

## ORIGINAL ARTICLE

# THE OLFACTORY SYSTEM REGULATES ACUTE MOUNTAIN SICKNESS

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## ABSTRACT

### OBJECTIVE:

Hyperventilation is the first response to hypoxia in high altitude (HA). Our study on rats was designed to establish an integrated hypothesis to include hyperventilation, increased activity of hypothalamic- pituitary- adrenocortical axis (HPA) in response to initial exposure to hypoxia and failure of adaptation to stress in olfactory bulbectomised rats.

### METHODS:

Albino rats whose olfactory lobes were removed were subjected to hypoxia and hypothermic conditions. Blood and urine samples were collected at various stages to measure biochemical parameters. Rats whose olfactory systems were intact were used as controls.

### RESULTS:

The results suggested that the olfactory system regulated pituitary function and that in rats whose olfactory lobes were removed failed to adapt to the stress created by hypoxia and hypothermia.

### CONCLUSIONS:

Acute Mountain Sickness (AMS) is a type of stress. Normal rats when subjected to stress such as AMS are able to adapt. This adaptation is lost when the olfactory bulbs are removed. It is postulated that serotonin receptors in the hypothalamus, through the splanchnic pathway regulate stress. This mechanism is independent of ACTH – Cortisol feed back system. Perhaps irregular and rapid respiratory rhythm simulates physiological Olfactory Bulbectomy during rapid climbing and AMS manifests as a failure of stress adaptation.

*Key words: Hyperventilation; Olfactory bulb; Hypothalamus; Endocrine dysfunction; AMS; Adaptation; Stress regulation.*

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## INTRODUCTION

The mechanism of the AMS is not fully understood. Between 25-85% of mountaineers suffer

from AMS depending on the altitude and rapidity of ascent (Basnayak et al., 2003). They have proposed a mechanism of AMS based on the concept that there is

an increase in cerebral blood flow and blood brain damage. This is based on their observation that at moderate high altitude (HA)(3500ms) there is increased pulse rate and systemic arterial pressure. To assume that there is increased CBF and damage to blood brain barrier (BBB) is inappropriate. BBB damage occurs if there is a pre existing brain damage. The levels of systolic arterial pressure recorded in the above study is insufficient to break the BBB in an undamaged brain. Cytotoxic oedema (damage to BBB) is unlikely to be associated with AMS or early HACE as the levels of hypoxemia are insufficient to impair cell-ion homeostasis and produce cellular swelling in an undamaged brain. Vasogenic oedema occurs in rat brains exposed to hypoxia. It is speculated that this may be induced by the vascular endothelial growth factors. However plasma vascular endothelial growth factors concentration in climbers who have suffered AMS has not been consistent.

The mechanism of AMS proposed by Roach and Hacket based on “increased cerebral blood flow” and “inadequate cerebrospinal fluid buffer capacity” is an attractive speculation not supported by evidence. (Roach and Hacket., 2001)

The authors during their expedition to the Himalayas observed a significant drop in the carotid blood flow of fellow climbers between the altitudes of 3500-6500ms (Casikar and Venkatesh. 2001& Casikar., 1984).

The Doppler evaluation by this group showed a significant reduction in carotid artery lumen and corresponding drop in flow, in spite of increased pulse pressure and systemic arterial pressure.

Grosney proposed a central neurogenic hypothesis to explain the effects of hypoxia at HA. (Grosney *et al.*, 1965). Marcilhac and Cairncross have shown the effects of olfactory bulbectomy on rats during stress conditions (Marcilhac *et al.* 1999 & Cairncross., 1971). We have tried to integrate all the three concepts namely hyperventilation in HA, influence of hypoxia on HPA function and effects of stress on rats. We propose that hyperventilation in

HA triggers dysfunction in the olfactory bulb and this in turn disturbs the HPA stress mechanism. AMS is a manifestation of such a stress.

## METHODS

A group of 106 male Whistler strain inbred albino rats weighing between 170-220gms fed on a laboratory diet (Hind–Liver) was selected. 46 of these were isolated for olfactory Bulbectomy (OB). They were subjected to sniffing response training. For a period of three days at intervals of fifteen minutes for 6 hours at stretch cotton pledgets soaked in 70% alcohol were used to record the response. Those that showed less than 80% aversion response were rejected. Six rats were excluded.

Forty rats were subjected to bilateral stereotactic olfactory bulbectomy.

The animals were anaesthetised with ether and fixed in a rat stereotaxy frame (Mfg. Ambala, India). Using a hand drill bilateral parasagittal burr holes were made. Using standard stereotactic co-ordinates (Nieuwenhys., 1998) with a plastic sheath connected to a 2cc syringe olfactory bulbs were aspirated bilaterally avoiding damage to the Superior Sagittal Sinus. Burr holes were sealed with bone wax and the scalps were sutured with 4<sup>0</sup> nylon sutures. There was no mortality at this stage.

On the first postoperative day they were subjected to sniffing test to ensure complete olfactory bulbectomy. Each rat was exposed to 70% alcohol for twenty five times at fifteen minute intervals. Those that showed more than 5% aversion response were excluded. Four rats were rejected. Thirty-six rats were finally selected for the study. Un operated rats (nos. 10) and those that had the bilateral burr-holes without stereotactic ablation (nos. 10) were used as controls. The latter group was used as controls to exclude the stress due to the surgical procedure. We do believe that the stereotactic procedure does not increase the operative stress in these animals.

The rats were exposed to three types of stress conditions - hypothermia, hypoxia and combination of hypoxia and hypothermia (HA). (Table. 1).

Table 1. **Exposure to stress.**

	Hypoxia	Hypothermia	High Altitude (Hypoxia + Hypothermia)
<b>Control</b>	10	10	10
<b>Sham</b>	10	10	10
<b>OB</b>	11 – 1 = 10	11 – 1 = 10	14 – 4 = 10

The rats were exposed to simulated conditions of hypothermia, hypoxia and high altitude (hypothermia and hypoxia) in a high altitude chamber developed in the laboratory. The rats were unrestrained. The ambient temperature of the chamber was maintained between 20-23 degrees C, during the exposure to Hypoxia. Pre cooled humidified room air was used during the exposure to Hypothermia. The temperature

of the chamber was maintained between 0-5 degrees C. Aneroid barometer (Compens N – 967514) was used to regulate the air extraction to simulate high altitude conditions. The chamber was covered with a black cloth to create nocturnal conditions, to eliminate stress factors outside of the conditions of the experiment. (Table 2).

Table 2. The protocol of exposure.

Altitude in mts	2400	3000	3500	4000	4500
<b>Hypoxia</b>	2 hrs	1 hr	1 hr	1 hr	1 hr
<b>Hypothermia</b>	6 hrs				
<b>Hypoxia &amp; Hypothermia</b>	2 hrs	1 hr	1 hr	1 hr	1hr

Of the 37 OB rats, 6 died during various stages of the experiment (Table3). These were excluded from the study, as they did not complete the protocol. There

was no mortality in the control and sham operated groups.

Table 3. Mortality.

Hour of Exposure	1	2	3	4	5	6
<b>Cerebral Haemorrhage</b>	1	0	2	0	0	0
<b>Cerebral Infarction</b>	1	1	0	0	0	0
<b>Infection</b>	0	0	1	0	0	0

The animals were killed by decapitation in a guillotine (Designed by Dr. J. David, Research consultant to St John's Medical college). The blood was collected and left for half an hour at room temperature for the serum

to separate. The liver and kidney were removed and processed immediately and tested for enzymes. Serum was collected and an estimation of glucose was done within an hour. The remaining tests were done the next

day after storing the serum at a temperature between 0 to -4 degrees C. The following biochemical parameters were measured by standard techniques (Acosta., 1941; Kearney, 1972) - serum levels of urea, uric acid,

glucose and creatinine, proteins, lactic dehydrogenase, succinyl dehydrogenase, creatinine, blood urea, serum uric acid serum glucose and serum electrophoresis

Table 4. The experimental data.  
a: Significant from CONTROL

Biochemical Parameters	Levels	CONTROL	SHAM	OLFACTORY BULBECTOMISED	P value
Lactate Dehydrogenase (Liver)	Hypoxia	1808.59 ±119.49	1892.83 ±111.61	1658.77 ±142.83	0.0174
	Hypothermia	2043.96 ±118.99	2179.74 ±266.04	1720.15 <sup>ab</sup> ±146.60	<b>0.0013</b>
	Hypoxia + Hypothermia	1801.44 ±125.42	1986.69 ±243.59	1467.31 <sup>ab</sup> ±151.49	<b>0.0002</b>
Lactate Dehydrogenase (Kidney)	Hypoxia	1800.97 ±162.34	1948.92 ±158.15	2092.88 ±161.67	0.0212
	Hypothermia	1890.88 ±189.16	2042.26 ±155.14	2180.52 ±180.01	0.049
	Hypoxia + Hypothermia	1927.17 ±166.34	2005.05 ±173.01	2280.67 ±312.74	0.0216
Liver Weight (grams)	Hypoxia	5.23±0.38	5.68±0.34	5.35±0.32	0.0453
	Hypothermia	5.38±0.63	5.12±0.59	5.05±0.68	0.4471
	Hypoxia + Hypothermia	4.98±0.52	5.74±0.35 <sup>a</sup>	5.11±0.38 <sup>b</sup>	<b>0.0108</b>
Succinate Dehydrogenase (Liver)	Hypoxia	123.69±9.18	100.03±8.55 <sup>a</sup>	77.68±8.13 <sup>ab</sup>	<b>&lt;0.001</b>
	Hypothermia	84.45±12.87	86.66±11.49	84.56±43.97	0.022
	Hypoxia + Hypothermia	66.76±8.32	62.23±6.88	50.21±8.29 <sup>ab</sup>	<b>0.0069</b>
Succinate Dehydrogenase (Kidney)	Hypoxia	262.84±64.29	255.00±24.16	199.53±21.95	0.0311
	Hypothermia	184.42±9.88	227.90±21.46 <sup>a</sup>	166.39±13.94 <sup>b</sup>	<b>0.0036</b>
	Hypoxia + Hypothermia	220.90±19.28	274.47±15.97 <sup>a</sup>	213.48±15.20 <sup>b</sup>	<b>0.0013</b>
Serum Creatinine	Hypoxia	1.25±0.09	1.86±0.32 <sup>a</sup>	2.06±0.27 <sup>a</sup>	<b>0.0007</b>
	Hypothermia	0.93±0.27	2.26±0.22 <sup>a</sup>	2.44±0.28 <sup>a</sup>	<b>0.0033</b>
	Hypoxia + Hypothermia	1.69±0.27	2.16±0.21 <sup>a</sup>	2.23±0.31 <sup>a</sup>	<b>0.0001</b>

b: Significant from SHAM

Serum Calcium	Hypoxia	10.96±1.29	12.58±0.65 <sup>a</sup>	13.42±0.95 <sup>a</sup>	<b>0.0007</b>
	Hypothermia	6.92±0.51	7.83±0.55 <sup>a</sup>	8.23±0.58 <sup>a</sup>	<b>0.0033</b>
	Hypoxia + Hypothermia	12.92±0.47	15.13±1.09 <sup>a</sup>	15.93±0.90 <sup>a</sup>	<b>0.0001</b>
Total protein (Liver)	Hypoxia	97.17±15.14	102.71±23.45	145.40±37.17	0.0232
	Hypothermia	103.53±41.91	65.75±14.30	112.99±61.96	<b>0.1092</b>
	Hypoxia + Hypothermia	120.45±23.12	128.68±42.93	153.87±35.52	0.2155
Total protein (Kidney)	Hypoxia	104.57±7.92	101.09±18.92	134.18±12.75 <sup>ab</sup>	<b>0.004</b>
	Hypothermia	83.17±4.04	103.33±12.31	95.96±10.26	0.2618
	Hypoxia + Hypothermia	127.07±10.80	154.22±16.46 <sup>a</sup>	210.08±28.19 <sup>ab</sup>	<b>0.0003</b>
Serum Electro-phoresis	Hypoxia	0.92±0.03	0.94±0.04	1.00±0.04	0.0584
	Hypothermia	0.94±0.03	0.98±0.03	0.98±0.03	0.019
	Hypoxia + Hypothermia	0.94±0.04	0.99±0.03	1.04±0.05 <sup>ab</sup>	<b>0.0023</b>
Loss of weight	Hypoxia	6.80±2.04	9.90±1.11 <sup>a</sup>	12.18±0.98 <sup>ab</sup>	<b>&lt;0.001</b>
	Hypothermia	4.70±0.95	6.10±1.19 <sup>a</sup>	8.90±1.37 <sup>ab</sup>	<b>&lt;0.001</b>
	Hypoxia + Hypothermia	8.10±1.29	12.40±1.58 <sup>a</sup>	17.70±1.42 <sup>ab</sup>	<b>&lt;0.001</b>
Blood Urea	Hypoxia	29.86±4.41	39.74±4.76 <sup>a</sup>	50.60±5.19 <sup>ab</sup>	<b>&lt;0.001</b>
	Hypothermia	27.04±3.54	34.94±3.61 <sup>a</sup>	39.35±1.95 <sup>ab</sup>	<b>&lt;0.001</b>
	High Alt	33.05±5.39	71.78±10.56 <sup>a</sup>	120.03±5.94 <sup>ab</sup>	<b>&lt;0.001</b>
Uric Acid	Hypoxia	2.04±0.19	2.62±0.17	2.38±0.11	<b>&lt;0.001</b>
	Hypothermia	2.37±0.15	2.54±0.27	2.83±0.39	0.044
	High Alt	2.65±0.24	3.19±0.25	2.67±0.23	<b>0.0025</b>

**RESULTS**

The results are shown in table 4. As the sample size was small we have used the non-parametric Kruskal Wallis to determine the level of significance among the three groups- Control, Sham and OB. To overcome

Type 1 error, Bonferroni correction was used. (0.05/3 = 0.0167).

Pair wise comparisons were made (where mean ranks were similar) between control and Sham, Control

and OB and Sham and OB.using Mann-Whitney test. (The \* are used to indicate the results).

This was done to determine the effect of sham surgery. The results indicated that while sham surgery did produce some level of stress it was not significant when compared with control rats. There was a significant difference in the response between the sham and the OB group of rats.

- The loss of body weight and increase in the levels of Blood Urea were very significant, compared with the Sham and Control groups.
- Decrease in LDH in liver and kidney in the OB group was significant. Hypothermia had less effect on kidney LDH than hypoxia.
- Drop in the SDH in liver and kidney was significant in the OB group. Hypothermia alone in the absence of hypoxia did not alter the level in the liver.
- Total protein levels in kidney and liver increased in hypoxia. Hypothermia alone did not have any effect on the levels.
- Increase in the uric acid and A-G ratio (electrophoresis) was more significant in hypoxic conditions than in hypothermia.
- There were no significant changes in the weight of the liver.
- Serum creatinine and calcium levels increased significantly in both Sham and OB groups.

## DISCUSSION

We believe that AMS is a type stress due to hypoxia and hypothermia. Rats are not known to suffer from AMS and were therefore selected for this study. There are many studies, which have indicated the relationship between Ob and stress response in rats (Brown.,1984 ). In our study rats whose Obs were removed suffered from stress reaction when subjected to conditions which are known to be associated with AMS – lowered atmospheric pressure, reduced atmospheric oxygen concentration and low temperatures. Comparisons of responses to stress were

made between those whose Ob's were intact with those Ob's were removed. The experiments show the regulatory role of olfactory system in acute stress situations, which are prone to produce AMS.

## Stress adaptation failure

### Weight Loss

Very significant loss of weight was seen in Ob rats at Hypoxia, hypothermia and hypoxia and hypothermic conditions. Starvation was not a factor as the serum glucose levels were within normal limits (3.107-5.2m Moles/litre.)

The loss of weight in the control group at hypothermic atmosphere could be due to shivering, diuresis caused by anaesthetic ether and decrease in vasopressin. The loss of weight in sham-operated rats was perhaps due to postoperative stress coupled with hypothermia and hypoxia.

Subjecting rats to moderate high altitude causes natriuresis and diuresis through chemoreceptors (Marcilhac et al., 1999). There is an inhibition of renal tubular sodium reabsorption with natriuresis and diuresis in the kidneys. This is possible even if cortisol levels are increased. Olfactory bullectomy also results in natriuresis and diuresis possibly through a decrease in secretion of aldosterone. Olfactory bulb regulates the amount of aldosterone secreted through the pituitary (Childers., and Schneider., 1982 & Claydon et al., 2004)). In the absence of this mechanism in Ob rats, aldosterone is secreted through the splanchnic connections between the hypothalamus and adrenals (see diagram).

### Serum Urea

A marginal increase in serum urea levels in the control rats subjected to both hypoxia and HA was probably due to exhaustion. Starvation was not a factor as the blood glucose levels were within normal limits. Urea synthesis is under the control of nutritional status especially the protein intake and its catabolism. Since the rat feed was constant the increase in urea was probably due to increased

shivering (muscular activity). Hypoxic and hypothermic stress results in increased adrenal hormonal status (both medullary and cortical). Significant increase in the OB rats suggests a serious endocrine disturbance. This is probably through the hypothalamic-splanchnic pathway.

The reason for the increase in uric acid content is possibly related to defective renal tubular reabsorption in hypoxia and high altitude. It is not clearly understood if this is a protective mechanism or the primary cause (Robinson et al., 1998).

#### **LDH-Liver**

LDH in liver was significantly reduced in Ob rats (Grzybowski et al., 2002). It is not clear if this is ACTH-Cortisol feed back mechanism or a serotogenic response. There is no clear evidence to support the former though it is a commonly held belief. Goddard et al observed that the activity of LDH was not correlated with the plasma concentration of cortisol, a widely used physiological measure of stress. LDH level is also influenced by hepatic blood flow and may indicate a drop in systemic blood pressure.

The increase in LDH levels in sham-operated rats is due to an adaptation response to stress (Goddard et al., 1997 and Jaeschke., 1990).

There is a genetically determined variation to stress (LDH levels) in different species. The role of lactate dehydrogenase – B gene expression has been reported (Schulte et al., 2000). We do not know if a similar mechanism is in operation in the mountaineers. If it does it would explain the susceptibility of certain individuals to the stress of high altitude activity.

#### **LDH –Kidney**

The high levels of LDH in the kidney indicate renal damage, an indication of high plasma concentration. This is indicative of stress (Anand et al., 1993).

#### **SDH – Liver and Kidney**

Significant reduction in SDH levels in kidney and liver in Ob rats is a reflection of mitochondrial activity

which is oxygen dependent.. SDH in liver reduced significantly in both hypoxia and in combination with hypothermia (HA). However there was no significant drop under hypothermic conditions. It is possible that hypoxia was the principal cause (Shertzer.,1972). Sensitivity of mitochondrial enzymes to low oxygen concentrations was responsible for these changes.

#### **Serum Creatinine**

The increase in the levels of serum creatine in Ob rats is a reflection of stress response failure (Koga et al., 2005).

#### **Serum Calcium**

Increase in serum calcium levels occur during psychogenic stress. Hypoxic exposure also releases membrane bound calcium. Vasopressin levels are increased secondary to Hypothalamic-Hypophyseal reaction to stress. The former induces hypertonic urine and stimulates adrenocorticotrophin. The latter increases the serum calcium levels by stimulating hyperthyroid (Walters., 1986). It is not clear why the sham-operated rats also had increased levels similar to the OB group.

#### **Serum Electrophoresis**

Changes in the A/G ratio are probably due to two reasons:

1. A reflection of loss of body weight;
2. Direct effect of psychological stress (Van Hunsel et al., 1998)

It is well known that all animals suffer from psychological stress in adverse conditions (Kawahara et al., 1993). We are unable to determine if our rats suffered such psychological stress in laboratory conditions.

Total Proteins in liver and kidney increased in hypoxia. Hypothermia seemed to have less influence. Acute necrosis secondary to hypoxia was responsible for the increase. Liver protein is also known to increase in acute stress. (Majumdar et al., 1967)



### Olfactory bulb and respiratory rhythm

Very early during climbing hypoxia induces hyperventilation. At this stage the levels of hypoxia are not sufficiently high to trigger BBB either by vasogenic or cytotoxic oedema.

The delicate balance between respiratory cycle and olfactory neurons are well recognised (Bounvicio et al., 2006). It has been shown that irregularities of respiratory rhythm induces phase locking of the mitral cells of the olfactory bulb. The temporal and spatial organisations of the responses are influenced by the frequency variability of the sniffing responses in the rats. These respiratory oscillations of the mitral cells are influenced by the glutamate receptor function (antagonistic).

We postulate that the respiratory rhythm might function as a modulator for olfactory-limbic-hypothalamic structures. There could be a limbic orientated memory function during periods of phase matching between olfactory and limbic-hypothalamic structures.

### Serotonin in hypothalamus during stress

The responses to the olfactory bulbectomy in unstressed and stressed rats differ.

Hyper corticosteronemia in unstressed OB is independent of the serotonergic system in Hypothalamus. Marcilhac has shown hypothalamic Serotonin (5-HT) changes in response to stress in rats whose olfactory bulbs have been removed (Marcilhac et al., 1999).

Hypothalamic serotonin changes in HPA activity of OB rats is in response to stress. However the relationship between ACTH and corticosterone peaks in OB rats is not clear. Plasma concentration of corticosterone increases without changing ACTH concentration suggesting a dissociation of synchronisation of ACTH and corticosterone.

Hypothalamus also regulates adrenal glands via the splanchnic nerve, independent of the pituitary–adrenal axis. (Fig. 1) Hypophysectomized rats under cold exposure stress react through 5 $\alpha$ -Deiodinase –

splanchnic pathway and not through the pituitary (Anguiano et al., 1999). Corticosterone- independent mechanism overriding glucocorticoid feed back occurs in chronically stressed rats (Marcilhac et al., 1999).

5-HT<sub>1A</sub> receptor activation probably reflects feedback inhibition of HPA system. In view of the controversy regarding the serum levels of ACTH and corticosterone in Ob rats (as indicated above) we did not estimate the levels of these hormones.

### Olfactory bulb regulates AMS

We propose that AMS is a type of stress reaction during rapid ascent to heights. OB dysfunction induced by changes in respiratory rhythm due to hypoxia influences HPA axis dysfunction through serotonergic system (Van Hunsel et al., 1998). The phase locking of the mitral cells of the olfactory bulb induces a physiological olfactory Bulbectomy. (Fig. 1)

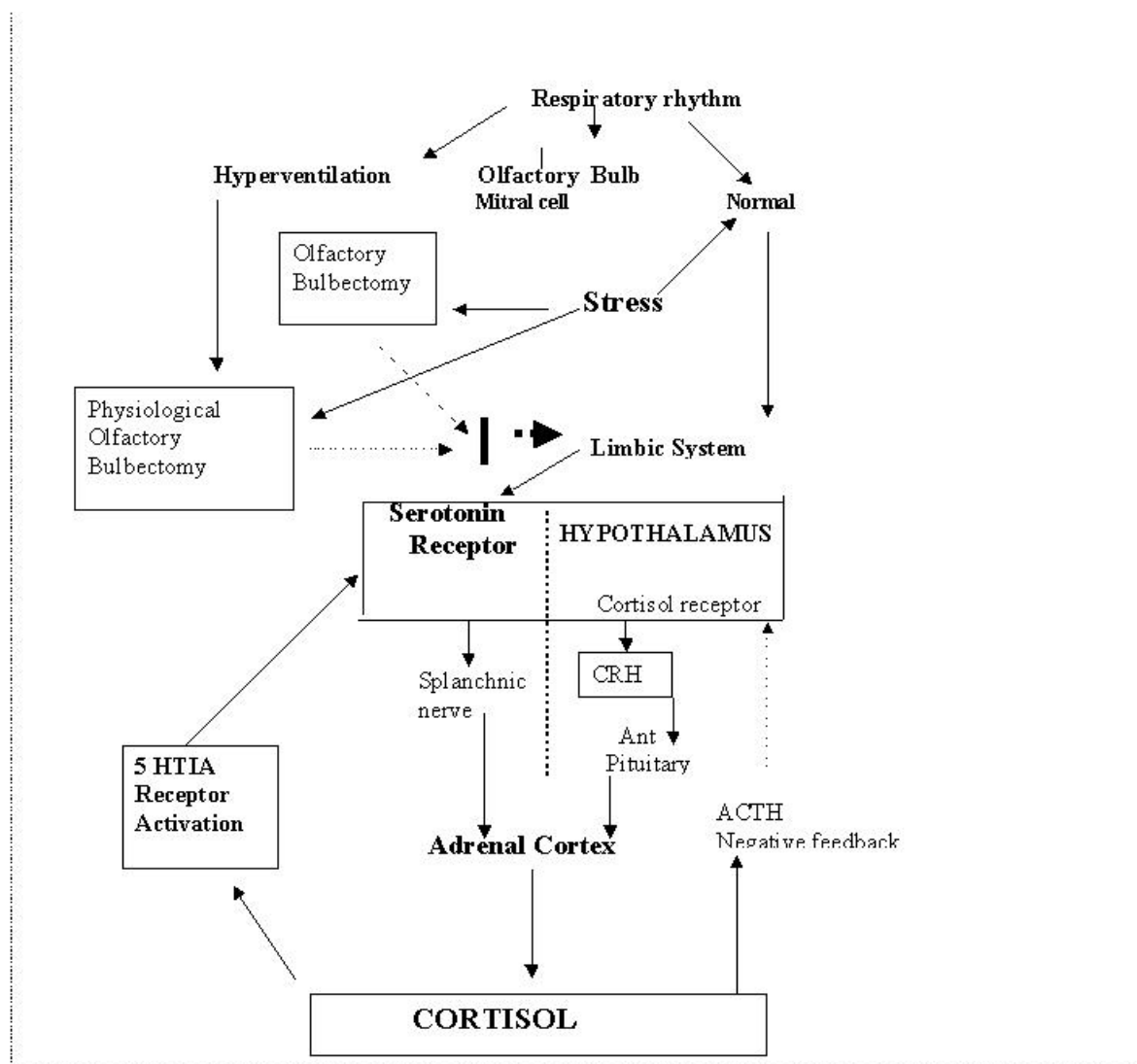
Serotonergic related dysfunction varies in individuals. Perhaps this explains the unpredictable response to HA in climbers. HPA axis responds to even small increases in serotonin stress sensitive individuals, a reflection of decreased central serotonergic neural activity (Bethea et al., 2005 & Konstandi et al., 2000).

Prolonged and repeated exposure to HA often reduces the severity of AMS commonly described as acclimatisation. A single exposure to stress in rat is known to increase serotonergic response in the hypothalamus to a significant degree. However a second application of stress after an interval does not induce further changes in the levels of 5-HT and serotonin responses. Further the response to second stress varies between mice and rats (De Souza., 1986). The central neurotransmission triggered by stress appears to be species specific and may trigger very discrete changes in the homeostatic mechanisms (Bethea et al., 2005).

It appears that many of the features of AMS are explainable if it is seen as a response to stress and not as a consequence of hypoxia and cerebral autoregulation failure due to hypoxia.

AMS is a type of stress. Normal rats when subjected to such stress, as AMS are able to adapt. This adaptation is lost when the olfactory bulbs are removed. It is postulated that serotonin receptors in the hypothalamus, through the splanchnic pathway regulate stress. This is independent of the ACTH-Cortisol feed back mechanism. Perhaps irregular and rapid respiratory rhythm triggers mitral cell dysfunction in the olfactory system. A phase locking mechanism occurs. This simulates a physiological olfactory Bulbectomy. This leads to serotogenic

This response is variable and occurs in individuals susceptible to serotogenic triggered hypothalamic dysfunction (Vogel et al., 2001). The correction procedures for AMS should be directed towards the serotogenic dysfunction and not at pituitary dysfunction modulated by corticosterone. The acute distress during rapid ascent to HA does not lead to BBB failure during early stages of AMS. The key to the AMS is in the olfactory system.



**Fig. 1. Proposed pathophysiology of AMS**

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stereo frame without which it would not have been possible to conduct the experiments. We are grateful for their help. Mr. Kadam spent time beyond the normal hours to maintain the animals in the HA

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