UiT
THE ARCTIC UNIVERSITY OF NORWAY

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Breathtaking brains

Intrinsic neural adaptations to hypoxia

Samuel J Geiseler

A dissertation for the degree of Philosophiae Doctor – January 2016

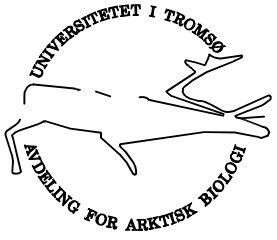
UNIVERSITY OF TROMSØ

Breathtaking brains

Intrinsic neural adaptations to hypoxia

Samuel J. Geiseler

A dissertation for the degree of Philosophiae Doctor – January 2016



Title picture

Recording from a hippocampal slice taken from a hooded seal (*Cystophora cystata*): Bottom-right double-wire is stimulating electrode (S1), placed in the *stratum radiatum* to stimulate Schaffer Collaterals; bottom-left glass-recording electrode (R1), placed in the *stratum radiatum* to record field excitatory post-synaptic potentials (fEPSP) evoked by S1. Top-left double-wire is stimulating electrode (S2), placed in the *alveus* layer to stimulate axons of pyramidal cells; top-right glass-recording electrode (R2), located in *stratum pyramidale* to record population spike evoked by S2.

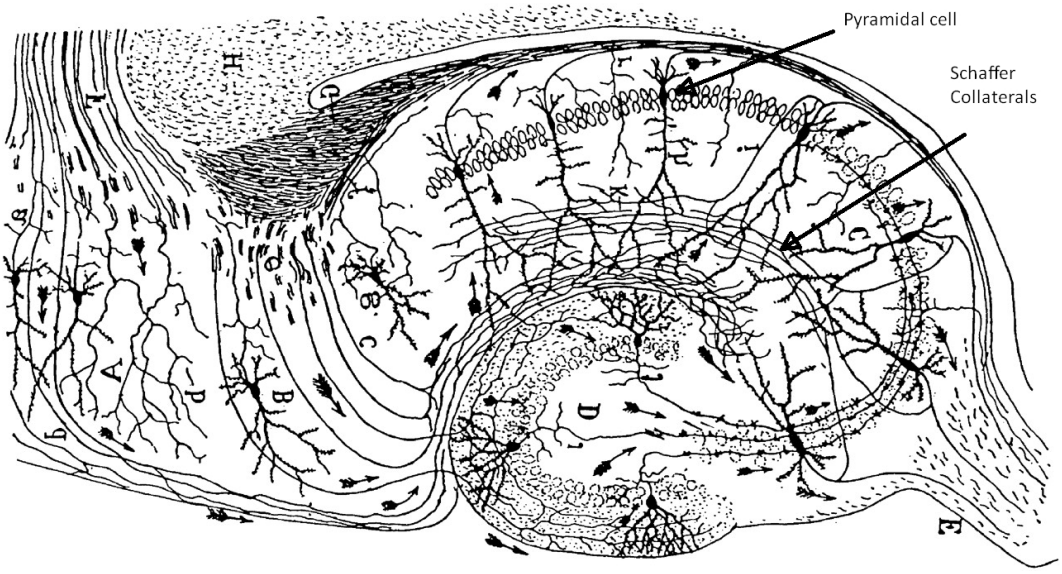


Figure 0.1: Neural circuitry of the rodent hippocampus. Modified after Cajal (1909-1911).

Table of Contents

List of papers

Paper I

Czech-Damal NU, Geiseler SJ, Hoff ML, Schliep R, Ramirez JM, Folkow LP, Burmester T (2014) **The role of glycogen, glucose and lactate in neuronal activity during hypoxia in the hooded seal (*Cystophora cristata*) brain**. Neuroscience 275:374-383.

Paper II

Geiseler SJ, Ludvigsen S, Folkow LP (2015) **KATP-channels play a minor role in the protective hypoxic shut-down of cerebellar activity in eider ducks (*Somateria mollissima*)**. Neuroscience 284:751-758.

Paper III

Geiseler SJ, Larson J, Folkow LP (2016) **Synaptic transmission despite hypoxia in hippocampal slices of the deep-diving hooded seal.** Neuroscience, submitted.

Summary

To maintain and restore the membrane potential that is crucial for normal function of neurons, the brain requires a constant and high energy supply. This supply is mainly covered by oxidative metabolism. When oxygen supply is impaired on a cellular level (hypoxia), such as in the case of stroke, neural energy levels become inadequate to maintain ion balance, leading to excessive ion flux and ultimately to neural death.

Despite the brain's high vulnerability to hypoxia, a number of animals are exposed to and survive hypoxia on a regular basis. Consequently, these animals must possess intrinsic adaptations to hypoxia. An overview of some of these neural adaptations is presented here, with focus on two diving animal species, the hooded seal (*Cystophora cristata*) and the eider duck (*Somateria molissima*).

To cover their energy requirements, mammalian neurons generally seem to aerobically metabolize lactate provided by astrocytes. Energy metabolism in the seal brain, however, appears to be organized differently with regard to the roles of astrocytes and neurons, possibly reducing oxidative stress in neurons and enhancing neuronal anaerobic capacity during hypoxia. In paper I we investigated the role of glucose and lactate as fuel sources in hooded seal neurons. We confirmed that spontaneous neuronal activity is maintained in hypoxia, both in the presence of glucose, but also in lactate and aglycemia. While the first implies an increased anaerobic capacity, the latter is possibly due to the enhanced neural glycogen reserves that we found, which are also found in other hypoxia-tolerant species.

To cope with the reduced energy available during hypoxia, many hypoxia tolerant species reduce brain activity to decrease energy demand. In accordance with this strategy, some neurons in the hooded seal and eider duck brain display a

shutdown response to hypoxia. In paper II we showed that K_{ATP}-channels are present in the eider duck brain and may contribute to reduce neuronal activity during hypoxia.

In paper III we investigated the effect of hypoxia on neuronal communication in the hooded seal. We showed that hippocampal slices *in vitro* are able to maintain synaptic transmission for at least 3 h in severe hypoxia. We found attenuated paired pulse facilitation in the seal hippocampus and suggest that this may reflect an altered presynaptic calcium regulation in the seal neurons to mitigate the detrimental effects of excessive calcium influx.

The presented results contribute to our understanding of intrinsic neural adaptations in hypoxia tolerant animals. The underlying mechanisms seem to contribute to a reduction in neural energy demand by attenuating activity, maintain energy balance by adapting metabolism, and employ various molecular mechanisms to protect the brain from hypoxic damage.

Introduction

We take for granted many things in life, only missing them when they are gone. In some cases this is less dramatic, but sometimes the consequences can be quite severe, such as when the brain loses blood supply (ischemia) in the case of cardiovascular diseases. Stroke is still one of the major causes of human death in the western world, occurring on average every 40 seconds and leading to one death every 4 min in the USA while cardiovascular diseases kill over 2150 Americans every day (Mozaffarian et al., 2015).

The pathophysiology of stroke-induced cerebral ischemia has been extensively investigated (for review see e.g. Dirnagl et al., 1999 and Lipton, 1999). In short: ischemia restricts substrate supply to the brain, in particular glucose and oxygen. This impairs the brain's energy supply (adenosine triphosphate, ATP) necessary to maintain neural integrity, ultimately leading to cell death.

Most of the brain's ATP consumption is used for ion pumping to maintain neuronal membrane potential during synaptic transmission (Harris et al., 2012). When ATP levels decrease, the Na^+/ K^+- ATPase ion-pump will eventually not be able to maintain the ion-balance and neurons will depolarize. This will lead to the release of neurotransmitters, including the excitatory neurotransmitter glutamate, which activates N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Choi, 1988, Novelli et al., 1988). The continuous excitation will lead to an uncontrolled flood of Ca^{2+} from extra- and intracellular stores, which leads to calcium overload in the neurons (Lipton and Whittingham, 1979). The excessive intracellular messenger Ca^{2+} then over-activates several enzyme systems, leading to the generation of free radicals which damage cell membranes, DNA and mitochondria, subsequently triggering apoptosis (Dirnagl et al., 1999; fig 1).

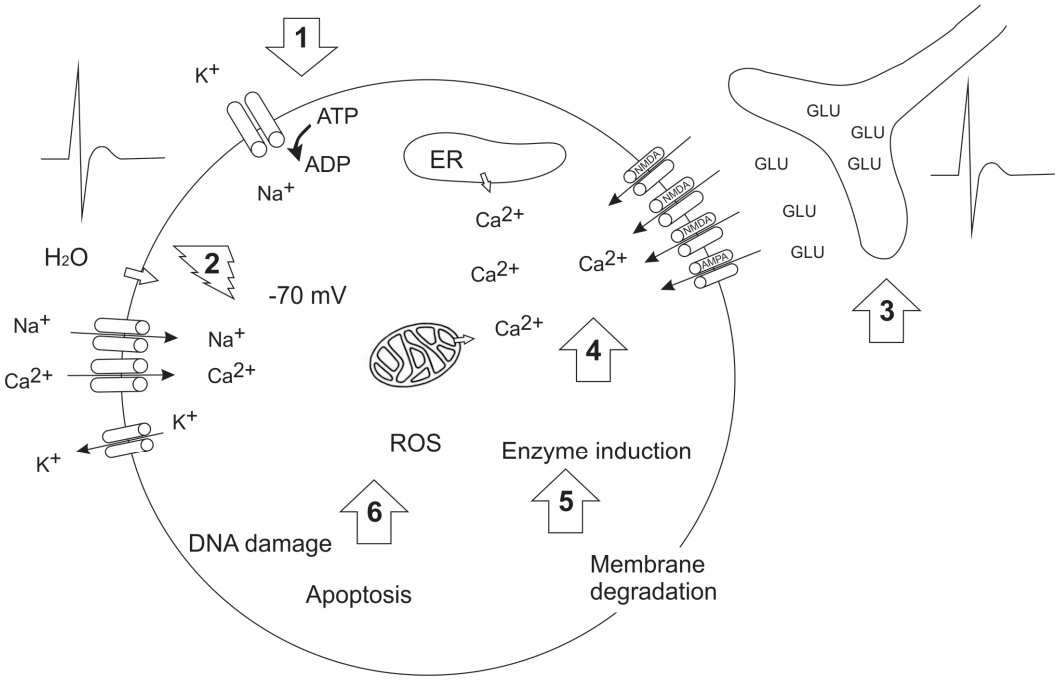


Figure 1: Detrimental steps resulting from hypoxia: **1** energy failure, **2** disruption of ion homeostasis/depolarization, **3** (uncontrolled) release of neurotransmitters (glutamate) and over activation of NMDA/AMPA receptors, **4** excessive influx of Ca^{2+} and release from intracellular stores leading to Ca^{2+}-overload, **5** over activation of enzyme systems leading to production of free radicals, **6** damage of cell components, DNA damage and apoptosis. Modified after Dirnagl et al. (1999) and Drew et al. (2004).

Most of the brain's ATP supply is derived from aerobic respiration in mitochondria, which yields 19 times more ATP from each metabolized glucose molecule than the alternative anaerobic metabolism. Under normoxic conditions, more than 95% of brain glucose utilization is based on oxidative phosphorylation (Erecińska and Silver, 1994). Even though it represents only 2 % of the body mass (BM), the human brain consumes ~20 % of body oxygen use (Mink et al., 1981, Rolfe and Brown, 1997).

Despite extensive research on the pathophysiology of insufficient oxygen supply (hypoxia), related diseases such as stroke remain one of the leading causes of

human death in the western world (Mozaffarian et al., 2015); Novel clinical approaches might therefore be desirable.

Animal models and aim of thesis

The Nobel Prize winning Danish physiologist August Krogh (1874 –1949) once wrote “For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied.“ (Krogh, 1929).

There is a large number of animals which are exposed to hypoxia on a regular basis and survive it without obvious damage. These organisms must possess intrinsic defense mechanisms against the above-described detrimental effects of hypoxia and consequently these animals should be particularly well suited to study adaptations to hypoxia. A number of such adaptations have been discovered in these organisms and the understanding of their intrinsic hypoxia tolerance might be of high clinical relevance (Bickler, 2004, Ramirez et al., 2007, Larson et al., 2014).

This thesis aims at summarizing the research on this topic with focus on adaptations to acute neural hypoxia, as experienced by diving animals. In this context I will present my own contribution to this field, which focussed on the deep diving hooded seal (*Cystophora cristata*) and the common eider duck (*Somateria mollissima*). Neurons of the hooded seal are able to generate action potentials after up to 1 h in severe hypoxia (Folkow et al., 2008). Similarly, eider duck neurons survive 1 h episodes of hypoxia as well as chemical anoxia (Ludvigsen and Folkow, 2009). This display of exceptional neuronal hypoxia tolerance makes both species worthy study objects to shed light on intrinsic adaptations. In addition, the relatively large size and complexity of the seal brain (Graham and

Hickie, 1986, Eisert et al., 2014) makes the hooded seal a very relevant model in a clinical context.

Intrinsic adaptations to hypoxia

Two main strategies are involved in hypoxia tolerance of adapted animals: I) increased body oxygen reserves to prevent, or at least delay, hypoxia during times of low oxygen availability; II) reduced oxygen demand by decreased metabolic processes. Both strategies are supplemented by various defense and repair mechanisms to prevent and/or relieve hypoxia induced damage (for review see e.g. Ramirez et al. 2007 and Larson et al. 2014).

Oxygen stores

Increased body oxygen stores are the most expressed in diving animals. The deep diving hooded seal for example has oxygen reserves of almost 90 ml O_2/kg BM (Burns et al., 2007) compared to 20 ml O_2/kg BM in humans (Kooyman, 1989). Among birds the deep diving emperor penguin (*Aptenodytes forsteri*) possesses 53 ml O_2/kg BM (Kooyman and Ponganis, 1998). For the largest part, those stores are based on high levels of the oxygen-binding molecules hemoglobin in blood and myoglobin in muscles (Kooyman and Ponganis, 1998, Burns et al., 2007). More specific to the brain, Burmester et al. (2000) discovered a third protein which possibly increases brain-specific oxygen stores, namely neuroglobin (Ngb). Indeed, the brain of minke whales *(Balænoptera acuto-rostrata)*, harbor porpoises (*Phocoena phocoena*) and the subterranean mole-rat (*Spalax sp*) have high expression rates of Ngb (Avivi et al., 2010, Schneuer et al., 2012). Surprisingly, Ngb expression in seals is no higher than in terrestrial mammals (Schneuer et al., 2012). The distribution of Ngb, however, differs: Most mammals express Ngb in neurons, where most mitochondria are also found. In seals mitochondria seem to be

primarily located in astrocytes instead of neurons and the same was found for Ngb expression (Mitz et al., 2009), suggesting a different hypoxia adaptation strategy in the seal brain.

Adapted neural metabolism

The main fuel for the mammalian brain is glucose (Clarke and Sokoloff, 1999), which is metabolized anaerobically in astrocytes. There is growing evidence that astrocytes then provide lactate to neurons via an astrocyte-neuron lactate shuttle (ANLS) for aerobic metabolism (Pellerin and Magistretti, 1994, Schurr et al., 1997b, Kasischke et al., 2004, Bergersen, 2007, Pellerin and Magistretti, 2012) even though this hypothesis was challenged by Chih and colleagues (Chih et al., 2001, Chih and Roberts, 2003). Recent research suggests a beneficial role of lactate in the brain (Bergersen, 2015), there is evidence that neurons in mice and rats prefer lactate over glucose as a fuel source in normoxia, and that lactate even supports neuronal recovery after hypoxia (Schurr et al., 1988, Schurr et al., 1997a, Schurr, 2002). In seals, however, the ANLS mechanism might be reversed: due to the localization of mitochondria and Ngb expression in astrocytes (as opposed to neurons) it seems that oxidative metabolism is located primarily in astrocytes (Mitz et al., 2009, Schneuer et al., 2012). This would have the advantage that seal neurons are less dependent on oxygen and avoid oxidative stress from mitochondrial activity (Turrens, 2003, Halliwell, 2006, Mitz et al., 2009), e.g. upon re-oxygenation. If such a reverse ANLS is present in hooded seals, we would expect seal neurons to be well adapted to anaerobic glycolysis and possibly display differences in lactate utilization compared to mice. In **paper I** we therefore compared spontaneous neuronal activity during hypoxia in cortical brain slices from hooded seals and mice *in vitro*, providing them with glucose (10 mM) or the equimolar amount of lactate (20 mM) as fuel. In this context, it is worth noting that post-dive plasma lactate levels of 14-25 mM have been recorded in live seals (Scholander, 1940, Kooyman et al., 1980, Kooyman et al., 1983). In addition, we investigated the role of stored glycogen in the hooded seal brain.

The results of this study confirm the hypoxia tolerance of hooded seal neurons. The observed survival of neurons in hypoxia in presence of glucose might be explained with increased anaerobic capacity in the seal neurons in accordance with a reversed ANLS (Mitz et al., 2009), but surprisingly lactate and aglycemia were equally well tolerated. Lactate levels above 4 mM are usually detrimental (Broder and Weil, 1964, Phypers and Pierce, 2006) which is likely the reason why mice neurons in 20 mM lactate did not survive even in normoxia. The maintenance of activity in the seal neurons in the presence of 20 mM lactate suggests an increased tolerance to lactic acidosis, even though recent research does not confirm increased buffering capacity in the hooded seal brain (Müller-Hoff et al., 2016). The same study, however, suggests an increased aerobic capacity of seal astrocytes, which is in accordance with a reverse ANLS, even though low general levels of LDH dismiss a generally increase anaerobic capacity (Müller-Hoff et al., 2016). The enduring activity of seal neurons in hypoxia might at least partly be explained with the ~4-fold higher glycogen levels that we found in the seal brain compared to in mice, a value which is similar to in other hypoxia-tolerant vertebrates (Kerem et al., 1973, Lutz et al., 2003). Glycogen is mainly found in astrocytes (Cataldo and Broadwell, 1986), where it is thought to support energy production in hypoglycemia (Choi et al., 2003). In accordance with a reversed ANLS hypothesis in seals, we would expect glycogen to be located mainly in neurons, and active neuronal glycogen metabolism has indeed recently been shown to increase hypoxia tolerance even in mice (Saez et al., 2014). The exact location of seal glycogen stores remains to be investigated.

My own preliminary results from *in vitro* studies on hooded seal hippocampal slices seem to contradict the results described above: slices provided with lactate as a fuel-source lost synaptic transmission around 20 min into hypoxic conditions, and did not recover when reoxygenated (n=4, Geiseler, unpublished observations). Hypoxia in a glucose substrate was tolerated for any lengths of time tested, i.e. at least 3 h (paper III) and in one experiment almost 5 h (Geiseler,

unpublished observation). While these results fit well with the suggested reversed ANLS, it is surprising that some seal neurons maintain activity during hypoxia in lactate and aglycemia (cortex) much longer than others do (hippocampus). A possible explanation might be a selective vulnerability of certain brain regions to hypoxia (Cervos-Navarro and Diemer, 1991) and indeed neurons of the CA1 region in the hippocampus seem to be especially vulnerable (Schmidt-Kastner and Freund, 1991, Bartsch et al., 2015).

Protective shutdown response

When oxygen becomes scarce and anaerobic energy production reaches its limits, neurons might survive by reducing activity to prevent energy depletion as suggested by Hochachka some 30 years ago (Hochachka and Dunn, 1983, Hochachka, 1986). A number of animals reduce systemic energy demand by reducing their body metabolism e.g. during hibernation (for review e.g. Heldmaier et al., 2004). Some vertebrates such as freshwater turtles (*Trachemis scripta* and *Chrysemis picta*), crucian carp (*Carassius carassius*) and frogs (*Rana spp*), survive anoxia for hours to months at a time by entering a state of deep hypometabolism (Lutz and Nilsson, 2004, Jackson and Ultsch, 2010). Diving animals usually experience much shorter episodes of hypoxia than hibernating animals. Furthermore, since diving activity is usually connected to foraging, a systemic shutdown as observed in hibernating animals (e.g. Drew et al. 2013), would not be possible. Some systemic reductions in energy demand, however, are also present in diving seals, albeit at a much more modest scale. Seals display bradycardia and vasoconstriction, during dives, thereby reducing perfusion and hence metabolism in body parts not essential for immediate survival (Scholander, 1940, Blix et al., 1983, Folkow and Blix, 2010). This reserves the available resources for critical organs such as the brain, which seems to receive increased perfusion during episodes of hypoxia (Blix and Folkow, 1983). A similar increase in cerebral blood flow was also observed in turtles and crucian carp during shorter episodes and the initial phase of anoxia (Hylland et al., 1994, Nilsson et al., 1994). Adjusted brain

perfusion during hypoxia seems to be also responsible for brain cooling in seals and ducks, which is suggested to reduce cerebral oxygen demand by up to 25% in seals (Caputa et al., 1998, Odden et al., 1999, Blix et al., 2010). There is evidence for reduced neuronal activity in response to hypoxia in the turtle (Fernandes et al., 1997), eider duck (Ludvigsen and Folkow, 2009) and hooded seal (paper I). This suggests that a protective shutdown response is not only present on a systemic level but also specifically in some neurons in hypoxia-tolerant animals (Ramirez et al., 2007).

K_{ATP}-channels

In **paper II** we studied the mechanisms for a depression of activity in response to hypoxia in the eider duck. ATP sensitive potassium channels (K_{ATP}-channels) open when ATP levels are low. The resulting hyperpolarization attenuates neuronal excitability and thereby reduces activity (Hansen, 1985, Fujimura et al., 1997, Mironov and Richter, 2000, Ballanyi, 2004). In addition it was shown that K_{ATP}-channels attenuate excitatory neurotransmitter release (Tanaka et al., 1995) and reduce AMPA receptor currents in anoxic turtle neurons, contributing further to a reduction in neuronal excitability (Zivkovic and Buck, 2010). Looking into the effect of K_{ATP}-channels on spontaneous activity in the cerebellum *in vitro*, we found that K_{ATP}-channels are indeed present in the eider duck cerebellum, even though they do not seem to account alone for the observed shutdown response to hypoxia (paper II).

Adenosine

The neuromodulator adenosine could be an additional protective agent in the shutdown response. Besides activating K_{ATP}-channels, ATP depletion leads to a proportionally greater release of adenosine than other ATP breakdown products, indicating that energy depletion is both a potent and selective stimulus for adenosine formation and release (Nilsson and Peter, 1992, Lloyd et al., 1993). An increase in adenosine in response to hypoxia is well described (Van Wylen et al., 1986, Phillis et al., 1987) and also its presence in the anoxia-tolerant brain of the

freshwater turtle (Nilsson and Peter, 1992, Hylland et al., 1994). Adenosine is commonly associated with neuroprotective functions during hypoxia (Rudolphi et al., 1992, Wardas, 2002, Cunha, 2005). It increases cerebral blood flow (Coney and Marshall, 1998) and modulates neuronal activity by acting on various receptors, leading to a decrease in excitatory transmitter release and inhibition of neuronal activity (Prince and Stevens, 1992, Dunwiddie and Masino, 2001, Andoh et al., 2006). In the freshwater turtle, adenosine seems to promote neuronal ion channel arrest by decreasing NMDA receptor activity and ion permeability (Pek and Lutz, 1997, Buck and Bickler, 1998, Buck, 2004) which further contributes to reduce neuronal excitability. In the naked mole-rat (*Heterocephalus glaber*), however, Larson and Park (2009) found that synaptic transmission seems to be less affected by adenosine as in mouse and suggests this might be due to an altered presynaptic calcium regulation in the naked mole-rat (discussed below).

Inhibitory and excitatory amino acids/neurotransmitters

Another candidate mechanism to protect from excitotoxicity is the regulation of neurotransmitters. In the anoxia tolerant painted turtle, the inhibitory neurotransmitter GABA seems to reduce electrical activity in response to anoxia, both by inhibiting depolarizing ion current and suppressing excitatory glutamate release (Pamenter et al., 2011, Pamenter et al., 2012). In addition, both the freshwater turtle and crucian carp display an increase of GABA concentration in response to hypoxia, while glutamate is downregulated (Nilsson, 1990, Nilsson et al., 1990, Nilsson and Lutz, 1993).

In further experiments, we therefore looked into the effect of glutamate and GABA on spontaneous activity for the same *in vitro* model of spontaneous activity in the cerebellum of the eider duck, which we used in paper II. The observed responses were, however, varying to a large degree in the Purkinje cell layer we recorded from. This was possibly due to the complexity of functional circuits for this area,

which receives multiple inputs of both excitatory and inhibitory nature (Womack and Khodakhah, 2002).

In a further effort to investigate the possible role of neurotransmitters in hypoxia adaptation, we investigated the intrinsic changes of their concentrations in response to hypoxia. Kirschner et al. (2009) showed that hippocampus glutamate and the NMDAR coactivator D-serine are elevated in the rat *in vitro* in response to oxygen glucose deprivation, as expected due to the excitotoxic cascade in the non-hypoxia-tolerant brain. In contrast, the hypoxia-tolerant hibernating arctic ground squirrel (*Spermophilus parryii*), displayed a delayed and attenuated increase in hippocampal glutamate concentration (Drew et al., 2013). In a similar micro-perfusion setup, using cerebellar slices of eider duck and chicken (*Gallus gallus*) *in vitro* we compared the efflux of glutamate, serine and in addition the possibly neuroprotective amino acid glycine. The latter is upregulated in the freshwater turtle in response to anoxia and might be involved in attenuating neuronal activity (Nilsson, 1990, Nilsson et al., 1990). When exposed to hypoxia, the efflux of all compounds from both eider duck and chicken brain slices tended to increase slightly, but the variation in our results was too high to draw any conclusions (Ragazzi et al., 2014).

Synaptic transmission

Synaptic transmission consumes most of the brain's ATP (Harris et al., 2012). To be able to investigate directly how synaptic transmission is affected by hypoxia, we adapted the widely used method of evoked field excitatory post synaptic potential (fEPSP) recordings in the CA1 region of the hippocampus *in vitro* (e.g. Lipton and Whittingham, 1979, Croning and Haddad, 1998, Larson and Park, 2009). This was applied to our hooded seal model as shown in **paper III**.

The hippocampus is a widely studied part of the mammalian brain consisting of several distinct layers and areas. Due to the laminar and highly organized structure, the hippocampus is a well suited organ to study synaptic activity, such

as the fEPSP at the synapses of the Schaffer collateral fibers and pyramidal cell neurons (Andersen, 1960). The selective vulnerability to brain ischemia of the hippocampus, and especially the CA1 region (Schmidt-Kastner and Freund, 1991, Ying et al., 1997), makes this organ a well-suited study object for intrinsic adaptations to hypoxia.

Besides adapting the method for the first time to large mammals, we were not only able to confirm that seal neurons tolerate severe hypoxia for an extended time, but we also showed that they are able to maintain synaptic transmission for at least 3 h in severe hypoxia (paper III). Notably, also for the synaptic transmission, we observed a depression in activity (fEPSP) in response to hypoxia. The signal, however, always remained at around 30 % of the initial strength. Possible mechanisms for the reduction in neuronal activity in response to hypoxia are discussed above but remain to be investigated in the hooded seal. As mentioned, adenosine has an inhibitory effect in non-hypoxia-tolerant animals and is also upregulated in the turtle in response to hypoxia. Since synaptic transmission in the naked mole-rat is much less inhibited by adenosine than in rats (Larson and Park, 2009), adenosine might not be directly involved in the immediate shutdown of activity. The underlying reason is possibly an altered presynaptic calcium regulation in hypoxia tolerant neurons in mammals, as discussed below.

Calcium regulation

Even though moderate increase of intracellular calcium seems to be neuroprotective (Bickler and Fahlman, 2004), the excessive glutamate-mediated influx of calcium is one of the principal causes of hypoxic damage (Lutz et al., 2003). Reduced Ca^{2+} influx was indeed observed in hypoxia-tolerant animals (Bickler and Gallego, 1993, Peterson et al., 2012a) but the exact mechanisms behind the protection from extensive Ca^{2+}-influx remain to be elucidated. Interestingly, the hypoxia-tolerant naked mole-rat lacks the otherwise ubiquitous

short-term neuroplastic phenomenon of paired pulse facilitation (PPF) (Zucker, 1989, Larson and Park, 2009) and also in the hooded seal we found significantly attenuated PPF compared to reindeer or mouse. According to the residual calcium theory (e.g. Katz and Miledi, 1968 and Zucker, 1989) the saturation of calcium binding proteins, such as calbindin d28k, might be responsible for a transient increase in Ca^{2+} in synaptic terminals immediately after excitation (Blatow et al., 2003). It was shown that an increase in calbindin d28k or the presence of parvalbumin suppresses PPF (Chard et al., 1995, Caillard et al., 2000). In this context, we investigated the hypothesis of increased Ca^{2+} buffering ability in hooded seals and compared calbindin d28k and parvalbumin gene-expression in response to normoxia and hypoxia in hippocampal slices from seals compared to mice. Even though we did not find a difference between normoxia and hypoxia in either species, we do not exclude the calcium buffering theory, since the calcium buffering capacity of the seal could be constitutively enhanced in the hypoxia-tolerant seal neurons, which would also explain why we see attenuated PPF *in normoxia*. To clarify this question, however, a detailed quantitative analysis of the actual protein levels would be needed to investigate the Ca^{2+} buffering capacity in synaptic terminals of hooded seals.

Additional intrinsic hypoxia adaptations

Besides the described mechanisms, a number of additional repair and protective mechanisms have been investigated in hypoxia-tolerant animals. Excitability seems to be generally reduced, indicated by beta-opioid mediated suppression of NMDA receptor currents in the turtle brain (Pamenter and Buck, 2008) and decreased NMDA receptor function in the arctic ground squirrel (Zhao et al., 2006). Heat shock proteins (HSP) are ubiquitous protectors against stress-induced denaturation (Feder and Hofmann, 1999) and high levels of HSP were found in the turtle in response to anoxia (Lutz and Milton, 2004). Further repair mechanisms are upregulated in the turtle (Milton et al., 2008, Nayak et al., 2011) and might be active in the arctic ground squirrel (Dave et al., 2009). Both Ngb and adenosine

seem to protect the turtle brain from reactive oxygen species (Milton et al., 2007, Nayak et al., 2009).

Neonates and preconditioning

Limited intrinsic hypoxia tolerance exists also in terrestrial mammals. Neonate terrestrial mammals experience intrauterine oxygen levels corresponding to 8000 m altitude and correspondingly express a similar neural tolerance to hypoxia as some of the above described animals (Singer, 1999, Bickler, 2004, Folkow et al., 2008). Neonatal rats exhibit decreased glutamate mediated calcium influx in response to hypoxia (Friedman and Haddad, 1993, Bickler and Hansen, 1998) similar to turtles or naked mole-rat (Bickler and Gallego, 1993, Peterson et al., 2012a). More parallels in the naked mole-rat to neonate terrestrial mammals are a retention of neonatal NMDA subunits (Peterson et al., 2012b) and the absence of PPF (Larson and Park, 2009). The maintenance of ion balance and delay in hypoxic depolarization is similar in turtles and neonates (Hansen, 1977, Sick et al., 1982, Trippenbach et al., 1990), possibly due to low membrane permeability and ion channel density (Edwards et al., 1989, Xia and Haddad, 1994).

Leading back to the clinical relevance of the research described in this thesis, I will briefly mention ischemic preconditioning (IPC). Exposing the brain to short durations of ischemia (i.e. IPC) seems to trigger intrinsic neural protective mechanisms (e.g. Gilchrist and Gidday, 2013). Those protective mechanisms are strikingly similar to what we observe in “true” hypoxia-tolerant animals: IPC suppresses for example excitotoxicity due to increased GABA and decreased glutamate release (Dave et al., 2005) and activates neuroprotective pathways (Raval et al., 2007, Dave et al., 2008). The detailed mechanisms of IPC are, however, beyond the scope of this thesis and are, together with the clinical relevance of IPC, extensively reviewed e.g. by Dirnagl et al. (2009) and Narayanan et al. (2013).

Conclusions and further research

Summarizing the research done on intrinsic hypoxia tolerance, it seems that adapted brains have several defense mechanisms against the detrimental steps described in figure 1. Hypoxia-tolerant neurons 1) maintain adequate energy levels by focusing energy supply or switching to alternative metabolism and by decreasing energy demand due to a reduction of neuronal metabolism and activity; 2) maintain ion balance and membrane potential by reducing ion flux; 3) attenuate release of neurotransmitters (glutamate) and activation of NMDA/AMPA receptors; 4) attenuate influx of Ca^{2+} and release from intracellular stores and thereby prevent Ca^{2+}-overload, and possibly buffer excessive intracellular Ca^{2+} concentrations; 5) inhibit detrimental enzyme systems and prevent production of free radicals and 6) employ various repair and maintenance systems to prevent neuronal degradation (fig 2).

This thesis sheds new light on intrinsic neural adaptations to hypoxia of diving animals. It was shown that K_{ATP}-channels are present in the eider duck brain and possibly involved to some extent in a protective shutdown response to hypoxia (paper III). Furthermore, it was shown that the hooded seal is able to maintain neuronal activity even in the absence of glucose, possibly due to increased neuronal glycogen reserves and that its neurons also tolerate exposure to high lactate levels (paper I). Not only was it possible to confirm the neuronal hypoxia tolerance in the seal brain, but also to show that the usually particularly hypoxia sensitive CA1 region of the hippocampus maintains synaptic transmission for at least 3 h in severe hypoxia (paper III). Both the results from paper I and III are in accordance with the reverse ANLS hypothesis. The attenuated paired pulse facilitation points into the direction of an altered calcium regulation in the hooded seal. By establishing hippocampal evoked fEPSP recording in large mammals (both seal and reindeer) for the first time, we enable further specific research using the

possibilities of a reliable electrophysiology method in a well-studied part of the mammalian brain. It would be for example possible to perform live calcium imaging (Lattarulo et al., 2011) while performing evoked fEPSP experiments in hypoxia, similar to what we did in paper III, simultaneously (Pozzo Miller et al., 1993). This model could then be used to further investigate the hypothesis of blunted calcium influx in hooded seal neurons and the underlying mechanisms.

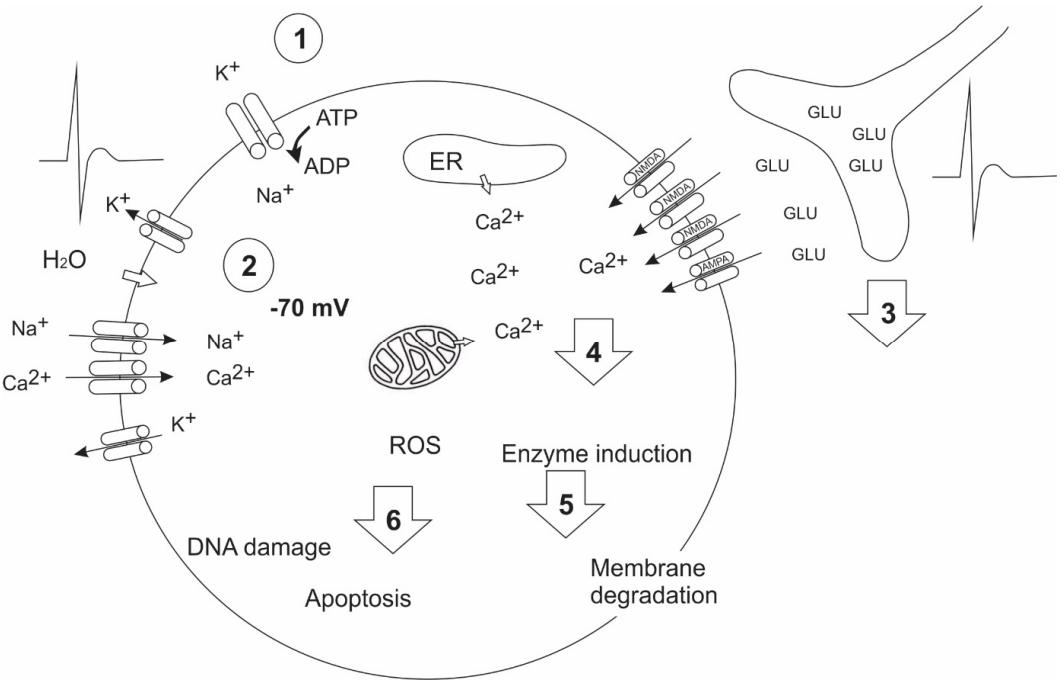


Figure 2: Hypoxia tolerant-neurons in hypoxia: **1** energy levels remain adequate, **2** ion balance and membrane potential is maintained, **3** attenuated release of neurotransmitters (glutamate) and activation of NMDA/AMPA receptors, **4** attenuated influx of Ca^{2+} and release from intracellular stores, preventing Ca^{2+}-overload, **5** inhibition of detrimental enzyme systems and reduction of free radicals, **6** various repair and maintenance systems prevent neuronal degradation.

Methodological considerations

Methods

Studying spontaneous neuronal activity gives a good indication of general viability of neurons during experimental manipulation. This method has, however, its limitations concerning specificity of neuronal activity, which is why we adapted the method of hippocampal evoked fEPSP to the hooded seal. Despite this method being widely used, it was never previously applied to large mammals. Scaling the method up to a hippocampus roughly 100 times the size of the commonly used rodent hippocampus proved to be a significant challenge, not least due to limited availability of tissue (see below).

In paper I, the response of spontaneous activity to hypoxia in cortical seal slices was not consistent, some of the slices displayed sustained activity throughout, some showed a slight reduction in activity and some went completely silent in response to hypoxia (see e.g. fig 3 in paper I). Ramirez et al. (2011) hypothesized that intrinsically oscillating persistent activity was observed in seal cortical slices due to unusually thick slices (680 µm), allowing larger networks to remain intact. The use of much thinner slices (400 µm) in our experiments might have compromised the integrity of such networks to some extent, which might explain the differing results.

Animal model

The hooded seal displays impressive diving performance (Folkow and Blix, 1999) and shows a high intrinsic neural tolerance to hypoxia. Together with its relatively large and complex brain, this makes it a relevant model species to study adaptations to such tolerance. Those animals are, however, difficult to come by due to their distribution in the north Atlantic (Folkow and Blix, 1995) and access to them is very limited. We were fortunate to have access once a year during the

breeding season and were able to collect some animals to keep them at our animal facilities in Tromsø. To be able to study not only juvenile and captive animals, we also brought a mobile laboratory, which we put on the deck of a research vessel. This enabled us to perform some of the electrophysiology at the breeding site of hooded seals on location in the pack-ice offshore East Greenland (West-Ice), but represented an obvious large logistic challenge. To supplement the seal data we studied also the eider duck, which is more easily accessible than the hooded seal. Since there is very little knowledge/comparative research about mechanisms of avian neural hypoxia tolerance, studying the regular diving eider duck may provide valuable comparative data.

Ethics

All experiments were performed in accordance with the Norwegian Animal Welfare Act and current Norwegian Regulations on the Use of Animals in Experimentation. Animal suffering was consequently kept to an absolute minimum (catching and handling of the animals) and all electrophysiology experiments on living tissue were performed post-mortem. All relevant approvals were obtained as listed in the papers. The number of animals used were limited to a minimum.

Overview of abbreviations and terms

AMPA(R)	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)
BM	Body mass
NMDA(R)	N-methyl-D-aspartate (receptor)
ANLS	Astrocyte neuron lactate shuttle
ATP	Adenosine triphosphate
fEPSP	Field excitatory post synaptic potential
GABA	γ-Aminobutyric acid
HSP	Heat shock protein
IPC	Ischemic preconditioning
K_{ATP}-channels	ATP dependent potassium channels
LDH	Lactate dehydrogenase
Ngb	Neuroglobin
OGD	Oxygen glucose deprived
PPF	Paired pulse facilitation
ROS	Reactive oxygen species

Anoxia	Absence of oxygen supply to an organ or a tissue
Hypoxia	Lower than normal / inadequate oxygen supply to an organ or a tissue
Ischemia	Insufficient supply of blood (perfusion) to an organ or a tissue

Source: medical dictionary (http://medical-dictionary.thefreedictionary.com/)

Acknowledgements

First and foremost I want to thank my main supervisor Lars Folkow to give me the possibility to pursuit this PhD. You gave me great opportunities to fulfil my interest in exciting fieldwork and provided me with lab-space many other PhD students can only dream of. You provided me with academic freedom and kept me grounded when I got too ambitious. Most importantly, you believed I could actually do research on a PhD level and write a thesis, or at least you gave me the impression you did. Either way, here it is, and I might not have written it without your excellent guidance throughout my studies!

I also would like to thank my co-supervisor Nicole Czech-Damal. Even though you were not as present during my PhD time, I got a lot of valuable help from you. You were among the first I dared to show the first drafts of my papers and you always managed to reignite my enthusiasm about my work.

Furthermore I would like to thank Dr. John Larson for hosting me in his lab in Chicago and introducing me to the electrophysiology of the hippocampus. Your wealth of knowledge and experience with electrophysiology was incredibly helpful to develop the methods for paper III. I really appreciate your help on this project!

The technicians at the “Avdeling for Arktisk Biologi” also helped me a lot by taking care of the research animals in Tromsø, providing me with a mobile laboratory and Chandra Ravuri by supporting me with gene expression analyses. In this context, I would also like to thank the crew of R/V Helmer Hanssen and cruise leader Erling Nordøy for their support during field trips.

I am thankful for statistical advice from Mikko Vihtakari and of course I am grateful for all the support I got from my colleagues and friends throughout the last 4 years!

References

Andersen P (1960) Interhippocampal impulses. II. Apical dendritic activation of CAI neurons. Acta Physiol Scand 48:178-208.

Andoh T, Ishiwa D, Kamiya Y, Echigo N, Goto T, Yamada Y (2006) A(1) adenosine receptor-mediated modulation of neuronal ATP-sensitive K channels in rat substantia nigra. Brain Res 1124:55-61.

Avivi A, Gerlach F, Joel A, Reuss S, Burmester T, Nevo E, Hankeln T (2010) Neuroglobin, cytoglobin, and myoglobin contribute to hypoxia adaptation of the subterranean mole rat Spalax. Proc Natl Acad Sci U S A 107:21570-21575.

Ballanyi K (2004) Protective role of neuronal K-ATP channels in brain hypoxia. J Exp Biol 207:3201-3212.

Bartsch T, Dohring J, Reuter S, Finke C, Rohr A, Brauer H, Deuschl G, Jansen O (2015) Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. J Cereb Blood Flow Metab 35:1836-1845.

Bergersen LH (2007) Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle. Neuroscience 145:11-19.

Bergersen LH (2015) Lactate transport and signaling in the brain: Potential therapeutic targets and roles in body-brain interaction. J Cereb Blood Flow Metab 35:176-185.

Bickler PE (2004) Clinical perspectives: neuroprotection lessons from hypoxia-tolerant organisms. J Exp Biol 207:3243-3249.

Bickler PE, Fahlman CS (2004) Moderate increases in intracellular calcium activate neuroprotective signals in hippocampal neurons. Neuroscience 127:673-683.

Bickler PE, Gallego SM (1993) Inhibition of brain calcium channels by plasma proteins from anoxic turtles. Am J Physiol 265:R277-281.

Bickler PE, Hansen BM (1998) Hypoxia-tolerant neonatal CA1 neurons: relationship of survival to evoked glutamate release and glutamate receptor-mediated calcium changes in hippocampal slices. Brain Res Dev Brain Res 106:57-69.

Blatow M, Caputi A, Burnashev N, Monyer H, Rozov A (2003) Ca2+ buffer saturation underlies paired pulse facilitation in calbindin-D28k-containing terminals. Neuron 38:79-88.

Blix AS, Elsner RW, Kjekshus JK (1983) Cardiac output and its distribution through capillaries and A-V shunts in diving seals. Acta Physiol Scand 118:109-116.

Blix AS, Folkow B (1983) Cardiovascular adjustments to diving in mammals and birds. In: Handbook of Physiology (Shepherd, J. T. and Abboud, F. M., eds), pp 917-945 Bethesda: American Physiological Society.

Blix AS, Walloe L, Messelt EB, Folkow LP (2010) Selective brain cooling and its vascular basis in diving seals. J Exp Biol 213:2610-2616.

Broder G, Weil MH (1964) Exess lactate: An index of reversibility of shock in human patients. Science 143:1457-1459.

Buck LT (2004) Adenosine as a signal for ion channel arrest in anoxia-tolerant organisms. Comp Biochem Physiol B Biochem Mol Biol 139:401-414.

Buck LT, Bickler PE (1998) Adenosine and anoxia reduce N-methyl-D-aspartate receptor open probability in turtle cerebrocortex. J Exp Biol 201:289-297.

Burmester T, Weich B, Reinhardt S, Hankeln T (2000) A vertebrate globin expressed in the brain. Nature 407:520-523.

Burns JM, Lestyk K, Folkow LP, Hammill MO, Blix AS (2007) Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. J Comp Physiol B 177:687-700.

Caillard O, Moreno H, Schwaller B, Llano I, Celio MR, Marty A (2000) Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Natl Acad Sci U S A 97:13372-13377.

Cajal SR (1909-1911) Histologie du Systeme Nerveux de l'Homme et des Vertebretes. Paris: Maloine, A.

Caputa M, Folkow L, Blix AS (1998) Rapid brain cooling in diving ducks. Am J Physiol 275:R363-371.

Cataldo AM, Broadwell RD (1986) Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. II. Choroid plexus and ependymal epithelia, endothelia and pericytes. J Neurocytol 15:511-524.

Cervos-Navarro J, Diemer NH (1991) Selective vulnerability in brain hypoxia. Crit Rev Neurobiol 6:149-182.

Chard PS, Jordán J, Marcuccilli CJ, Miller RJ, Leiden JM, Roos RP, Ghadge GD (1995) Regulation of excitatory transmission at hippocampal synapses by calbindin D28k. Proc Natl Acad Sci U S A 92:5144-5148.

Chih C-P, Lipton P, Roberts Jr EL (2001) Do active cerebral neurons really use lactate rather than glucose? Trends Neurosci 24:573-578.

Chih C-P, Roberts EL (2003) Energy Substrates for Neurons during Neural Activity: A Critical Review of the Astrocyte-Neuron Lactate Shuttle Hypothesis. J Cereb Blood Flow Metab 23:1263-1281.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Choi I-Y, Seaquist ER, Gruetter R (2003) Effect of hypoglycemia on brain glycogen metabolism in vivo. J Neurosci Res 72:25-32.

Clarke DD, Sokoloff L (1999) Substrates of cerebral metabolism. In: Basic Neurochemistry: Molecular, Cellular and Medical Aspects (G.J., S. et al., eds) Philadelphia: Lippincott-Raven.

Coney AM, Marshall JM (1998) Role of adenosine and its receptors in the vasodilatation induced in the cerebral cortex of the rat by systemic hypoxia. J Physiol 509:507-518.

Croning MD, Haddad GG (1998) Comparison of brain slice chamber designs for investigations of oxygen deprivation in vitro. J Neurosci Methods 81:103-111.

Cunha RA (2005) Neuroprotection by adenosine in the brain: From A(1) receptor activation to A(2A) receptor blockade. Purinergic Signal 1:111-134.

Dave KR, Anthony DeFazio R, Raval AP, Dashkin O, Saul I, Iceman KE, Perez-Pinzon MA, Drew KL (2009) Protein kinase C epsilon activation delays neuronal depolarization during cardiac arrest in the euthermic arctic ground squirrel. J Neurochem 110:1170-1179.

Dave KR, DeFazio RA, Raval AP, Torraco A, Saul I, Barrientos A, Perez-Pinzon MA (2008) Ischemic preconditioning targets the respiration of synaptic mitochondria via protein kinase C epsilon. J Neurosci 28:4172-4182.

Dave KR, Lange-Asschenfeldt C, Raval AP, Prado R, Busto R, Saul I, Perez-Pinzon MA (2005) Ischemic preconditioning ameliorates excitotoxicity by shifting glutamate/gamma-aminobutyric acid release and biosynthesis. J Neurosci Res 82:665-673.

Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. Lancet Neurol 8:398-412.

Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391-397.

Drew KL, Harris MB, LaManna JC, Smith MA, Zhu XW, Ma YL (2004) Hypoxia tolerance in mammalian heterotherms. J Exp Biol 207:3155-3162.

Drew KL, Zuckermann JA, Shenk PE, Bogren LK, Jinka TR, Moore JT (2013) Hibernation: A Natural Model of Tolerance to Cerebral Ischemia/Reperfusion. In: Innate Tolerance in the CNS (Gidday, J. M. et al., eds), pp 241-335 London: Springer.

Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24:31-55.

Edwards RA, Lutz PL, Baden DG (1989) Relationship between energy expenditure and ion channel density in the turtle and rat brain. Am J Physiol 257:R1354-1358.

Eisert R, Potter CW, Oftedal OT (2014) Brain size in neonatal and adult Weddell seals: Costs and consequences of having a large brain. Mar Mamm Sci 30:184-205.

Erecińska M, Silver IA (1994) Ions and energy in mammalian brain. Prog Neurobiol 43:37-71.

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243-282.

Fernandes JA, Lutz PL, Tannenbaum A, Todorov AT, Liebovitch L, Vertes R (1997) Electroencephalogram activity in the anoxic turtle brain. Am J Physiol 273:R911-919.

Folkow LP, Blix AS (1995) Distribution and diving behavior of hooded seals. In: Whales, seals, fish and man (Blix, A. S. et al., eds), pp 193-202 Amsterdam: Elsevier Science.

Folkow LP, Blix AS (1999) Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian seas. Polar Biol 22:61-74.

Folkow LP, Blix AS (2010) Air breathers under water: Diving mammals and birds. In: Respiratory physiology of vertebrates – Life with and without oxygen (Nilsson, G. E., ed), pp 222-264 Cambridge: Cambridge University Press.

Folkow LP, Ramirez J-M, Ludvigsen S, Ramirez N, Blix AS (2008) Remarkable neuronal hypoxia tolerance in the deep-diving adult hooded seal (Cystophora cristata). Neurosci Lett 446:147-150.

Friedman JE, Haddad GG (1993) Major differences in Ca2+i response to anoxia between neonatal and adult rat CA1 neurons: role of Ca2+o and Na+o. J Neurosci 13:63-72.

Fujimura N, Tanaka E, Yamamoto S, Shigemori M, Higashi H (1997) Contribution of ATP-Sensitive Potassium Channels to Hypoxic Hyperpolarization in Rat Hippocampal CA1 Neurons In Vitro. J Neurophysiol 77:378-385.

Gilchrist RD, Gidday JM (2013) Hypoxic Preconditioning in the CNS. In: Innate Tolerance in the CNS (Gidday, J. M. et al., eds), pp 183-212: Springer New York.

Graham AJW, Hickie JP (1986) Relative brain size in marine mammals. Am Nat 128:445-459.

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634-1658.
Hansen AJ (1977) Extracellular potassium concentration in juvenile and adult rat brain cortex during anoxia. Acta Physiol Scand d99:412-420.
Hansen AJ (1985) Effect of anoxia on ion distribution in the brain. Physiol Rev 65:101-148.
Harris Julia J, Jolivet R, Attwell D (2012) Synaptic energy use and supply. Neuron 75:762-777.
Heldmaier G, Ortmann S, Elvert R (2004) Natural hypometabolism during hibernation and daily torpor in mammals. Respir Physiol Neurobiol 141:317-329.
Hochachka PW (1986) Metabolic arrest. Intensive Care Med 12:127-133.
Hochachka PW, Dunn JF (1983) Metabolic arrest: The most effective means of protecting tissues against hypoxia. In: Hypoxia, exercise, and altitude: Proceedings of the third banff international hypoxia symposium, pp 297-309 New York: Alan R. Liss, Inc.
Hylland P, Nilsson GE, Lutz PL (1994) Time course of anoxia-induced increase in cerebral blood flow rate in turtles: Evidence for a role of adenosine. J Cereb Blood Flow Metab 14:877-881.
Jackson DC, Ultsch GR (2010) Physiology of hibernation under the ice by turtles and frogs. J Exp Zool A Ecol Genet Physiol 313:311-327.
Kasischke KA, Vishwasrao HD, Fisher PJ, Zipfel WR, Webb WW (2004) Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. Science 305:99-103.
Katz B, Miledi R (1968) The role of calcium in neuromuscular facilitation. J Physiol 195:481-492.
Kerem DH, Hammond DD, Elsner RW (1973) Tissue glycogen levels in the Weddell seal (Leptonychotes weddelli): A possible adaptation to asphyxial hypoxia. Comp Biochem Physiol A Mol Integr Physiol 45:731-736.
Kirschner DL, Wilson AL, Drew KL, Green TK (2009) Simultaneous efflux of endogenous D-ser and L-glu from single acute hippocampus slices during oxygen glucose deprivation. J Neurosci Res 87:2812-2820.
Kooyman GL (1989) Diverse divers: Physiology and behavior. Berlin: Springer-Verlag.
Kooyman GL, Castellini MA, Davis RW, Maue RA (1983) Aerobic diving limits of immature Weddell seals. JCompPhysiol 151:171-174.
Kooyman GL, Ponganis PJ (1998) The physiological basis of diving to depth: Birds and mammals. Annu Rev Physiol 60:19-32.
Kooyman GL, Wahrenbrock EA, Castellini MA, Davis RW, Sinnett EE (1980) Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. J Comp Physiol B 138:335-346.
Krogh A (1929) The Progress of Physiology. Am J Physiol 90:243-251.
Larson J, Drew KL, Folkow LP, Milton SL, Park TJ (2014) No oxygen? No problem! Intrinsic brain tolerance to hypoxia in vertebrates. J Exp Biol 217:1024-1039.
Larson J, Park TJ (2009) Extreme hypoxia tolerance of naked mole-rat brain. Neuroreport 20:1634-1637.
Lattarulo C, Thyssen D, Kuchibholta KV, Hyman BT, Bacskaiq BJ (2011) Microscopic imaging of intracellular calcium in live cells using lifetime-based ratiometric measurements of Oregon Green BAPTA-1. Methods Mol Biol 793:377-389.

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79:1431-1568.
Lipton P, Whittingham TS (1979) The effect of hypoxia on evoked potentials in the in vitro hippocampus. J Physiol 287:427-438.
Lloyd HG, Lindstrom K, Fredholm BB (1993) Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical stimulation or energy depletion. Neurochem Int 23:173-185.
Ludvigsen S, Folkow LP (2009) Differences in in vitro cerebellar neuronal responses to hypoxia in eider ducks, chicken and rats. J Comp Physiol A 195:1021-1030.
Lutz PL, Milton SL (2004) Negotiating brain anoxia survival in the turtle. J Exp Biol 207:3141-3147.
Lutz PL, Nilsson GE (2004) Vertebrate brains at the pilot light. Respir Physiol Neurobiol 141:285-296.
Lutz PL, Nilsson GE, Prentice HM (2003) The brain without oxygen. Dordrecht, NL: Kluwer Academic Publishers.
Milton SL, J Dirk L, F Kara L, Prentice HM (2008) Adenosine modulates ERK1/2, PI3K/Akt, and p38MAPK activation in the brain of the anoxia-tolerant turtle Trachemys scripta. J Cereb Blood Flow Metab 28:1469-1477.
Milton SL, Nayak G, Kesaraju S, Kara L, Prentice HM (2007) Suppression of reactive oxygen species production enhances neuronal survival in vitro and in vivo in the anoxia-tolerant turtle Trachemys scripta. J Neurochem 101:993-1001.
Mink JW, Blumenschine RJ, Adams DB (1981) Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. Am J Physiol 241:R203-212.
Mironov SL, Richter DW (2000) Intracellular signalling pathways modulate K-ATP channels in inspiratory brainstem neurones and their hypoxic activation: involvement of metabotropic receptors, G-proteins and cytoskeleton. Brain Res 853:60-67.
Mitz SA, Reuss S, Folkow LP, Blix AS, Ramirez JM, Hankeln T, Burmester T (2009) When the brain goes diving: glial oxidative metabolism may confer hypoxia tolerance to the seal brain. Neuroscience 163:552-560.
Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER, 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB (2015) Heart disease and stroke statistics-2015 update: a report from the American Heart Association. Circulation 131:e29-322.
Müller-Hoff ML, Fabrizius A, Folkow LP, Burmester T (2016) An atypical distribution of lactate dehydrogenase isoenzymes in the hooded seal (Cystophora cristata) brain may reflect a biochemical adaptation to diving. J Comp Physiol B in press.
Narayanan SV, Dave KR, Perez-Pinzon MA (2013) Ischemic preconditioning and clinical scenarios. Curr Opin Neurol 26:1-7.
Nayak G, Prentice HM, Milton SL (2009) Role of neuroglobin in regulating reactive oxygen species in the brain of the anoxia-tolerant turtle Trachemys scripta. J Neurochem 110:603-612.

Nayak GH, Prentice HM, Milton SL (2011) Neuroprotective signaling pathways are modulated by adenosine in the anoxia tolerant turtle. J Cereb Blood Flow Metab 31:467-475.

Nilsson E, Peter LL (1992) Short communication: adenosine release in the anoxic turtle brain: a possible mechanism for anoxic survival. In: J exp Biol: Citeseer.

Nilsson GE (1990) Long-term anoxia in crucian carp: Changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. J Exp Biol 150:295-320.

Nilsson GE, Alfaro AA, Lutz PL (1990) Changes in turtle brain neurotransmitters and related substances during anoxia. Am J Physiol 259:R376-384.

Nilsson GE, Hylland P, Lofman CO (1994) Anoxia and adenosine induce increased cerebral blood flow rate in crucian carp. Am J Physiol 267:R590-595.

Nilsson GE, Lutz PL (1993) Role of GABA in hypoxia tolerance, metabolic depression and hibernation possible links to neurotransmitter evolution. Comp Biochem Phys C 105:329-336.

Novelli A, Reilly JA, Lysko PG, Henneberry RC (1988) Glutamate becomes neurotoxic via the N-methyl-d-aspartate receptor when intracellular energy levels are reduced. Brain Res 451:205-212.

Odden A, Folkow LP, Caputa M, Hotvedt R, Blix AS (1999) Brain cooling in diving seals. Acta Physiol Scand 166:77-78.

Pamenter ME, Buck LT (2008) delta-Opioid receptor antagonism induces NMDA receptor-dependent excitotoxicity in anoxic turtle cortex. J Exp Biol 211:3512-3517.

Pamenter ME, Hogg DW, Gu XQ, Buck LT, Haddad GG (2012) Painted turtle cortex is resistant to an in vitro mimic of the ischemic mammalian penumbra. J Cereb Blood Flow Metab 32:2033-2043.

Pamenter ME, Hogg DW, Ormond J, Shin DS, Woodin MA, Buck LT (2011) Endogenous GABA(A) and GABA(B) receptor-mediated electrical suppression is critical to neuronal anoxia tolerance. 108:11274-11279.

Pek M, Lutz PL (1997) Role for adenosine in channel arrest in the anoxic turtle brain. J Exp Biol 200:1913-1917.

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci 91:10625-10629.

Pellerin L, Magistretti PJ (2012) Sweet sixteen for ANLS. J Cereb Blood Flow Metab 32:1152-1166.

Peterson BL, Larson J, Buffenstein R, Park TJ, Fall CP (2012a) Blunted neuronal calcium response to hypoxia in naked mole-rat hippocampus. PloS one 7:e31568.

Peterson BL, Park TJ, Larson J (2012b) Adult naked mole-rat brain retains the NMDA receptor subunit GluN2D associated with hypoxia tolerance in neonatal mammals. Neurosci Lett 506:342-345.

Phillis JW, Walter GA, O'Regan MH, Stair RE (1987) Increases in cerebral cortical perfusate adenosine and inosine concentrations during hypoxia and ischemia. J Cereb Blood Flow Metab 7:679-686.

Phypers B, Pierce JT (2006) Lactate physiology in health and disease. CEACCP 6:128-132.

Pozzo Miller LD, Petrozzino JJ, Mahanty NK, Connor JA (1993) Optical imaging of cytosolic calcium, electrophysiology, and ultrastructure in pyramidal neurons of organotypic slice cultures from rat hippocampus. Neuroimage 1:109-120.

Prince DA, Stevens CF (1992) Adenosine decreases neurotransmitter release at central synapses. Proc Natl Acad Sci USA 89:8586-8590.

Ragazzi L, Geiseler SJ, Folkow LP (2014) Efflux of neurotransmitters from brain slices during O2 deprivation - comparing diving and non-diving species. Acta Physiol Scand 211 (Suppl. 696):68.

Ramirez J-M, Folkow LP, Blix AS (2007) Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. Annu Rev Physiol 69:113-143.

Ramirez JM, Folkow LP, Ludvigsen S, Ramirez PN, Blix AS (2011) Slow intrinsic oscillations in thick neocortical slices of hypoxia-tolerant deep diving seals. Neuroscience 177:35-42.

Raval AP, Dave KR, DeFazio RA, Perez-Pinzon MA (2007) epsilonPKC phosphorylates the mitochondrial K(+) (ATP) channel during induction of ischemic preconditioning in the rat hippocampus. Brain Res 1184:345-353.

Rolfe DF, Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 77:731-758.

Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB (1992) Neuroprotective role of adenosine in cerebral ischaemia. Trends Pharmacol Sci 13:439-445.

Saez I, Duran J, Sinadinos C, Beltran A, Yanes O, Tevy MF, Martinez-Pons C, Milan M, Guinovart JJ (2014) Neurons have an active glycogen metabolism that contributes to tolerance to hypoxia. J Cereb Blood Flow Metab 34:945-955.

Schmidt-Kastner R, Freund TF (1991) Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 40:599-636.

Schneuer M, Flachsbarth S, Czech-Damal NU, Folkow LP, Siebert U, Burmester T (2012) Neuroglobin of seals and whales: Evidence for a divergent role in the diving brain. Neuroscience 223:35-44.

Scholander PF (1940) Experimental investigations on the respiratory function in diving mammals and birds. Hvalrådets Skrifter, Norske Videnskamps Akademi Oslo 22:1-131.

Schurr A (2002) Lactate, glucose and energy metabolism in the ischemic brain Int J Mol Med 10:131-136.

Schurr A, Payne RS, Miller JJ, Rigor BM (1997a) Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: Further in vitro validation. J Neurochem 69:423-426.

Schurr A, Payne RS, Miller JJ, Rigor BM (1997b) Glia are the main source of lactate utilized by neurons for recovery of function posthypoxia. Brain Res 774:221-224.

Schurr A, West C, Rigor B (1988) Lactate-supported synaptic function in the rat hippocampal slice preparation. Science 240:1326-1328.

Sick TJ, Rosenthal M, LaManna JC, Lutz PL (1982) Brain potassium ion homeostasis, anoxia, and metabolic inhibition in turtles and rats. Am J Physiol 243:R281-288.

Singer D (1999) Neonatal tolerance to hypoxia: A comparative-physiological approach. Comp Biochem Physiol A Mol Integr Physiol 123:221-234.

Tanaka T, Yoshida M, Yokoo H, Mizoguchi K, Tanaka M (1995) The role of ATP-sensitive potassium channels in striatal dopamine release: An in vivo microdialysis study. Pharmacol Biochem Behav 52:831-835.

Trippenbach T, Richter DW, Acker H (1990) Hypoxia and ion activities within the brain stem of newborn rabbits. J Appl Physiol 68:2494-2503.

Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552:335-344.

Van Wylen DGL, Park TS, Rubio R, Berne RM (1986) Increases in cerebral interstitial fluid adenosine concentration during hypoxia, local potassium infusion, and ischemia. J Cereb Blood Flow Metab 6:522-528.

Wardas J (2002) Neuroprotective role of adenosine in the CNS. Pol J Pharmacol 54:313-326.

Womack M, Khodakhah K (2002) Active contribution of dendrites to the tonic and trimodal patterns of activity in cerebellar Purkinje neurons. J Neurosci 22:10603-10612.

Xia Y, Haddad GG (1994) Postnatal development of voltage-sensitive Na+ channels in rat brain. J Comp Neurol 345:279-287.

Ying HS, Weishaupt JH, Grabb M, Canzoniero LM, Sensi SL, Sheline CT, Monyer H, Choi DW (1997) Sublethal oxygen-glucose deprivation alters hippocampal neuronal AMPA receptor expression and vulnerability to kainate-induced death. J Neurosci 17:9536-9544.

Zhao HW, Ross AP, Christian SL, Buchholz JN, Drew KL (2006) Decreased NR1 phosphorylation and decreased NMDAR function in hibernating Arctic ground squirrels. J Neurosci Res 84:291-298.

Zivkovic G, Buck LT (2010) Regulation of AMPA receptor currents by mitochondrial ATP-sensitive K+ channels in anoxic turtle neurons. J Neurophysiol 104:1913-1922.

Zucker RS (1989) Short-term synaptic plasticity. Annu Rev neurosci 12:13-31.

Original papers