

**The protective profile of argon, helium and xenon in a model of neonatal asphyxia  
in rats**

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**Running title:** Neuroprotection by the noble gases

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**Abstract:**

OBJECTIVE: Xenon provides neuroprotection in multiple animal models however little is known about the other noble gases. The aim of the current study was to compare xenon, argon and helium neuroprotection in a neonatal asphyxia model in rats.

DESIGN: Randomized controlled trial.

SETTING: Laboratory.

SUBJECTS: Seven day old postnatal Sprague-Dawley rats.

INTERVENTIONS: 70% argon, helium, xenon or nitrogen balanced with oxygen following hypoxic-ischemic brain injury.

MEASUREMENTS AND MAIN RESULTS: Control animals undergoing moderate hypoxic-ischemia endured reduced neuronal survival at 7 days with impaired neurological function at the juvenile age compared with naïve animals. Severe hypoxic-ischemic damage produced a large cerebral infarction in controls. Following moderate hypoxic-ischemia, all three noble gases improved cell survival, brain structural integrity and neurological function on post-natal day 40 compared to nitrogen. Interestingly argon improved cell survival to naïve levels while xenon and helium did not. When tested against more severe hypoxic-ischemic injury only, argon and xenon reduced infarct volume. Furthermore post-injury body weight in moderate insult was lower in the helium treated group compared to the naïve, control and other noble gas treatment groups while in severe injurious setting it is lower in both control and helium treated group than other groups. In the non-directly injured hemisphere argon, helium and xenon increased the expression of Bcl-2 while helium

and xenon increased Bcl-xL. In addition, Bax expression was enhanced in the control and helium groups.

**CONCLUSIONS:** These studies indicate that argon and xenon provide neuroprotection against both moderate and severe hypoxia-ischemic brain injury likely via prosurvival proteins synthesis.

**Keywords:** Argon; Helium; Xenon; Hypoxia-ischemia; Neuroprotection; Noble gas

**Introduction:**

Perinatal hypoxic-ischemic encephalopathy (HIE) endures a high mortality with 20-40% of the survivors having severe neuropsychological impairment (1-3). The effect on patient quality of life and the social and financial burden incurred are significant warranting research into novel neuroprotective strategies. Hypothermia is now an established neuroprotective therapeutic for HIE (2, 4-6) although the neuroprotection is far from absolute with approximately half of patients still enduring neurological sequelae. Combination strategies are therefore required to augment hypothermic neuroprotection.

The noble gas xenon has demonstrated organoprotective properties when administered alone (7-14) or in combination with hypothermia (15-18). Similar to xenon, argon and helium have shown neuroprotective qualities *in vitro* (11-14) and *in vivo* (13, 19) while other members of noble gas family, neon and krypton, lack effect (11). Interestingly helium has also been reported to exhibit both detrimental as well as protective effects (11-13). However, a study to comparing the neuroprotective potency of these three gases argon, helium and xenon *in vivo* is not available to date.

There are notable differences between the three noble gases. Xenon is an anesthetic at atmospheric pressure, argon and helium are not, making their potential roles and side effects very different. Furthermore compared with the scarceness and excessive cost of xenon, helium and argon are relatively inexpensive. Despite these differences

some important core attributes are shared: good blood-brain barrier penetration and fast onset making them good candidates for neuroprotection. Our aim herein was to compare the neuroprotective effects of argon, helium and xenon against brain injury in a model of neonatal asphyxia in rats. Ultimately, we wish to identify the best candidate noble gas to treat HIE patients.

### **Materials and Methods:**

The study protocol was approved by the Home Office (United Kingdom), and all efforts were made to minimize the animals' suffering and the number of animals used to detect a difference of at least 30% between the control and the trial arm. Sprague-Dawley rat pups were used for experiments. Twelve pups with their mother were used and housed with a 12-hour light/dark schedule in a temperature and humidity controlled colony room. The pups were housed with their dams until weaning on postnatal Day 21 (PND 21), and then were housed in groups of four per cage based on different sex.

### **Animal Model of Neonatal Asphyxia**

Seven day old postnatal Sprague-Dawley rats underwent right common carotid artery ligation under surgical anesthesia (2% isoflurane) as reported previously (9) . After ligation, the animals were returned to their dams at a constant temperature (23°C) and humidity (48%) for recovery. One hour after surgery, neonatal rats were placed in purpose-built multi-chambers in which 8% O<sub>2</sub> balanced with N<sub>2</sub> was supplied continuously for 90 minutes or 120 min. The body temperature was

maintained by submerging the exposure chambers into a water bath as reported previously (15). The experimental protocol is presented in Fig. 1. Two hours after hypoxic insult, animals were randomly exposed to either argon, helium, xenon (70% noble gas balanced with O<sub>2</sub>) or nitrogen (known as control group, 70% N<sub>2</sub> balanced with O<sub>2</sub>) for 90 minutes, using a purpose-built, closed-circle delivery system which was made up with six multi-chambers, one gas reservoir bag and one gas monitor with a built in gas pump. The total volume of the system is about 3.5 L. The system was flushed with more than 4 times of system volume of the mixed gases before the closed gas delivery circle system was established. Soda lime and silica gel (Merck, Leicestershire, United Kingdom) was used in the chambers to eliminate carbon dioxide and water generated by animals. The mixed gases were refilled into the system for every 20-30 min to compensate the gas lost from the system. Gas concentration of oxygen was measured continuously by an in-line gas analyzer (Air Products Model No. 439Xe). Other cohort of untreated pups were included as naïve group (n = 30).

### **Determination of Cell Survival *In Vivo***

Seven days after receiving 90 min hypoxic insult (n = 5/group) or 14 days after receiving 120 min hypoxic insult (n = 7/group), rats were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally) and perfused transcardially with paraformaldehyde (4%) in phosphate buffer (PB) (0.1 M). The brains were removed and placed in 4% paraformaldehyde in 0.1 mol/L PB overnight. The blocks of brain were dehydrated, embedded in wax, sectioned into 5 mm slices and then stained

with 0.5% cresyl violet. The coronal sections (5 mm) from rats received 90 min hypoxic insult were harvested around -3.6 mm bregma relative to adult brain. The CA1 area of the hippocampus from the bilateral side of each brain was chosen for data analysis and the microphotograph was taken at 40X using a BX-60 light microscope (Olympus, Southall, United Kingdom) attached with a digital camera (Zeiss, Gottingen, Germany). The total number of healthy cells that appeared in the CA1 area of hippocampus in cresyl violet staining slices was counted from 4 sections/brain in a blind manner (T.Y.) and their mean value was used for data analysis.

The following morphological criteria were used to analyze apoptotic or necrotic cell death in cerebral CA area of hippocampus. Viable cells were regularly shaped with pale cytoplasm and a clearly visible, darker nucleus (Fig 2A). Apoptotic cells had dark stained, shrunken nuclei that were spherically shaped and an intact cell membrane, often with a surrounding area of vacuolation (20). Necrotic cells were identified by intense cresyl violet staining of the cytoplasm with irregularly shaped, enlarged nuclei and loss of nuclear membrane integrity (21).

The coronal sections (5 mm) from rats received 120 min hypoxic insult were selected from each pup to match predefined brain regions relative to the bregma (+2 mm, +1, 0, -2, -4 and -5) relative to adult brain. Once identified, each slice was photographed and the size (arbitrary unit) of the healthy matter of both hemispheres was calculated with data analysis software (ImageJ v1.31, NIH image software, USA) by one author



who was blinded to the treatment that the pups had received. The infarction size was calculated with a formula of (left hemisphere – right hemisphere)/left hemisphere (%). Then the data were used to plot curves and the area under curve was calculated to indicate the infarction volume (arbitrary unit).

### **Neurologic Function Assessment**

Other cohorts with 90 min hypoxic insult were allowed to live up to PND 49 days after the experiments for neurologic functional test. The body weight of each rat was recorded before following behavior assessments.

#### *Rotarod test*

Animals (n = 6/group) underwent rotarod test according to an established protocol (22). Motor function was tested by placing rats on a 2.5 cm diameter plastic rod, rotating at 30 r.p.m. (rounds per minute) and 50 r.p.m. on the next day, and the latency to fall off the rod was assessed (maximal latency was 300 seconds). The apparatus was set up in an environment with minimal stimuli such as noise, movement or changes in light or temperature. For each of the functional assay, the rat was tested three times with a 10 minute interval between each assessment, and the mean value of three assessments was used for each rat in the analysis. The assessments were performed by an investigator who was blinded to the experimental protocol.

### *Morris Water Maze (WM)*

After rotarod test, neurocognitive outcome was evaluated daily (starting from PND 40) using Morris water maze (WM) by using a computerized video tracking system (EthoVision®; Noldus, Wageningen, The Netherlands) (n = 6/group). Briefly, WM consisted of a circular pool 110 cm in diameter and 60 cm in height, filled to a depth of 30 cm with water ( $24 \pm 0.5^{\circ}\text{C}$ ). A hidden submerged platform was placed in one quadrant 2.5cm below water surface. Rats were placed in the water in a dimly lit room with visual clues around room wall. The time of locating the submerged platform (defined as the latency and cut off time 90 sec) was measured. The longer time required to locate the platform indicates the more impairment in spatial learning and memory. Four trials of testing were performed for each rat everyday. Each trial started from a different quadrant. Testing was consecutively repeated for 5 days.

### **Western Blot Analysis**

Rats were killed under pentobarbitone anesthesia two hours after gas exposure (n = 5/group). Left hemispheres were harvested separately, lysed in lysis buffer (pH 7.5, 20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L Na<sub>2</sub>DTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L  $\beta$ -glycerophosphate, 1 mmol/L Na<sub>3</sub>VO<sub>4</sub>, 2 mmol/L DL-dithiothreitol, 1 mmol/L phenylmethanesulphonyl and 1 ug/ml leupeptin), and centrifuged at 3,000g for 30 mins. Protein concentration in the supernatant was determined by Dc protein assay (Bio-Rad, Herts, UK). Protein extracts (30 ug/sample) and a biotinylated molecular weight marker (New England

Biolab, Hitchin, UK) were denatured in Laemmli sample loading buffer (Bio-Rad, Herts, UK) at 100°C for 5 mins, separated by 10.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Amersham Biosciences, Little Chalfont, UK). The membrane was treated with blocking solution (5% nonfat dry milk in Tween-containing Tris-buffered saline (10mmol/L Tris, 150mmol/L NaCl, 0.1% Tween, pH 8.0)) and incubated with rabbit monoclonal antibody directed against Bax (1:1500; Delta Biolabs, Cambridge, United Kingdom), Bcl-xL (1:1000, Cell Signaling Technology, Danvers, USA), Bcl-2 (1:500, Cell Signaling Technology, Danvers, USA) or mouse monoclonal antibody against  $\alpha$ -tubulin (1:2000, Sigma, Poole, UK) overnight at 4°C. The horseradish peroxidase-conjugated goat antibody to rabbit or mouse IgG (1:1000, New England Biolab, Hitchin, UK) was used to detect the primary antibodies. The bands were visualized with the enhanced chemiluminescence system (ECL, Amersham Biosciences, Little Chalfont, UK) and intensities were quantified by densitometry. Results were normalized with  $\alpha$ -tubulin.

### **Statistical Analysis**

Data are expressed as mean +/- SD or box-and-whisker plot. The significance of differences was determined by analysis of variance (ANOVA) followed by Newman-Keuls test or Mann-Whitney test wherever appropriate. A value  $p < 0.05$  was considered to be statistical significance.

## **Results:**

No pups died during or after the 90 minute hypoxic insult but following the 120 minute hypoxic insult 3 and 4 pups died respectively in the helium and nitrogen gas treatment groups.

### **Cell Viability in the Hippocampus**

Healthy cells in both right and left CA area of hippocampus were readily to be seen in naïve animals (Fig 2A). No differences in viable cell count were observed in the left hemisphere ( $F = 1.7$ ;  $P=0.16$ ). The thickness of healthy layer in the right CA area (that underwent hypoxic-ischemic injury) of the control group was significantly reduced when compared with that of naïve, argon, helium and xenon group (Fig 2B). Quantitative analysis revealed that argon, helium and xenon treatment significantly increased the healthy cell number of cells in the right CA area of hippocampus from  $37 \pm 8$  in control group to  $54 \pm 6$ ,  $48 \pm 5$  and  $47 \pm 5$  respectively ( $F= 25$ ;  $P < 0.001$ ). Argon treatment restored the cell number to that of naïve rats while that number of viable cells in the xenon and helium was still reduced compared to the naïve and argon groups ( $P < 0.001$ ) (Fig 2F).

### **Infarction size**

We then determined whether the protective profile is also evident following severe insult (with 120 min hypoxia). Xenon and Argon significantly decreased infarction volume by 42% ( $F = 4.4$ ,  $p < 0.05$ ) and 38% ( $p < 0.05$ ) when compared to control. Interestingly, helium did not offer any protection in this severe insult setting (Fig. 3).

## **Neurological Function and Body Weight**

Hypoxic (for 120 min) -ischemic injury reduced the body weight on PND 21 from  $39 \pm 2$  g (naïve) to  $33 \pm 1.3$  g (control) ( $p < 0.01$ ) and  $30 \pm 3.9$  g (helium) ( $F = 22$ ;  $P < 0.0001$ ) (Fig 4A). Hypoxic-ischemic injury for 90 min reduced the body weight on PND 38 from  $123 \pm 20$  g (naïve) to  $110 \pm 7$  g (control). However in the helium group the body weight was further reduced to  $85 \pm 12$  g ( $F = 6.6$ ;  $P = 0.0009$ ). There was no significant difference between the body weight of argon, xenon and control group (Fig 4B).

On PND 38 the animals underwent neurological testing. There was no difference between the five groups when motor function was tested at 30 rpm ( $F = 0.7$ ;  $p = 0.5996$ ; Fig 5A) or 50 rpm ( $F = 2.5$ ;  $p = 0.0774$ ; Fig 5B).

On PND 40 neurological function was tested in the Morris Water maze. The line graph showed that the latencies to find the platform of five groups were similar on the first day. All the groups, except the control group, showed gradually reduced latency in following four days (Fig 6A). We used the area under curve (AUC) to denote the cumulative time of four trials based on the five testing day. There was a significant difference between noble gas groups and control group ( $F = 8.2$ ;  $P = 0.0002$ ; Fig 6B). There was no difference in the average swimming speed between groups (Fig 6C).

## **Bax, Bcl-2 and Bcl-xL**

Apoptotic cell death induced by ischemia/hypoxia insult in neonates is mediated via the intrinsic (mitochondrial) pathway (23). We chose Bax, Bcl-2 and Bcl-xL as target

proteins to examine whether they were involved mechanistically in the neuroprotection effect provided by noble gases. Bax, Bcl-2 and Bcl-xL exert pivotal roles in apoptosis; Bax promotes cell death and latter two promote survival. Because the right hemisphere is injured, the effects of gas treatment on protein expression can be distorted. We, therefore, chose to investigate these protein expression in the left hemisphere following gas exposure. Two hours after gas exposure, Bax expression was enhanced in the control and helium groups ( $F = 2.7$ ;  $p = 0.0624$ ; Fig 7A) although it did not reach to a statistical significance. Bcl-xL expression was increased by helium and xenon in comparison with control and naïve groups ( $F = 5.9$ ;  $p = 0.0025$ ; Fig 7B). Bcl-2 expression was significantly increased by argon compared with control and naïve groups and increased to lesser degree by helium and xenon ( $F = 5.7$ ;  $P = 0.003$ ; Fig 7C).

#### **Discussion:**

There are five major findings from our work: (i) Argon, helium and xenon treatment significantly restored the cell morphology from mild ischemic/hypoxic insult, though only argon restored cell viability to naïve levels; (ii) Argon and xenon treatment reduced infarct volume from severe ischemia but helium did not; (iii) Neurological function assessed with Morris Water maze was improved in all noble gases treated groups; (iv) Animals treated with helium had reduced body weight compared to the other groups; (v) The neuroprotection afforded by these three gases was associated with increased Bcl-2 levels while xenon and helium were also associated with increased Bcl-xl levels.

It is intriguing that the so called “inert” noble gases attract attention as putatively organ-protective agents; indeed the most widely studied, xenon, shows protective effects *in vitro* and *in vivo* in several different organs (7-9, 11, 12). We have repeated our observation that xenon is a potent neuroprotective agent in this model and extended these findings to include evidence that long-term neurocognition, measured by the Morris Water maze, is enhanced by xenon therapy. However our aim was to compare the different noble gases to discern whether cheaper alternatives to xenon may be identified. Therefore it is of interest that in our study, argon and xenon showed comparable neuroprotection with more healthy cells in hippocampus, reduced infarct volume and long-term maintenance of brain tissue integrity and neurological function. Furthermore argon was the only noble gas able to restore the viable cell counts to naïve levels. In contrast while helium was protective against 90 minutes of hypoxic-ischemia, its protection was lost against a more severe insult of 120 minutes of hypoxic-ischemia.

Argon has also been shown to exert significant neuroprotective effect in *in vitro* models of injury and in an adult model of hypoxic-ischemic injury *in vivo* (11, 14). As far as we are aware this is the first report describing argon neuroprotection against hypoxic-ischemic injury in young animals. Furthermore this is the first report showing that argon effects long-term cognition, suggesting it is worth pursuing into other models of injury. It is encouraging that argon treatment following hypoxia-ischemia increased the number of viable neurons to the same value as naïve

animals (**Fig 2**); we speculate that an argon induced upregulation of Bcl-2 increased cell survival (**Fig 7**).

In previous studies helium has been shown to be protective in brain slices (12) and middle cerebral artery occlusion animal model (13); however we observed that helium had a detrimental effect on oxygen-glucose deprivation injury in cell culture (11). Herein helium significantly improved morphology and neurologic function from 90 minutes of hypoxic-ischemia in line with a number of previous studies (12, 13, 24, 25) however this protection was lost against the more severe insult of 120 minutes of hypoxic-ischemia. Simultaneously rats of helium group had lower body weight. The reason for this lower body weight is unclear, nonetheless this suggests that helium may exert some harmful as well as protective effects.

It is of interest that argon and xenon both activate anti-apoptotic signaling shown by increased Bcl-2 and Bcl-xL levels respectively. Apoptotic neuronal cell death induced by ischemia/hypoxia results from activation of both the extrinsic and intrinsic pathways. In the process of activation of the intrinsic (mitochondrial) pathway, the increase in intracellular free calcium concentration, following excessive release of glutamate and activation of the NMDA receptor (26), triggers Bax translocation to and competition with Bcl-2 and other members of the Bcl-2 family in the mitochondria membrane evoking cytochrome C release to the cytosol (27-30). Bcl-2 promotes cell survival, whereas Bax accelerates apoptotic cell death. The Bcl-2-related gene Bcl-x encodes 2 proteins: Bcl-xL which inhibits cell death, and



Bcl-xS which inhibits cell survival (31, 32). Our data suggests that the mechanism of neuroprotection afforded by noble gases may be due to the upregulation of genes and synthesis of the prosurvival proteins, Bcl-2 as well as Bcl-xL.

Little is known about the direct molecular targets of the noble gases especially as the drugs may affect both intracellular and extracellular effectors. Xenon is known to antagonize NMDA receptors (33) and activate ATP-sensitive potassium channels (34) and the two-pore potassium channels (35). Argon may effect, gamma-aminobutyric acid type-A receptors (GABA) though further data is required to assess whether this is the mechanism for cytoprotection (36). Similarly the mechanisms of helium-induced organ-protection remain unclear. As a preconditioning agent, helium reduced infarct size via activating prosurvival kinases (24, 25) as well as mitochondrial ATP regulated potassium channel (24) and calcium-sensitive potassium channel (37), ultimately inhibiting mitochondrial permeability transition pore opening. Furthermore, the high thermal conductivity makes helium produce hypothermia in animals which is known to be a neuroprotective intervention (13) however in our model brain temperature was kept in the normal range.

There are several limitations in our study. Notably in the rotarod test we only observed a trend to differences in neurological function and statistical significance was not achieved ( $p = 0.08$ ). We suspect this is a type two statistical error due to lack of power to identify the effect especially given the multiple statistical tests required with so many groups. Unfortunately we are not able to repeat the experiment to

increase the statistical power. However we did observe a difference in the Morris water maze that more accurately measures neurocognitive function than the rotarod and is more likely a more sensitive test for detecting neurological impairment in this model. In concert, the neurological follow up data show that the noble gases provided functional neuroprotection. In addition, we cannot exclude that the lower body weight of helium group is attributable to poor maternal nurturing. However, from our experience, the variation between mother animals could not be expected to produce such a profound physiological difference. While all three noble gases proved protective in our model, we may not have been able to identify subtle differences between each gas in their neuroprotective potency. Only argon was able to restore viable cell counts to naïve levels, proving superior to helium and xenon, however we were unable to show that argon also improved neurological function above the other two gases. Nonetheless these data do provide rationale for testing argon in further models of neuronal injury and in combination with other neuroprotective therapeutics such as hypothermia in future studies. lastly, our conclusions could be compromised by the neurotoxic effect of isoflurane in the developing brain that has been well documented in the literature (38). However, 2% isoflurane exposed to the pups in our study was for less than 10 min which unlikely caused neuroapoptosis unless the exposure time reached up to 1 hour reported previously (39).

In summary, this study showed that all three gases show effective neuroprotective properties *in vivo*. However while helium afforded morphological and behavioral

protection against moderate hypoxic-ischemic injury it was not protective against severe hypoxic-ischemia and showed detrimental effects on physiologic development. On the contrary both xenon and argon provided neuroprotection against both moderate and severe hypoxic-ischemic injury without gross adverse effects. Our observation that argon may be superior or at least equally efficacious to xenon is of interest as argon is significantly cheaper than xenon. In turn, this increases the breadth of potential applications for argon and the possibility of helping more sufferers of hypoxic-ischemic brain injury.

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### **Titles and legends to figures:**

Figure 1. Experimental protocol. PND = postnatal day; HI = Hypoxic-ischemia.

Figure 2: **Hippocampal neuroprotection of noble gases against ischemia/hypoxia insult in rat neonates.** Example microphotographs of the CA area of hippocampus taken from an animal with (A) no treatment (naïve), (B) nitrogen (control), (C) argon, (D) helium and (E) xenon, stained with 0.5% cresyl violet. (F) Argon, helium and xenon treatment significantly increased healthy cell number in the right CA area of hippocampus from  $37 \pm 8$  in control group to  $54 \pm 6$ ,  $48 \pm 5$  and  $47 \pm 5$  ( $P < 0.01$ ) respectively. Mean  $\pm$  SD ( $n = 5/\text{group}$ ). RH in the top panel = right hippocampus; LH in the bottom panel = left hippocampus. \* $p < 0.05$ , \*\* $P < 0.01$  vs naïve; \*\* $P < 0.01$  vs control, † $p < 0.05$  vs argon.

Figure 3: **Nissl stained of the coronal sections of brain 14 days after experiments.** Representative brain micrograph from a rat treated with (A) nitrogen (control), (B) argon, (C) helium and (D) xenon. (E) Plot with mean data of the brain matter in the right healthy hemisphere against left hemisphere vs relative distance from Bregma. (F) Mean  $\pm$  SD ( $n = 7/\text{group}$ ). \* $P < 0.05$  vs control.

Figure 4: **Body weight of rats 14 days after experiments (120 min hypoxic insult) (A) and 31 days after experiments (90 min hypoxic insult) (B).** Across all groups, hypoxic-ischemia for 120 min or hypoxic-ischemia for either 120 min or 90 min followed with helium treatment caused a significant decrease of body weight. Mean  $\pm$  SD ( $n = 6/\text{group}$ ). \*\* $P < 0.01$  vs Naive.



Figure 5: **Neurologic function assessed with Rotarod at the juvenile age.** The time spent on rod at the speed 30 (A) and 50 (B) r.p.m. The data presented in a box-and-whisker plot (boxes are constructed with 25% and 75% confident intervals; the thick line in the box are medians; positive or negative bars are maximum or minimum individual values (n = 5/group).

Figure 6: **Neurocognitive behavior was determined by using Morris Water Maze at the juvenile age.** (A) The mean learning and memory formation data against time course across all groups for five consecutive days. (B) Area under curve (AUC) derived from Figure A. (C) The mean swimming speed across all groups for executive five days. Mean  $\pm$  SD (n = 6/group). D1-5 = Day 1-5. \*\*p < 0.01 vs naïve; + p < 0.05, ++p < 0.01 vs control.

Figure 7: **Argon, Helium or Xenon treatment induced changes of proapoptotic protein Bax and prosurvival proteins Bcl-xl and Bcl-2 expression in the left hippocampus.** Bax (A), Bcl-xL (B) and Bcl-2 expression (C) after ischemia/hypoxia for 90 min followed by 90 min gas exposure. Mean  $\pm$  SD (n = 5/group). N = naïve; Con = control; Ar = argon; He = helium; Xe = xenon. \*p < 0.05, \*\*p < 0.01 vs naïve; + p < 0.05, ++p < 0.01 vs control.

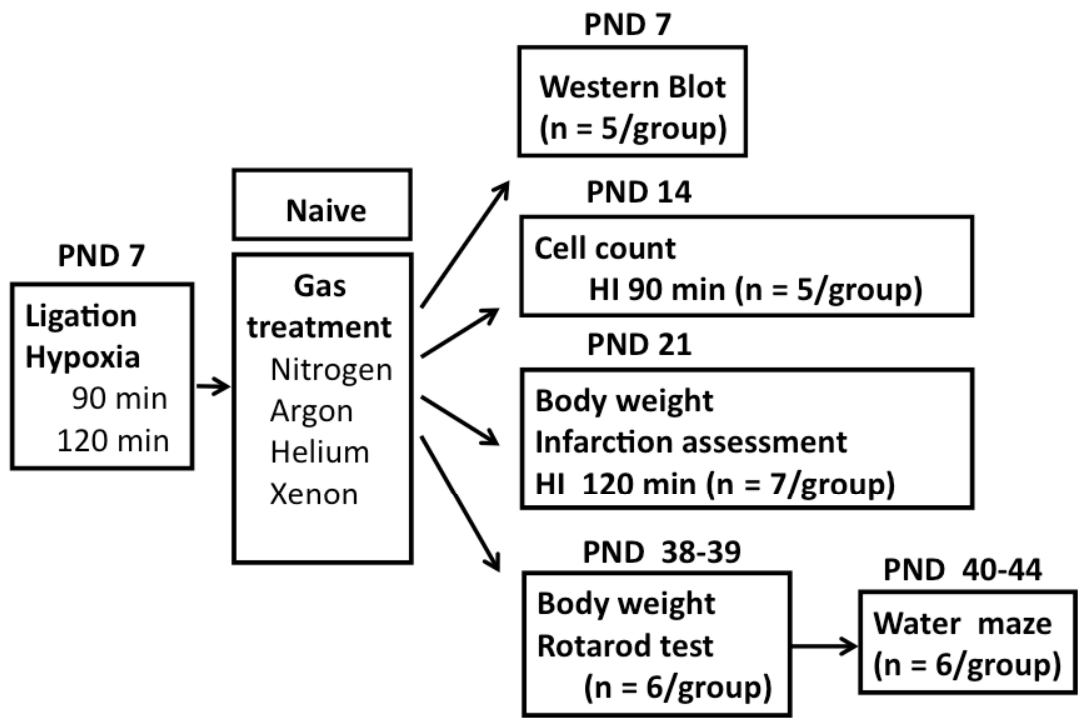
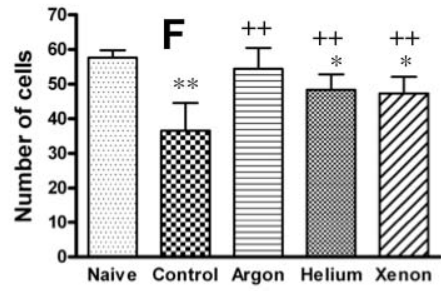
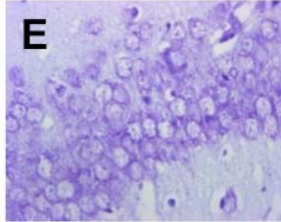
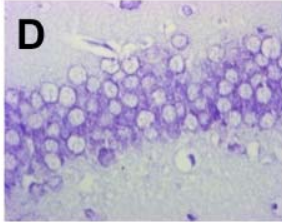
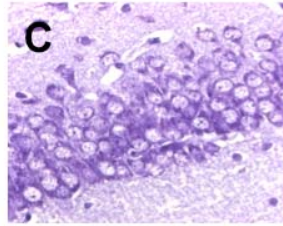
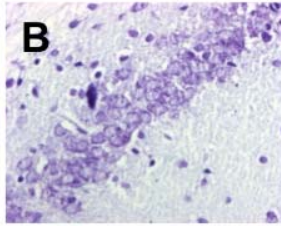
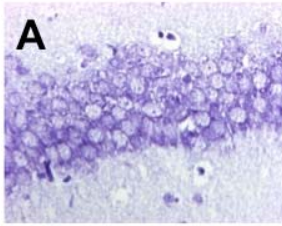


Figure 1

RH



LH

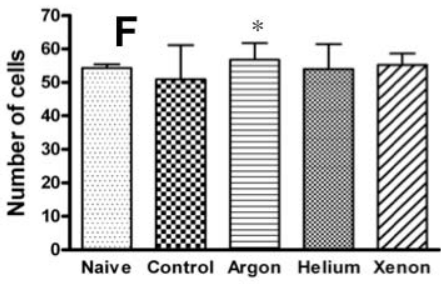
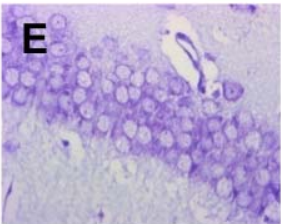
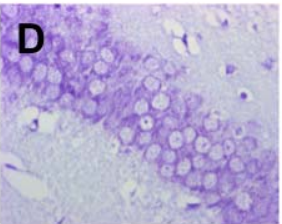
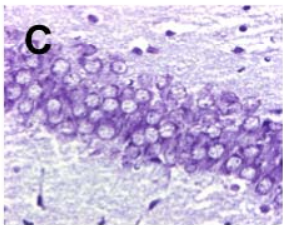
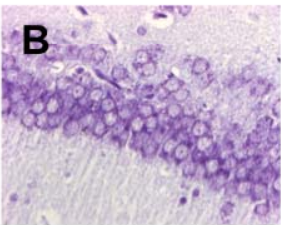
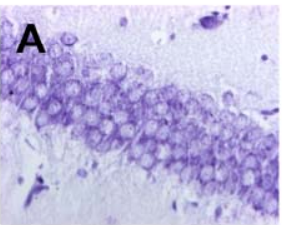


Figure 2

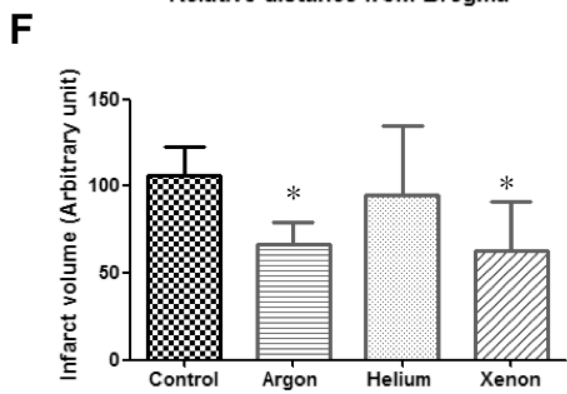
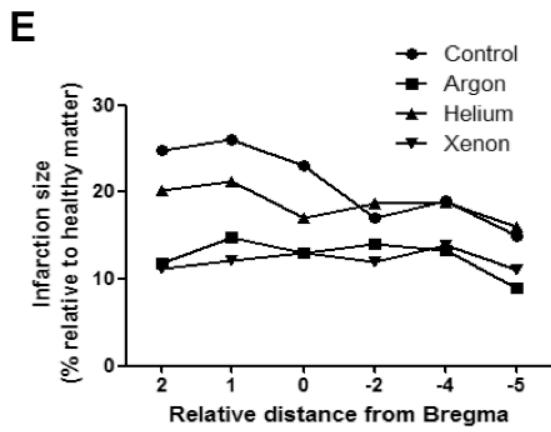
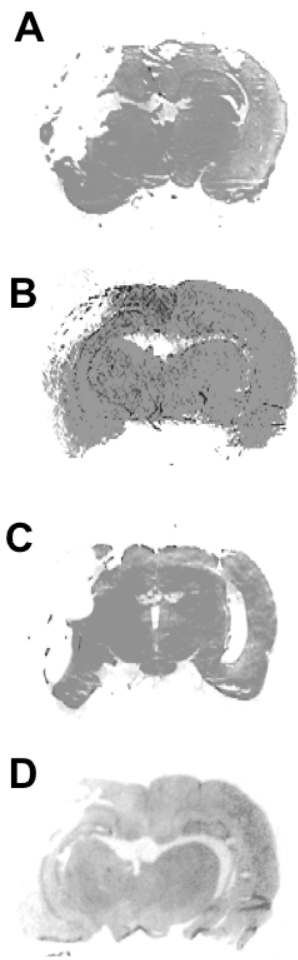


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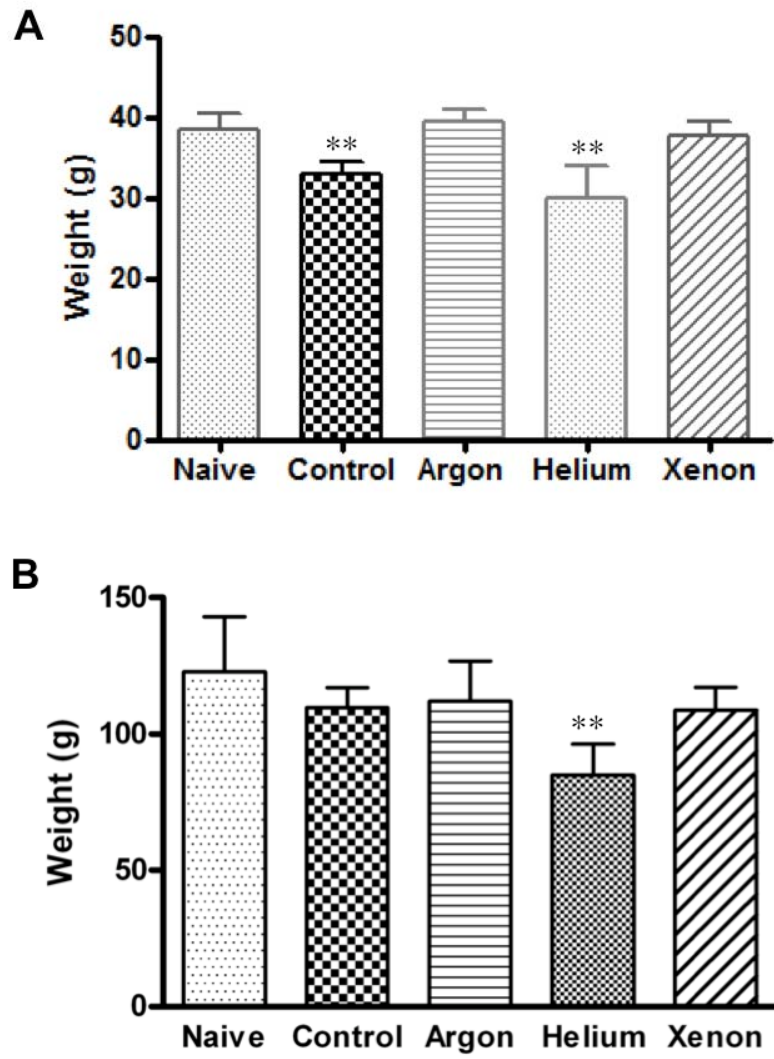


Figure 4

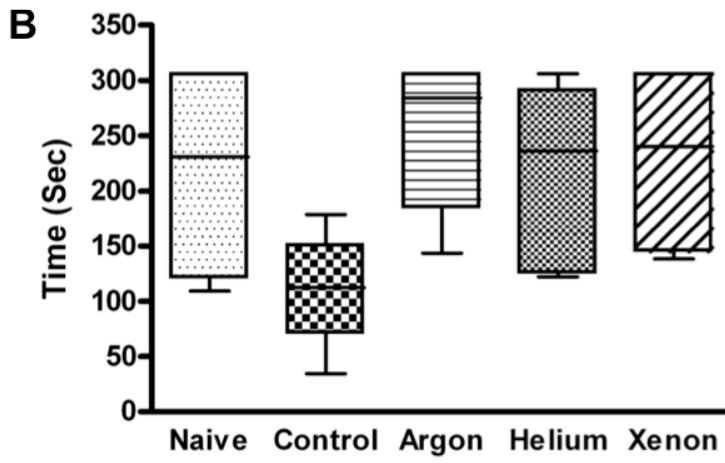
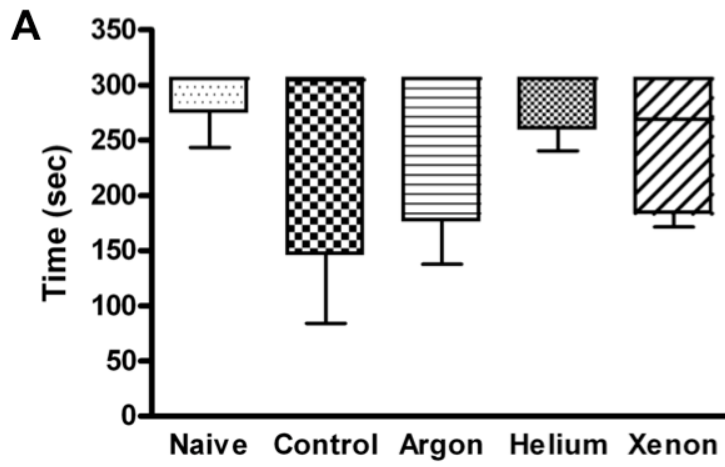


Figure 5

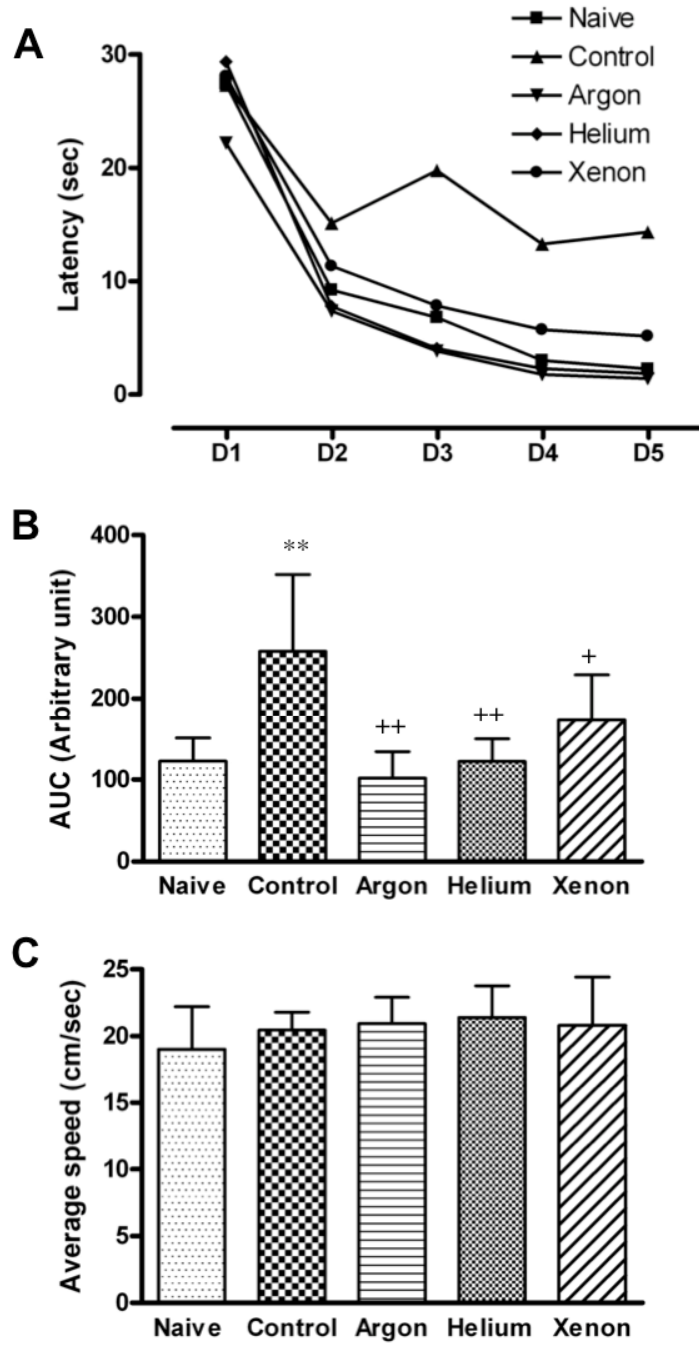


Figure 6