 6 h after TBI in both WT and Ngb-Tg mice compared to the sham mice, but the levels of Ngb increased significantly more in the Ngb-Tg mice than in the WT controls; (2) a significant reduction in the levels of the oxidative damage marker 3 NT was observed 6 h after TBI in Ngb-Tg mice compared to WT controls; (3) compared to WT mice, at 3 weeks, Ngb-Tg mice exhibited smaller lesion volumes; (4) no significant differences were observed in neurobehavioral deficits between the WT and Ngb-Tg mice for during the three-week period after TBI (Jin *et al*., 2011; Bederson *et al*., 1995).

Expression of Ngb in the human brain

During the study, an investigation was conducted to test whether the level of Ngb expression play a role in global neuroprotection (Hundahl *et al*., 2012a). Whether plays an important role in total brain protection, the requirement of both global and overt expression is essential. Observations by researchers have shown that both the co-localization and localization of Ngb in the human brain are identical to those in the brains of rats and mice, particularly in the regions heavily involved in sleep (DellaValle *et al*., 2010; Hundahl *et al*., 2012a; Nornes, 1973; Schubert *et al*., 2011). There is still some controversy regarding the Ngb expression patterns found in the brains of rodents. Several studies have reported that Ngb has a ubiquitous expression pattern (Tiso *et al*., 2011). Human stroke predominantly affects the middle territory of the cerebral artery (MCA), which supplies blood to almost all of the neocortex (Hundahl *et al*., 2011). In relation to phylogenetics, the neocortex and the MCA are considerably than the diencephalic and mesencephalic brain structures where Ngb expression is found (Hundahl *et al*., 2011). The Ngb distribution thus results in the majority of the brain lacking protection against ischemic injury (Hundahl *et al*., 2011). Despite its anatomical distribution, Ngb plays a substantial role in general defense against ischemic cell death (Hundahl *et al*., 2011). If Ngb functions as a neuroprotectant, then an investigation is needed to determine why it is that only the specific neurons that highly express Ngb require protection (Hundahl *et al*., 2011). The anatomical function of Ngb could serve as a preliminary guide to begin this investigation (Hundahl *et al*., 2011). Except for the normal function of Ngb in the brain, studies have reported that Ngb may be involved in cellular processes related to basic homeostatic functions such as sleep, the circadian rhythm and the central regulation of appetite (Nornes, 1973; Schubert *et al*., 2011). Ngb-null mice showed an altered light-induced phase shift of the circadian rhythm (Capece *et al*., 2009), but no difference was observed in overt appearance, such as home-cage behavior and body weight (Hundahl *et al*., 2011).

CONCLUSION

This literature review show that despite the different methods used by researchers, Ngb was found to be widely expressed in the brains of mammals. The distribution of Ngb expression

and function in various regions differs. The expression levels in some areas of the brain are significantly higher than those in others, while in other areas, the expression levels are nearly identical. Studies on the expression of Ngb have indicated that Ngb is involved in neuroprotective functions or mechanisms in the brains of mammals and that its overexpression reduces tissue infarction volume and markers of oxidative stress in a focal stroke mouse model. Additionally, overexpression of Ngb in mouse brains decreased mechanical injury-induced neuronal death and reduced hypoxia/ischemia-induced oxidative cell damage in cultured neurons and focal cerebral ischemia. The expression of Ngb in mammalian brains is presented in this study and the patterns and levels of expression vary among different mammals. In addition, diverse conditions can increase or decrease the proportion of Ngb-positive cells in mammals, so it is appropriate to assess the exact pattern of distribution and localization before conducting Ngb investigations in mammals.

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Conflict of interest

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Author contributions

James Blackar Mawolo and Caselia Akiti contributed to the literature search. James Blackar Mawolo and Caselia Akiti organized, investigated and interpreted the data. James Blackar Mawolo and Caselia Akiti wrote the first draft of the manuscript. James Blackar Mawolo and Caselia Akiti performed the study methodology and formal analysis. James Blackar Mawolo and Caselia Akiti designed the study concept. Both authors contributed to this manuscript revision and read and approved the submitted version.

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