able to regulate growth and, consequently, connectivity indicates that rather common simple molecules may also play prominent roles in regulating the pattern of neuronal connectivity.

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15. Serotonin significantly reduced the strength of the electrical connection between neurons 5 and 19 [$t(8) = 4.15$, $P < 0.002$; t-test] and increased the frequency of zero coupling values [$\chi^2(1) = 9.0$, $P < 0.01$].
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Extrapulmonary Gas Exchange Enhances Brain Oxygen in Pigeons

Abstract. *Blood in mouth, nose, and eye tissues of birds cools by evaporation, then flows to a cephalic vascular heat exchanger, the ophthalmic rete. There, acting as a heat sink, blood from the evaporative surfaces cools arterial blood flowing countercurrent to it toward the brain. The brain thus remains cooler than the body core. Data for unanesthetized domestic pigeons* (Columba livia) *suggest that in addition to losing heat, blood perfusing the evaporative surfaces also exchanges oxygen and carbon dioxide with air. In the heat exchanger, this blood apparently gives up oxygen to, and gains carbon dioxide from, arterial blood. The consequent increase in oxygen and decrease in carbon dioxide in the brain's arterial blood enhance diffusion of these gases in, and oxygen supply to, the brain. Such events may help birds maintain the brain's oxygen supply during the high systemic demand of exercise and at the reduced oxygen availability of high altitude.*

Evaporation from mouth, nose, and eye surfaces in birds cools the blood flowing just beneath these surfaces. The cooled blood then courses to the ophthalmic rete (OR), a network of small arteries and veins caudal to each eye (Fig. 1). Arterial blood in the OR conducts heat to venous blood flowing countercurrent to it, then proceeds to the brain at reduced temperature. This protects the brain from overheating when body temperature rises during exercise or high temperature exposure (*1*).

Oxygen and carbon dioxide tensions (P_{O_2} and P_{CO_2}) in avian arterial blood at sea level are about 85 and 30 torr, compared to 159 and 0 torr in air (*2*). Blood circulating in the dense vascular beds beneath the moist cephalic surfaces must therefore encounter large gradients for these gases, and it seemed reasonable to infer that air and blood at these surfaces might exchange O_2 and CO_2, as well as water vapor and heat. Venous blood flowing to the OR would then not only have a lower temperature but would also have an increased P_{O_2} and a reduced P_{CO_2}. Our preliminary calculations indicated that P_{O_2} and P_{CO_2} in the OR veins would be higher and lower, respectively, than in OR arteries, suggesting that the OR is a site of O_2 diffusion from venous to arterial blood, and of CO_2 diffusion in the opposite direction, as well as a site of heat exchange. Furthermore, as shown in Fig. 1, changes in temperature, P_{CO_2}, and pH would affect hemoglobin O_2 binding in a manner conducive to both O_2 loading at the moist surfaces and O_2 transfer from venous to arterial blood in the OR (*3*).

The data presented here support this idea. They show that P_{O_2} in cerebral arterial blood of domestic pigeons (*Columba livia*: mean mass, 0.34 kg) is greater than in carotid arterial blood and suggest that the P_{O_2} difference is correlated with the extent to which cephalic mucosal surfaces contact air. An elevated P_{O_2} in cerebral arteries would increase the P_{O_2} in brain capillaries, improving O_2 diffusion to tissue. If arterial O_2 saturation also increases, total O_2 flow to brain would also increase.

To test the idea, we compared gas tensions in arterial blood before it entered the OR (pre-OR) and after it left the OR (post-OR) to determine whether enhancement of gas exchange occurs in the OR itself. Pre-OR arterial gas tensions were measured in carotid blood. However, post-OR arteries were inaccessible for sampling. We therefore reasoned that gas tensions in cerebrospinal fluid (CSF) sampled near the lateral ventricle's choroid plexus, a site of CSF formation (*4*), would represent corresponding values in choroid plexus blood, at least some of which comes from the OR. Accordingly anaerobic samples of CSF and arterial blood were simultaneously sampled from unanesthetized birds, and P_{O_2}, P_{CO_2}, and pH were measured (*5*).

Figure 2 shows results for 11 control birds at room temperature (experiment 1). Variances for mean differences were not significantly different from each other; we therefore used t-tests for comparing means. The mean P_{O_2} of the CSF was 114 torr (±3 standard deviation). This is 39 percent greater [$t(10) = 24.8$; $P < 0.0001$] than the mean P_{O_2} in carotid blood [82 torr (±3)]. We also found that P_{CO_2} was significantly lower in CSF [23.3 torr (±2.2)] than in carotid blood [31.4 torr (±2.3)], a 26 percent decrease [$t(10) = -8.5$, $P < 0.0001$]. The changes in cerebral arterial P_{O_2} and P_{CO_2} make possible increases in O_2 diffusion to and CO_2 diffusion from brain tissue.

Our results may also be explained by possible carbonic anhydrase activity in the ependymal cells of the choroid plexus. It has been suggested that the ependymal carbonic anhydrase reaction produces HCO_3^-, which moves into CSF, and H^+, which enters choroid capillary plasma (*6*). Erythrocytic bicarbonate

also moves into plasma to maintain ionic balance, combining with H^+ to form CO_2. The CO_2 moves into erythrocytes where carbonic anhydrase catalyzes its conversion to HCO_3^-, replacing HCO_3^- lost to plasma. Meanwhile H^+ produced in the same reaction facilitates the release of O_2 from hemoglobin, which moves into CSF and increases its P_{O_2}. This is the mechanism most likely to operate in the brain's glial cells for direct oxygenation of interstitial fluid (*6*).

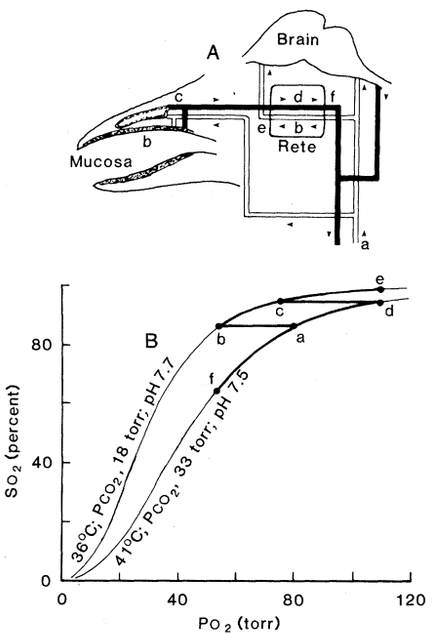

Fig. 1. (A) Simplified diagram of blood flow in pigeon head. Arrows show flow direction. Arterial blood (unshaded) flows to brain directly and via the bilateral ophthalmic retia (only one shown), and to the nasal and oral mucosa (stippled). Venous blood (shaded), cooled at the cephalic mucosa to about 36°C in this example, flows through the OR, where arterial blood at about 41°C is cooled by countercurrent heat exchange before flowing to brain. (B) Pigeon blood oxyhemoglobin dissociation curves showing changes in blood O_2 saturation (S_{O_2}) and tension (P_{O_2}) evoked by changes in temperature, CO_2 tension (P_{CO_2}), and pH (*3*). Letters a through f on curves correspond to locations on the diagram. The hypothesis presented here is as follows. Arterial blood at P_{O_2} = 80 torr and S_{O_2} = 85 percent [point a (*2*)] cools and loses CO_2 to air at mucosa, driving the curve leftward (point b). This blood also loads O_2 from air, raising P_{O_2} and S_{O_2} to point c. On returning to the rete the curve moves rightward (point d) as it obtains heat and CO_2 from arterial blood. The latter moves to point b because of its heat and CO_2 loss, establishing a retial O_2 gradient from point d to point b. If retial gas exchange is complete, arterial blood from the OR will enter the brain virtually saturated at P_{O_2} = 115 torr (point e), whereas venous blood from the OR will flow to the heart at point f. If retial exchange is not complete, points e and f for arterial and venous retial outflow will be closer together. Thus, final brain P_{O_2} depends on the proportioning of arterial flow between direct and retial routes, and on completeness of gas and heat exchange in the mucosa and OR.

To control for the possibility that this model may explain our CSF results in pigeons, we performed two additional experiments. In experiment 2, we plugged the nares with Plasticine and taped the beak to prevent contact between air and oronasal surfaces. Experiment 3 was identical to experiment 2, except that we also covered the closed eyelids with sheet paraffin to prevent contact between air and eye surfaces. Birds breathed freely via tracheostomies (*5*) in both experiments. The experimental treatments did not significantly affect arterial values of P_{O_2} and P_{CO_2} (Fig. 2). Cerebrospinal P_{O_2}, in contrast, fell significantly in experiment 2, although not all the way to the arterial level. In experiment 3, CSF P_{O_2} fell even farther and became indistinguishable from carotid arterial P_{O_2}. These results indicate that the nose, mouth, and eyes participate in the processes that determine CSF P_{O_2} in these animals.

Cerebrospinal P_{CO_2} increased from below the carotid level in experiment 1 to above the carotid level in experiments 2 and 3 (Fig. 2). We think that this finding resulted from our preventing gas exchange between the evaporative surfaces and air in experiments 2 and 3, so that venous P_{CO_2} in the OR was greater than P_{CO_2} in arterial blood. The arterial blood therefore must have gained rather than lost CO_2 in the OR before it flowed to the brain, contrary to the model in Fig. 1. We also found that pH in CSF was lower than in arterial blood in all the experiments—probably because of the absence of protein buffers in CSF—and did not change (Fig. 2). This suggests that CSF pH homeostasis is the probable end result of CSF HCO_3^- regulation caused by shifts in CA activity during disturbances in dissolved gas tensions.

Gas tension results from experiments 1, 2, and 3 are consistent only with the hypothesis that moist cephalic surfaces take up O_2 and release CO_2 and that O_2 and CO_2 exchange occurs within the OR. The results of experiments 2 and 3 would not have been observed if acid-base changes in the ependymal cells were the sole explanation for enhancement of CSF P_{O_2}.

Sealing the eyes against contact with air (experiment 3) affected P_{O_2} but not P_{CO_2} in the CSF (Fig. 2). Because CO_2 solubility in aqueous solutions is about 30 times greater than O_2 solubility, a large amount of CO_2 could diffuse from plasma to air without markedly altering the molar CO_2 content of the plasma. On the other hand, during O_2 diffusion from air to blood, a small increase of molar O_2 concentration in plasma, caused by diffusion from air, would lead to a large increase in plasma P_{O_2}. Because total surface area of the eyes is small compared to nasal and oral surfaces, the molar CO_2 content of blood might not be significantly reduced in the time of blood transit through the eyes, whereas CO_2 loss from blood flowing through nose and mouth tissues might be enough to reduce P_{CO_2} significantly. Therefore sealing the nose and mouth would evoke a significant reduction in the loss of blood CO_2 as well as in the uptake of O_2, whereas additionally sealing the eyes would reduce further the uptake of O_2 but not the loss of CO_2.

The physical properties of avian blood support the possibility of augmented cerebral oxygen supply. Because of the

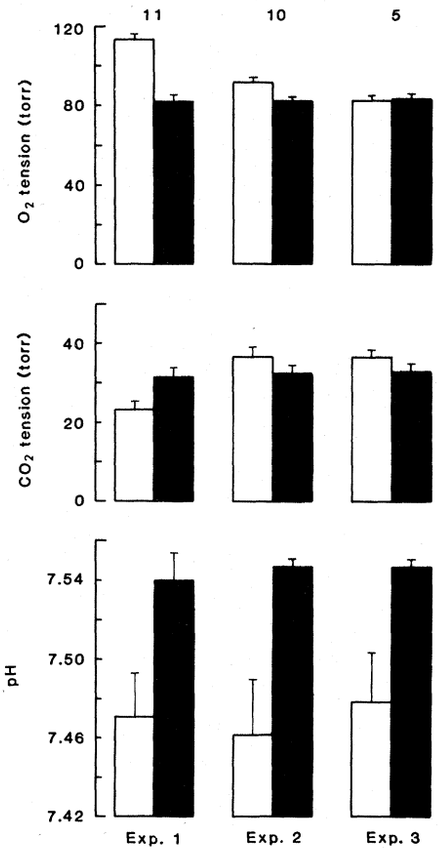

Fig. 2. Oxygen and CO_2 tensions and pH in anaerobic samples of cerebrospinal fluid (unshaded bars) and carotid arterial blood (shaded bars) obtained simultaneously from unanesthetized pigeons (environmental temperature, 23°C and barometric pressure, 665 torr). Bar heights represent means; vertical lines extend +1 standard deviation. Numbers across top represent numbers of individual birds used in each experiment. Each bird was used once. Experiment 1 was performed on intact control birds; experiment 2 was performed on tracheostomized birds with mouth and nose sealed against contact with air; and experiment 3 was identical to experiment 2, except that eyes were also sealed.

comparatively large size and nondeformability of avian erythrocytes, at equal tube diameters and hematocrits the relative viscosity of avian erythrocyte suspensions is four to six times greater than in mammalian erythrocyte suspensions (*7*). In apparent compensation, the fraction of tissue volume occupied by brain capillaries is about twice as high in birds as in mammals (*8*). In a tissue like the brain, contained in an inelastic skull, further increases in capillary density would increase O_2 delivery, but the resulting increase in tissue volume occupied by capillaries would reduce neuronal space. It therefore seems plausible that further evolutionary improvements in O_2 delivery would be brought about through increases in the diffusion gradient; this could be achieved by means of the proposed mechanism.

We conclude that the Po_2 is increased and the Pco_2 is decreased in a portion of the arterial supply to the pigeon brain, thereby facilitating diffusion of O_2 into and CO_2 out of brain tissue by increasing the respective gradients. Blood O_2 affinity is lower in birds than in similar-sized mammals, and arterial blood is not likely under most conditions to be saturated with O_2 (*9*). We therefore suggest further that, in raising arterial Po_2, the mechanism must also increase hemoglobin O_2 saturation. This would (i) sustain steady-state O_2 delivery at lower cerebral blood flows than would otherwise be necessary, and (ii) increase brain O_2 delivery under conditions in which an increase in cerebral blood flow alone could not meet the O_2 demand, such as at high altitudes.

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Induction of Pituitary Lactotrope Differentiation by Luteinizing Hormone α Subunit

Abstract. *Addition of gonadotropin releasing hormone to cultures of fetal rat pituitary induced differentiation of lactotropes as revealed by immunocytochemistry. Antiserum to luteinizing hormone (LH) (recognizing native LH), but not antiserum to LH-β (recognizing both native LH and its β subunit), inhibited this induction. Further addition of highly purified LH-α subunit in culture medium also induced lactotrope differentiation. Thus, the α subunit may have a specific biological activity of its own with probable practical use in clinical investigations.*

Induction of pituitary lactotrope differentiation by gonadotropin releasing hormone (GnRH) has been reported (*1*). This effect of GnRH is possibly subsequent to a primary action on gonadotropes and induces a further release of stimulating factor or factors. Such a chain of events has been described for the stimulation of the release of prolactin from adult rat lactotropes (*2*). Moreover in human anencephalic fetuses, where hypothalamic GnRH is lacking, lactotropes are numerous and active (*3*). In these types of fetuses, gonadotropes are known to store the α subunit almost exclusively (*4*, *5*). A possible relation between the abundance of this subunit and the proliferation of lactotropes was therefore considered. We now describe the role of the α subunit as mediator in the action of GnRH and in the differentiation of lactotropes.

Adenohypophysial primordia were explanted from 13-day-old rat fetuses and cultured in a synthetic medium as described (*1*, *6*). Cultures were maintained for 8 days to reach the expected term of gestation when the first lactotropes would normally appear in vivo (*7*). The culture medium was renewed every 2 days.

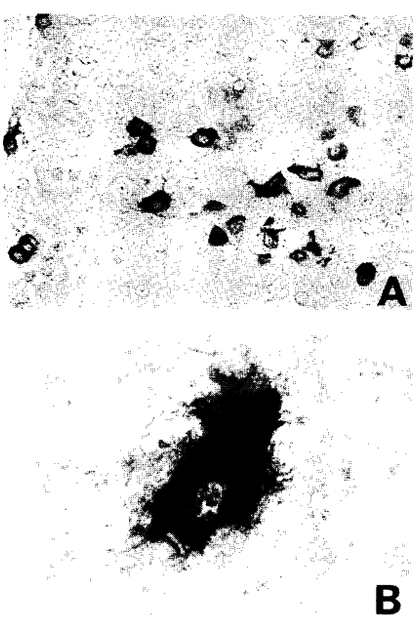

Fig. 1. Immunocytochemistry with antiserum to rat prolactin diluted 1:800 at 4°C overnight (light microscopy). This antiserum has been studied and used previously (*1*). Typical primordium cultured in medium containing LH-α ($10^{-7}M$) (the experiment was repeated four times, and a total of 20 primordia was studied). (A) Several immunoreactive cells are scattered in the cultured tissue (×250). (B) The immunoreactive cells are irregularly shaped and intensely stained (×1200).